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Automated Uniaxial Tension System for Mechanically Active Tissue Culture

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Development of an Automated, Uniaxial Tension System for Mechanically Active Tissue Culture

A Major Qualifying Project report submitted to the faculty of Worcester Polytechnic Institute
In partial fulfillment of the requirements for the Degree of Bachelor of Science, by:

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Abstract

Heart disease claims more lives worldwide than any other disease [1]. Victims of myocardial infarctions (heart attacks) suffer from permanently damaged cardiac muscle tissue and reduced heart function [2]. Stem cells offer a potential means of regenerating this damaged heart tissue, but researchers have struggled to efficiently deliver the cells to the heart [3]. The Gaudette Laboratory uses fibrin sutures to deliver these cells with high engraftment rate, yet the seeded cells remain immature and incapable of generating sufficient, contractile force [4]. Research suggests that cyclically stretching the seeded cells promotes their development into mature, aligned cardiomyocytes [5]. To address this problem, we designed a device to cyclically stretch seeded sutures in an incubated environment at a frequency of 0.5 - 3Hz. The modular design allows for live imaging without requiring experiment termination. This device opens doors for researchers to further study the effect of cyclic strain on cardiomyocyte maturation.
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1. Introduction

Every year, 620,000 Americans have a new coronary attack defined as first hospitalized myocardial infarction (MI), also known as a heart attack, or coronary heart disease death. In addition, 29,500 have a recurrent MI, and roughly 150,000 patients have silent first MIs every year [1]. Infarcts impact the heart’s function immensely. The death of cardiomyocytes is generally followed by formation of stiff scar tissue on the infarcted region. The stiff region causes higher stress concentrations to its surrounding, which decreases the mechanical function of the heart [2]. Natural regeneration of myocytes in infarcted areas is extremely limited, so stem cell delivery has been proposed as a way to increase cardiac function in infarcted regions of the heart [3].

Candidates for cell delivery include human mesenchymal stem cells (hMSCs), induced pluripotent stem cell derived cardiomyocytes (iPS-CMs), and neonatal ventricular cardiomyocytes (NVRMs). hMSCs originate in the bone marrow and can differentiate into cardiomyocytes. They are easy to harvest and have less ethical considerations, as they come from bone marrow, rather than healthy heart tissue [4]. iPS-CMs have similar contraction properties to cardiomyocytes, making them ideal for potential myocardial repair. However, both iPS-CMs and NVRMs require being seeded onto the infarcted regions of the heart, the cyclic stresses of which need to be considered [5]. Considering contraction of iPS-CMs and NVRMs across various strain concentrations on and surrounding the infarct region will provide a better understanding about the nature of iPS-CMs and NVRMs within the myocardial environment. How these cells react in such an environment will provide further insight on ways to maximize cardiac function within the stiff region using iPS-CMs and NVRMs [5]. Determining how mechanical stimulation and strain gradient affect the contractile mechanics of NVRMs and iPS-CMs will help advance myocardial heart regeneration and stem cell delivery research further [6].

Current methods of stem cell delivery to infarcted regions of the heart have only had minimal success in recovering the infarcted area. The most common methods currently being researched are through intravenous delivery or direct injection, or through surgical procedures [6]. Intravenous delivery of stem cells possesses relatively low risk to the patient, but has minimal success, with 99% of the cells cleared by first-passing through the lung. The remaining 1% that actually engraft are unable to mature into healthy cardiomyocytes. Direct injection of
cells is accomplished by either intracoronary or intramyocardial injection, which has an improved engraftment rate of 3% and 11-23%, respectively, compared to intravenous delivery. Even with the minimal improvement of adherence of stem cells on the infarct, maturation continues to be a challenge. Surgical methods have also been considered to deliver cells directly onto the infarcted region. Still, they have low engraftment rates, along with a high risk of side effects from invasiveness and increased cost. Due to the challenges of these basic cell delivery methods, tissue engineering is seen as the next approach to stem cell delivery for MIs [4].

Tissue engineering in the field of myocardial regeneration has gained a lot of traction in the past few years. Studies have shown that stem cells have a much higher percentage of engraftment on thread and scaffold structures made up of natural and/or synthetic materials. One such material that has been the cutting edge in this field is fibrin [7, 8]. Fibrin occurs naturally in the body as a major component of the formation of blood clots. Fibrin is formed by a protein found in blood plasma known as fibrinogen and the clotting enzyme thrombin. Single fibrin threads are bundled together to form sutures, which can then be seeded with stem cells and applied to the infarcted region. Due to its crucial role in the primary stages of wound healing, these fibrin sutures have been used as a biocompatible material because they allow for attachment and proliferation of cell types such as fibroblasts and hMSCs. Additionally, fibrin threads are able to maintain the multipotency of seeded hMSCs as well as act as an optimal substrate for contractile cells such as iPS-CMs [7]. The ongoing challenge for these constructs now is not only to maintain the multipotency of stem cells, but to encourage stem cells to differentiate into mature cardiomyocytes prior to the delivery of the suture into the body [9]. Thus, the sutures need to be electrically and/or mechanically stimulated to simulate the cyclic environment of the heart in order for cardiomyocyte maturation to occur. A device that induces the cyclic environment of the heart for this particular purpose is obsolete, albeit some labs have constructed their own to accomplish similar goals along with current gold standard devices to better understand myocardial regeneration.

Many devices exist to electrically and mechanically stimulate tissues and gels within a bioreactor. For example, Miklas et al. designed a bioreactor using compressed air into pistons to stretch the entire viscoelastic chamber made up of polydimethylsiloxane (PDMS) [10]. Electrical stimulation of the bioreactor was achieved through a pair of carbon rods connected to a power source. Devices such as these have yet to be applied to stimulating fibrin sutures, however, and
are necessary for the advancement of the field. Within the laboratories at WPI, the only device that can provide this type of stimulation is the Flexcell [11]. The Flexcell device uses vacuum pressure to apply both uniaxial and biaxial strain to a custom plate that has a soft silicon membrane on the bottom of the well instead of hard plastic surface of a standard plate. The rate and magnitude of strain is adjustable, making it versatile in a wide variety of experiments. The biggest drawback of the Flexcell for fibrin thread applications, however, is that it can only be used for monolayer cultures. This forces the user to make alterations to the plate in order for threads to be properly tested. Additionally, Flexcell wells are unable to be observed under a microscope due to depth, clarity, and opacity issues, which inhibits the user from checking on the progress of the cells during the experiment [11, 12]. Overall, the Flexcell device used in the labs at WPI has cost these labs valuable time and money that could have been saved with a new device, of which our group plans on designing and constructing.

Our team plans on constructing a device that both assists in the adherence and alignment of stem cells on biopolymer threads and provides stimulation to the cells in order to induce differentiation into mature cardiomyocytes. Our first step was to revise the initial client statement to highlight the main objectives both our team and our clients expect from the finished design through conducting thorough background research and gaining additional information through interviews with our client. After evaluating functions, constraints, and objectives for our device, the next stage was prototyping our design through brainstorming and further research. Next, we tested the prototypes and perfected our design to ensure our design met all the discussed specifications. Lastly, we presented our final design to the BME department at WPI along with a full report that contains qualitative and quantitative data regarding our finished product.
2. Literature Review

2.1 Heart Disease–The Problem

Heart disease and its resulting complications claim more lives than any other causes of death [13]. According to the American Heart Association, over 600,000 people die each year in the United States alone due to heart disease and related complications [1]. In addition, heart disease drains medical resources and costs tremendously. The American Heart Association states that from 2011 to 2012 heart disease cost a total of $207.3 billion due to medical care and loss of productivity [13].

2.2 Normal Cardiac Function

To study the disease, it is important to first understand the basics of normal cardiac function. Heart muscle, or myocardium, is composed of bundles of myocardial cells in both the atria and ventricles that attach to the upper and lower sections of the fibrous skeleton, respectively. Bundles are connected by conducting tissues that move action potentials from the atria to the ventricles. Myocardial cells are short, striated, branched cells that are connected at the ends by electrical synapses called gap junctions, which couple the myocardial cells both mechanically and electrically [14]. The stimulation of one myocardial cell results in the stimulation of neighboring myocardial cells, contributing to a whole muscle contraction, as opposed to a graded contraction found in skeletal muscle. The pacemaker region of the myocardium, which consists of specialized cells, automatically produces the action potential needed for contraction. Excitation-contraction coupling in myocardium, however, is not direct as in skeletal muscle, making cardiac muscle contraction slower than that of skeletal muscle.

2.3 Myocardial Infarctions

Heart disease comes in a variety of forms, and myocardial infarction (MI), commonly known as “heart attack,” is one of the deadliest. Approximately 720,000 people in the United States alone suffer heart attacks each year. Of that same group, nearly one third have experienced at least one MI previously [15]. Over time, many people develop atherosclerosis—a disease in which plaque buildups form within the arteries. The plaque consists of fats, cholesterol, and calcium, and as it progresses it begins to occlude the coronary arteries which supply the heart
muscle with blood [16]. If left untreated, the blockages will eventually prevent areas of the heart from receiving adequate perfusion. During this ischemic period, cardiomyocytes in the oxygen and glucose-deprived region are unable to contract and begin to die. Death of cardiomyocytes causes permanent tissue damage within thirty minutes of the ischemic onset [17]. Since mature cardiac cells lack the ability to reproduce, the myocardium is incapable of repairing itself post-MI. Instead, the heart forms scar tissue at the injury site in an attempt to heal the damaged region. The scar tissue is incapable of functioning as normal cardiac tissue, reducing the efficiency of the heart [17]. As a result, the heart no longer pumps as effectively as before. In addition, scar tissue induces a strain concentration on neighboring cardiomyocytes, causing further cell death and increases the risk of future MIs.

2.4 Heart Failure

Depending on the location and severity of the ischemic event, different sections of the heart may fail. Left-sided heart failure affects the left atrium and ventricle of the heart essential for moving blood throughout the body and can come in two types: systolic and diastolic failure. The former causes a decrease in the left ventricle’s contraction power, while the latter prevents the ventricles from complete relaxation between beats, causing the ventricle to not completely filled with blood from the left atrium [18]. Right-sided heart failure occurs as a result of left-sided heart failure since pressure from the blood in the left ventricle and atrium causes a blockage in pressure for the lungs, the right atrium, and right ventricle subsequently. This causes the right side to lose contractile ability as well, which makes it difficult to pump blood to the lungs and can cause blockages in the veins leading back to the heart [18, 19].

2.5 Post-MI Treatment

Following a myocardial infarction, clinicians often suggest the patient undergo a number of preventative or rehabilitative therapies. Preventative therapies include prescription of anticoagulants, such as aspirin, to reduce clotting in the blood or vasodilators to reduce the vascular network’s resistance to blood flow by increasing vessel diameter [20]. Patients may also receive coronary artery bypass graft surgery to the blocked artery to prevent the occurrence of a future MI [21]. As for rehabilitative treatment, exercise regimens are the only option clinicians currently have. Patients participate in exercise routines designed to build up cardiac endurance
and strengthen the living myocardium [21]. No standard procedures exist to replace the necrotic regions and return the heart to pre-MI contractile capability.

2.6 Stem Cell Treatment

Stem cells present an exciting new possibility in the realm of post-MI, rehabilitative therapy. These pluripotent cells can differentiate into cardiomyocytes under the proper conditions and may allow researchers to successfully replace cardiac scar tissue with functioning, contractile cardiac muscle.

To achieve this goal, researchers are studying methods of encouraging the stem cells to differentiate into phenotypically mature cardiomyocytes that can be delivered to the heart. When preparing cells or tissue grafts for delivery to the heart, it is critical that the synthetic myocardial tissue mimics in vivo cardiomyocytes in terms of activity and function to maximize implant survival. Delivery of cells with immature phenotypes reduces, or can even nullify, the intended therapeutic effect [22]. In order to consider a cardiac tissue mature, its cells must align and contract in unison.

Various means of encouraging cardiomyocyte maturity exist, including electrical stimulation, mechanical stimulation, rigidity and topology of the seeding substrate, and chemical direction [22]. Currently, most researchers focus primarily on two methods of encouraging maturation of in vitro cardiomyocytes—electrical stimulation and mechanical stimulation [23].

Researchers emphasize cardiomyocyte orientation and alignment when generating engineered, myocardial tissue. Cell alignment is critical for the development of striations within the heart tissue explant, which allow the cardiomyocytes to beat in unison [24]. Methods of maturation encouragement can be grouped into three primary categories: mechanical stimulation (both cyclic and non-cyclic), substrate manipulation, and stimulation via intracellular signaling.

2.7 Mechanical Stimulation

Many researchers believe mechanical stimulation plays a dramatic role in encouraging stem cell to differentiate into cardiomyocytes. In the last two decades, researchers have carefully studied the effects of cyclic stretching on stem cells. In 1996, Vandenburgh et al. developed a cardiomyocyte maturation model to test the effect of cyclic mechanical stimulation on cell alignment. The model used cyclic stretch to simulate the mechanical load placed on in vivo
cardiomyocytes resulting from a postnatal rise in blood pressure and volume. The cells were to uniaxially stretch for two to four days while receiving a steady supply of media. Vandenburgh et al. observed that the cardiomyocytes modeled in vitro displayed many of the same morphological changes displayed by in vivo cardiomyocytes. They noticed the in vitro tissue developed striations, expressed an increase in bi-nucleation of cardiomyocytes, showed longitudinal hypertrophy, and increased expression of myosin heavy chains [22, 25].

The cyclic stretching influences more than just the morphology. Mihic et al. revealed that mechanically stretching cardiomyocytes also allows for a faster spontaneous beating frequency [22]. In their study, hESC-CMs were seeded onto a gelatin scaffold and underwent cyclical stretching at a frequency of 1.25Hz for 72 hours. The mechanically stretched cells were able to achieve a spontaneous beating frequency approximately equal to the stretching frequency of 1.25Hz. Calcium imaging determined that the stretched cells possessed shorter calcium cycle durations than the un-stretched, control cells. The increased speed of the calcium cycle allows the cells to replenish their internal calcium levels more rapidly and beat at a higher frequency than the controls [22].

In addition, Gwak et al. observed the effects of embryonic stem cell (ESC)-derived cardiomyocytes under cyclic strain for an extended period of time [26]. In their study, ESCs were cultured onto a PLCL polymer and then placed under 10% cyclic strain in incubator conditions for two weeks. They found that cardiac specific genes were enhanced in ESCs subjected to cyclic strain, when compared to unstrained controls. Gwak et al. noted that the area of fibrotic tissue was smaller for scaffolds placed under cyclic strain before in vivo transplantation. This further demonstrates the importance of cyclic strain on the effectiveness of ESC-derived cardiomyocyte grafts.

As a part of the same study, Mihic et al. investigated the ion channel and by comparing ion channel expression in the stretched cells with expressions in the control cells. Mature cardiomyocytes express many ion channels which commence myocyte contraction through conduction of cardiac action potentials. Mihic et al. discovered that the stretched cells expressed 4.9 times as many L-type voltage-gated calcium channels as the control cells. They also reported 5.25-fold expression of voltage-gated sodium channel Nav1.5, 3.23-fold expression of inward-rectifier potassium channel Kir2.1, and a 1.48-fold expression of delayed-rectifier voltage-gated potassium hERG channels all compared to the un-stretched, control cells [22].
Although cyclic mechanical stimulation has taken priority, the effects of static mechanical stimulation should not be ignored. In static mechanical stimulation, the cells experience a steady axial load.

2.8 Substrate Properties and Morphologies

In addition to mechanical stimulation, research has emphasized the importance of substrate properties and morphology and their effects on stem cell maturation and alignment. An example of this method of non-cyclic testing includes the use of cell culture plates that contain microgrooves to incite cell alignment [27, 28]. Two studies investigated the use of cell cultures made from silicone containing microgrooves to induce cell alignment of tendon fibroblasts. The first study [29] investigating the microgroove structure’s effect on patellar tendon fibroblasts with addition of cyclic mechanical testing, while the second study [30] observed how mesenchymal stem cells behave under the microgrooves in terms of their alignment and maturity. Both studies found alignment in their studies, even in cases where the cell cultures were not put under cyclic testing. It is important to note that these studies were performed on mesenchymal stem cells with the intention on differentiation into tendon fibroblasts.

2.9 Intracellular Signal Techniques

A second method of inducing cardiac differentiation includes introducing molecules from the extracellular matrix of the heart, such as collagen and laminin, into stem cells growing in-vitro [31]. Because the extracellular matrix of the heart assists the growth of cells in-vivo, studies reported stem cells in-vitro becoming differentiated with a similar morphology to cardiomyocytes and greater myofibrillogenesis. It is also possible to achieve this effect when stem cells are co-cultured with mature cardiomyocytes [31]. Although there may be fewer intracellular signals between mature cardiomyocytes and stem cells while in-vitro, there have been observations that confirm that stem cells are able to induce differentiation into cardiomyocytes. Finally, reactive oxygen species can help stimulate growth factors and was reported to have a part in “early cardiac development” [31].
2.10 Experimental Bioreactors

Many useful ideas are found in the varying bioreactor designs produced by researchers around the world. Although none of the designs are particular to the mechanical stimulation of threads, many features and designs could be applied to a device designed to stimulate stem cell-seeded threads. Included in these designs are those for custom reaction chambers. Several papers describe custom-build, bioreactor reaction chambers. In their paper, “Bioreactor for Modulation of Cardiac Microtissue Phenotype by Combined Static Stretch and Electrical Stimulation,” Miklas et al. discuss the details of the device’s reaction chamber. They built their chamber using PDMS, a silicon-based, bio-friendly polymer, instead of designing their device to work around commercial plates and their specifications and limitations [36]. The custom build serves two main purposes. First, a custom chamber does not limit design possibilities. The use of commercial plates requires the designers to limit their design ideas to those which work around the particular specifications (size, shape, etc.) of their chosen plates. Building a custom chamber allows the design to focus on desired functionality; then the reaction chamber can be designed to work with the system. Secondly, the custom chambers are reusable thereby reducing the cost and waste generation of each experiment.

Additionally, the custom-built chambers can be designed to permit live imaging. In their paper, “The Use of a Novel Cardiac Bioreactor System in Investigating Fibroblast Physiology and its Perspectives,” Lu and Ravens discuss the design of the reaction chamber. They chose to build the chamber’s top and bottom using microscope slides. With this design, they imaged seeded cells mid-experiment without producing a significant disturbance [37].

Lu and Ravens continue to describe plans for a media reservoir and peristaltic pump system designed to provide the seeded cells with a constant flow of fresh media eliminating the need for manually changing media periodically. Their plans involved creating an inlet valve and an outlet valve in the PDMS chamber and calculating a media flow rate that would not dislodge the cells from the substrate [37].

In conjunction with these other features, clamp and motor systems or pneumatic systems are primarily used to apply mechanical stimulation to the seeded substrates. The clamp and motor systems generally consist of a set of parallel rods capable of sliding closer together and farther apart while remaining completely parallel. Each rod has a set of clamps which face inward toward the opposing rod and the seeded substrate is fastened between the clamps. A simple motor system
cyclically stretches the substrate by repeatedly pulling one rod away from the other before returning it to its starting position [37].

A variety of pneumatic systems exist, but the system described by Miklas et al provides a good example. In this case, they designed the PDMS chamber to contain a set of pneumatic pistons built into the chamber itself. A controller runs a compressor and causes it to pump compressed air into the pistons causing the whole chamber to expand and the substrate and cells to stretch. The compressed air is then released allowing the substrate and cells to return to the resting state. The controller repeats this process to produce the cyclic stretch [36].

In many cases researchers desire electrical stimulation to accompany the mechanical stimulation function. Miklas et al. accomplished this by placing pairs of carbon electrodes into the reaction chamber. The same microcontroller used to control the compressor can also power the electrodes allowing for electrical stimulation independent of or in conjunction with the mechanical stimulation [10].

2.11 Gold Standard: FlexCell

The previous literature survey demonstrates the need for cyclical, mechanical stimulation of stem cells. There are a number of commercial options available, including the ElectroForce by Bose, Strex by B-Bridge International, the TGT Bioreactor by Tissue Growth Technologies, and the Flexcell by Flexcell International [32].

The ElectroForce is a bioreactor that utilizes a perfusion pump to assist in cell culture and three-dimensional tissue culture [33]. Strex causes cyclic mechanical stress on cell culture by both stretch and compression of the cultures based on inputted parameters stretch ratio and stretch frequency [34]. The Strex device utilizes silicone film chambers that allow for proper stretching and laboratory analysis including cell fixation, fluorescent staining, and mounting for view under a microscope stage to allow analysis in real-time. The TGT Bioreactor provides in-vivo simulations to three-dimensional in-vitro cell cultures by utilizing compressive and hydrostatic stresses to cartilage where the in-vitro cultures are placed [35].

The Flexcell device utilizes vacuum pressure and is programmed to cyclically stretch the cell culture plates at a certain rate, which is useful when making adjustments. During past experiments in the Gaudette Laboratory, the device demonstrated success in placing cells under uniaxial cyclic stress. Because the Gaudette Laboratory at WPI utilizes the Flexcell device for
differentiating stem cells to a cardiomyocyte lineage, it was used as a Gold Standard for comparison during the course of the project.

The device, however, does come with limitations. In its current set-up at the Gateway Park Biomedical Engineering Facility at WPI, the device takes up an entire room with little space to complete experiments where the Flexcell is needed. It is also not possible to view the cells under a microscope while an experiment with the Flexcell is going on. The cells must first be fixed and have a fluorescent tag in order to observe the uniaxial effect. This is an issue because an experiment could be going wrong with no way to tell if the experiment should be terminated until the very end, which wastes time and money due to the loss of a Flexcell culture plate. In order for the Flexcell to use vacuum pressure, vacuum grease is needed in order to make an airtight seal with the rest of the environment. This causes problems with creating the slides without removing the grease, and it becomes another source of error in the event that grease enters the cell culture plate. Finally, the Flexcell is unable to mechanically stretch fibrin microthreads, which causes problems when these microthreads are tested in-vivo and may not operate properly when placed in the heart. Overall, while there are no functional issues with the Flexcell, the graduate students and undergraduate student volunteers have experienced several issues when operating the device as well as obtaining relevant data for experiments.

2.12 Future Work in the Field

While tissue engineering is not yet advanced enough to engineer a fully functional heart organ, there have been a number of advancements in recent years. Zimmermann et al. explained attainable goals in the next ten years for cardiac tissue engineering [24]. These goals included personalized drug screening, serum free cell cultures, and engineered tissue grafts for areas of the heart that has experienced myocardial infarction. Some of these goals, especially for the creation of scaffolds containing hMSCs or ESCs, have shown promise since the writing of the report in 2005. A number of laboratories have created a scaffold or sheet containing an ECM of either stem cells or grown cardiomyocytes using a number of different materials. For example, Murry et al. used 3D collagen scaffolds containing up to approximately two million ESCs and subjected to uniaxial cyclic stress before engraftment [38]. In addition, Kawamura et al. tested cell sheets using human induced pluripotent stem cells for delivery of stem cells to infarcted heart muscle as an alternative to injection of stem cells into heart muscle [39]. The result of this study found that
stem cells could be detected in the heart eight weeks after implantation of the cell sheet. According to Jackman et al., there are still other alternative methods in creating scaffolds or sheets containing ESCs or hMSCs including gelatin and fibrin based constructs [40].

New methods for placing scaffolds through cyclic mechanical or electrical stress are also being developed and tested. Riehl et al. explains that most of these devices, however, are either commercially available, such as the Flexcell, or specific to the laboratory performing the research [32]. Two-dimensional scaffolds are usually placed in a layer where they can later be stretched with the help of devices such as a Flexcell whereas three-dimensional scaffolds are held down using anchors. Electrical stimulation is another method of placing cell culture under cyclic stress and, according to Zimmermann et al., is “critical for functional maturation” [24]. Some examples of electrical stimulation of cyclic stress comes from Tandon et al. where they explain the process of applying pulsatile electrical fields to both a two-dimensional monolayer and a three-dimensional scaffold using carbon rod electrodes [41]. The results from the experiment showed that cells that were electrically stimulated had a seven-fold higher amplitude of contraction than cells that were not stimulated. The report also acknowledged that the myofibers became aligned orthogonal to the direction of the electrodes, and that there were greater numbers of mitochondria and glycogen, a higher volume fraction of sarcomeres, and an increase in intercalated discs and gap functions between the tissues.

The main goal of cardiac tissue engineering is the creation of a human heart that can be used in patients [24]. However, more research is needed before one can be created.

2.13 Conclusion

Every year, 620,000 Americans have a new coronary attack (defined as first hospitalized myocardial infarction or coronary heart disease death). In addition, 29,500 have a recurrent heart attack and roughly 150,000 patients have a silent first myocardial infarction each year. Myocardial infarction leads to the death of cardiomyocytes and replaces myocardial tissue with stiff scar tissue. This stiff region causes higher strain concentrations compared to the surrounding myocardial tissue, which decreases the mechanical function of the heart. Endogenous regeneration of myocardial tissue in the infarcted area is extremely limited and thus research in initiating this regeneration is currently undergoing. Stem cell delivery has been proposed as a way to increase cardiac function in infarcted regions of the heart.
3. Project Strategy

3.1 Initial Client Statement

The Gaudette Laboratory focuses much of its research on techniques for improving cardiovascular regeneration after MI through cell-based therapy techniques. Through the development of bio-polymer microthreads, the Gaudette Laboratory has been working to seed cells onto sutures made from these microthreads in order to facilitate the delivery of iPS-CMs directly to the damaged myocardium. Thus, much of the research performed in the lab is working towards the evaluation and improvement of this technique. Currently, the Gaudette Laboratory uses a FlexCell system to precondition threads, improve adherence and alignment of cells on the microthreads, and to induce contraction in premature cells so that they exhibit a mature phenotype. However, as discussed previously, there are inherent problems with the FlexCell system. Thus, the need for an improved system to perform this type of testing motivated this project. The following initial client statement was provided after meeting with Professor Gaudette, our main advisor, Joshua Gershak, our client, and members of the Myocardial Regeneration Laboratory who would benefit the most from the development of this device:

Design a device that can mechanically stimulate bio-polymer threads made by the Gaudette Laboratory and other labs in order to better understand the nature of stem cells on threads in a cyclically induced environment. The device needs to work in a standard cell culture incubator with minimal disturbance to other plates, and be user-friendly. If possible, the device should also allow for imaging without contamination of cells/threads.

After receiving the initial client statement, the team began performing research on experiments other laboratories have performed, as well as any specific design parameters and specifications. The team also began to analyze this client statement in order to determine the ultimate goal of the project in order for the Gaudette Laboratory and other laboratories at WPI to benefit from the design.
3.2 Design Requirements

From analysis of the above client statement, the team developed a list of technical design requirements in order to better determine the success of the final product. These technical requirements were divided into four different sections: functions, objectives, specifications, and constraints.

3.2.1 Functions

The functions of a device are defined as the actions that the device must complete in order to be operable. For this project, we aim to develop the following functions: enable experiments involving cell adhesion and alignment, live imaging of cells during the experiment, and operating for a length of at least thirty days.

3.2.1.1 Study of Cell Alignment and Adhesion

The ultimate goal of this project was to provide the Gaudette Laboratory with a device capable of placing cell-seeded microthreads under cyclic strain in order for the laboratory to investigate the capability of improving alignment and adhesion of cells on the threads. There are several methods used for inducing alignment in a cell culture, including chemical means and media to induce certain intracellular signals in the cells. For this project, however, the team plans to explore using either mechanical stimulation or electrical stimulation in order to cause alignment and adhesion.

Mechanical stimulation is the process of stretching the microthreads through mechanical means such as using motors, springs, or clamps. This involves manually stretching the microthreads in order to stimulate intracellular signals that will induce cell alignment and adhesion. Some aspects to keep in mind if mechanical stimulation is used in the design is the means by which the microthreads will be stretched, how the force and strain applied to the microthreads can be made consistent, and how parameters of the machine-like strain and frequency can be made adjustable. It is also important to keep in mind how the microthreads should be inserted into the machine and if such an insert and clamping method will damage the microthreads.

Electrical stimulation is similar to mechanical stimulation in that it induces the threads to undergo cyclic stretch. This will be achieved, however, by producing an electrical stimulus,
either from a pair of electrodes or placing the microthreads in a shifting magnetic field. The benefits of this set up is that it may be possible to load the microthreads without doing unintended damage to them; but electrical stimulation also requires extra materials to be placed alongside the cell culture. This includes electrodes and wiring that must be biocompatible in order to ensure the health of the cell culture being experimented on.

3.2.1.2 Live Imaging

One of the main features unavailable on the FlexCell device is the ability to view cells as they are undergoing cyclic stress. This causes problems with validating if the desired effect is occurring and if cells are still viable during the experiment. This device should be able to allow viewing of the cells during an experiment to monitor progress and to stop the device in the event of an emergency, such as device or experimental failure.

The Gaudette Laboratory currently has access to a Leica DFC420C Inverted Fluorescence microscope located in Professor Rolle’s laboratory. The dimensions needed in order for the device to fit underneath the microscope are a length of 21.2 cm, a width of 24.7 cm, and a height of 6 cm (the focal length to obtain a focus on a standard six-well culture plate is 3.6 cm away from the lens). Should the microscope from Professor Rolle’s laboratory be the designated method of performing live imaging, the device must be able to fit underneath the microscope, providing an additional constraint to the team’s design. It is also important to note that the device would need to be kept at a temperature of approximately 37°C so that the cells remain alive in a stable environment in the event that live imaging takes longer than expected.

The alternative to the microscope is to develop either a feature or additional device that allows for proper viewing of the experiment. This could include a camera placed above the device during an experiment that is capable of viewing at the correct magnification, or developing a separate viewing device that will not only keep the device sterile but also maintain a stable environment at an optimal temperature so that the cells used in the device do not become exposed to the outside environment.

3.2.1.3 Operating Duration

From most devices and experiments found in the team’s literature review, the length of the experiments typically lasted around seven days. Originally, this was the optimal length of the
experiment for the team’s prototype. Upon speaking with the client, however, the optimal length was extended to thirty days. This is because there may be additional experiments that can be performed for different cell types or for experiments other than alignment of cells on the microthreads. Methods the team developed in order to ensure the device can operate for at least thirty days includes the use of batteries, and an external power source. Both methods require electricity and so it is important for the device to have easy access to an electrical power source. Depending on which method is used, some electrical equipment may have to be inserted into the same environment as the device. In this case, it is important that the power source also remains biocompatible with the microthreads and any other experiments nearby in the event that the device is placed in a communal incubator.

3.2.2 Objectives

The objectives were defined as both the needs and wants of the client that would improve the functions of the device and make it preferable over the current gold standard. The targeted objectives developed include the device being easy to use, sustainable, versatile, safe for all stakeholders, and be able to measure parameters of the device. In each subsection, the objectives are explained in further detail.

3.2.2.1 Ease of Use

In order for a device to be easy to use, it must require minimal amount of training, have an understandable and responsive interface, and allow for viewing of results and data without confusion. If the device is not easy to use, then it is possible that the client will not want to use the device, and potentially opt for a different option that is simpler to use. The client anticipates that the individuals who may be working with the device include graduate student workers and undergraduate student volunteers.

The graduate student workers will be the main users of this device, so it is important that all information on this device is conveyed in a way that is easy to understand and operate. This includes the insertion of cell-seeded fibrin microthreads into the device, programming for proper strain rate, force, and frequency for the experiment, monitoring the device for maintenance and control of the experiment, removal of the microthreads from the device, and sterilization of the device after an experiment has concluded. Because this device may be used in future
experiments, it is important that these operations are conveyed in written form so other individuals can follow standard protocols.

In addition to graduate student workers, many laboratories at Gateway Park on the WPI campus allow undergraduate student volunteers to participate and assist in laboratory research. Students do not typically assist in major projects, but the client mentioned that it would be beneficial to have undergraduate students learn how to use this device. In order for this to be possible, there must be training that explains the operations of the device as explained above. The training should not be overly complicated so that undergraduate students can operate the device on their own. The standard protocols for this device should also be easily understandable in the event that a student volunteer has any questions.

3.2.2.2 Sustainability

Because of the high volume of waste developed by most laboratories, it is important to consider what materials are going to be used in the manufacturing of the device. A device that contains interchangeable parts, can be reused multiple times, and materials made from recyclable material can reduce the amount of waste a laboratory produces and have a more positive impact on the environment.

Interchangeable parts help in achieving sustainability; in the event that an individual part becomes inoperable, the whole device does not need to be disposed of. The piece can, instead, be replaced, preventing the need of a new device that could perform the same function. However, it is important to keep in mind that should the device have interchangeable parts, additional protocols and training will be required in order to fix or replace a part of the device in the event of a failure.

Laboratory equipment, such as six-well cell culture plates and microscope slides, can only be used once before they are thrown away as waste. Biohazardous waste can collect on these equipment, negatively impacting the results of the following experiments should they be reused. Part of making a device sustainable includes reducing the amount of waste produced from a piece of equipment and its experiments. A method of meeting the sustainability objective, therefore, comes from using materials for the device that can be used repeatedly in different experiments while also obtaining reliable data from these reusable parts. This comes from having
a prototype that can operate for longer than just one experiment and requires little to no replacement.

In order to make laboratory equipment of higher quality for optimal experiments, many labs use expensive materials that are not easily replaced, such as platinum wires in electrical equipment to be used for biocompatibility, or low quality materials such as plastic six-well cell culture plates that are easily disposable. Instead of using these types of materials, a method of making the device more sustainable is to manufacture it from recycled materials. This way, when a device is no longer operable, the parts can be recycled for use elsewhere. This is also beneficial when creating a device that is cheap and helps alleviate the constraint of the budget for this project, which is discussed later in the chapter.

3.2.2.3 Versatility

Versatility is defined as a device’s ability to perform under different parameters. This could be with testing different types of cells on the fibrin microthreads, extending the period of time the device is operational, and allowing the device to be exposed to different environments.

For this experiment, the team will use hMSCs for the experiment. There are other cell types that the client may also want to use, however. The Gaudette Laboratory has access to iPS-CMs, ESCs, and MSCs along with hMSCs that they use in their experiments with fibrin microthreads. In order to have the device be successful in terms of versatility, it should allow for the use of other cell types, such that these cell types can still experience adherence and alignment on the fibrin microthreads. This reduces limitations that the device has and reduces the amount of equipment a laboratory needs to purchase in order to run an experiment, making the device more valuable to the client.

One of the main functions for this device is running an experiment for a minimum of 30 days, despite other reports and devices researched performing experiments for shorter periods of time. With this in mind, in order for the device to be versatile, it should be able to run experiments for as long as a user needs, within an adjustable limit.

3.2.2.4 Safety

In order for a device to be safe to use, it should not pose a threat to the health or well-being of any stakeholders while using said device. This is important because if a device cannot
guarantee one’s safety, then a client will most likely choose to use an alternative device. Methods for maintaining a safe environment come mainly from the stakeholder’s perspective. This includes wearing appropriate gloves when handling the device and the fibrin microthreads inside, wearing eye protection when necessary, and working and preparing the device in a sterile environment, such as a fume or cell-culture hood. The device itself can also be designed with its own safety parameters, including biocompatibility, sterility, and minimizing basic hazards of the device such as sharp edges and stability.

Although the device is not going to be used for clinical needs, biocompatibility is still important for the device due to both the sake of the experiment and the sake of other projects that are located nearby. If the device is not biocompatible, the client will not be able to conduct experiments with said device. It can also cause issues for other experiments performed by other nearby laboratories that share equipment with the Gaudette Laboratory. The device should not cause problems for other experiments or result in harmful reactions that can be hazardous to the health of individuals working in the lab.

The safety of a device is also dependent on its sterility. If a device cannot be properly sterilized, then it is possible for biohazardous material left over from a past experiment to come into contact with a student worker or volunteer. In order to avoid this, the device should be easy to sterilize so individuals who work with the device are kept safe from biohazards and so future experiments can produce accurate data.

Finally, in order for a device to be considered safe, it should minimize any potential hazards that it may cause to an individual, even when they are using personal protective equipment. This can be done by designing the device itself to minimize risks. For example, reducing the amount of sharp edges on the device reduces the risk to both the personal protective equipment worn by an individual and the individual himself/herself. It is also important, however, that these design considerations do not interfere with the main functions of the device.

3.2.2.5 Parameter Measurements

For this project, parameter measurements is defined as actively measuring certain aspects of the device during an experiment. Parameter measurements are useful because it allows for an individual to more easily monitor the progress of an experiment, and to adjust the parameters when needed for a different experiment. Depending on whether mechanical or electrical
stimulation of the fibrin microthreads is performed, some of the parameters that can be measured include the strain applied to the microthreads, the force applied by the device on the microthreads, the frequency of the cyclic stress applied, and the voltage applied through the device.

Strain, force, and frequency are mechanical measurements; however, regardless of the method of cyclic stimulation of the microthreads, these three parameters need to be measurable when constructing the device. Based on research performed by other laboratories and from recommendations given by the advisors, cell stretching techniques generally applied a strain of 10 to 15% at a frequency of 0.5 to 3 Hz. The force varied for every design and was not the main focus of the experiment in the research of other laboratories. The client did, however, express interest in knowing and measuring the amount of force applied to the microthreads during an experiment. With these constant measurements in place, it will be possible for the client or another individual working with the device to observe if there are any discrepancies with the frequency, force, and strain during an experiment so an individual can alter the parameters on the device to keep the measurements within desired ranges. Voltage is another important parameter to measure if an electrical stimulus is decided as the mechanism for which the microthreads will be cyclically stretched. The voltage applied on the device will also be adjustable in the event that a stimulation through the use of electrodes applies a strain either too weak or too strong. The main purpose of actively measuring parameters is to monitor an experiment so the forces and strain applied are accurately being applied.

3.2.2.6 Pairwise Comparison Chart

In order to determine which objectives should be taken as a priority when designing the device, a pair-wise comparison chart of objectives was created by the team and filled out by all members of the team, the two project advisors, and the client. An example of the blank pair-wise comparison chart can be seen below in Table 1.
<table>
<thead>
<tr>
<th>Easy to use</th>
<th>Sustainable</th>
<th>Marketable</th>
<th>Versatile</th>
<th>Safe</th>
<th>Live imaging</th>
<th>Measures force</th>
<th>Measures frequency</th>
<th>Measures strain</th>
<th>Measures voltage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Easy to use</td>
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<tr>
<td>Sustainable</td>
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<td>Versatile</td>
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<td>Live imaging</td>
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<td>Measures force</td>
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<td>Measures frequency</td>
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<td>Measures strain</td>
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</table>

Table 1: Blank Pair-wise Comparison Chart with Device Objectives. This table shows the pair-wise comparison chart that the team created for the advisors and client to fill out so that the team could rank the objectives in order to determine which objectives are most important when moving to the design phase.

It is important to note that at the creation of the pair-wise comparison chart, it was still uncertain whether live imaging should be classified as a function or an objective. At the conclusion of everyone completing the pair-wise comparison chart, it became clear that live
imaging was very important for the design of the device and should be considered a function. The rankings of which objectives were deemed most important are found in Table 2, where the number one spot indicates the most important objective and the number seven spot indicates the least important objective.

<table>
<thead>
<tr>
<th>Rank</th>
<th>Objective</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Live Imaging</td>
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<tr>
<td>2</td>
<td>Measures:</td>
</tr>
<tr>
<td>1</td>
<td>Force</td>
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<tr>
<td>2</td>
<td>Frequency</td>
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<tr>
<td>3</td>
<td>Strain</td>
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<td>4</td>
<td>Voltage</td>
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<tr>
<td>3</td>
<td>Versatility</td>
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<tr>
<td>4</td>
<td>Marketability</td>
</tr>
<tr>
<td>5</td>
<td>Safety</td>
</tr>
<tr>
<td>6</td>
<td>Easy to Use</td>
</tr>
<tr>
<td>7</td>
<td>Sustainability</td>
</tr>
</tbody>
</table>

Table 2: Ranked objectives from the Pairwise Comparison Chart. This table shows the rankings that the team assigned to each objective using the information gathered from our Pairwise Comparison Chart and discussions with our client.

Our client indicated that live imaging, measuring various parameters such as force, frequency, strain, and voltage, and versatility were the most important objectives for the device. The ability to image the device without having to terminate any current experiments was a top priority when discussing device functions and objectives with the client, as this is a function that the current gold standard lacks. The measurement of various testing parameters was secondary to live imaging, but still ranked as a necessary function of the device, as these measurements can be used to not only validate that the device is performing well, but live measurements can be
utilized to help automate the device. Versatility was considered important to our client, as well as to the team, as versatility allows for expanded use of our device in other laboratories that may be working with various cell lines or threads that may require adjustable testing conditions. Initially, the client had ranked marketability relatively low, however the team decided that marketability was important, as the team hopes the device will be useful not just in other WPI laboratories, but in laboratories outside of the WPI community. Safety was ranked fifth by our client, as the device must inherently be safe for the user, and additional safety precautions are not necessary so long as the device functions properly. Ease of use was ranked similarly, as our client’s only objective was that the device is as easy as or easier to use than the current gold standard. Our client ranked sustainability last, as they felt that sustainability was a feature that was unnecessary to the overall performance of the device. This ranked chart will be used to develop conceptual designs for a device, as well as help in the validation of the device once a prototype has been built.

3.2.3 Specifications

The project specifications include specific values and quantitative parameters by which the device should be able to operate. These specifications were determined through literature review, where the team researched similar designs and basic information on the subject of heart tissue and anatomy. The specifications determined by the team include applying between 10% to 15% strain on the cell-seeded bio-polymer threads, cyclically stretching cell-seeded threads at a frequency of between 0.5 and 3 Hertz (Hz), and operating at 37°C and 5% CO₂ conditions in order to simulate the environment of native heart tissue in vitro.

3.2.4 Constraints

Constraints of the project are defined as parameters that limit design concepts developed by the team. Some of these constraints include keeping the device sterile during the experiment and allowing the device to be sterilized after an experiment is concluded, determining the dimensions of the device so live imaging can be performed on an available viewing device, such as a microscope, and the stabilizing the device so it does not interfere with any nearby experiments. The MQP budget of $1000 is also a constraint, because it limits the amount of material that the team can purchase. The last constraint to consider is the time frame of the
project – the team only had one academic year to perform background research, design, build, and test this device, requiring the team to think critically about certain steps of the process and manage our time accordingly.

3.3 Design Standards

Design standards exist in order to ensure that devices, products, services, and facilities are safe, dependable, and in applicable cases, well maintained. Design standards aid companies, manufacturers, and researchers in improving safety of products, protocols, and facilities, increasing production, and preventing practices that could put the business or an individual at risk. Additionally, these standards help to protect clients, customers, and consumers of these products and services by ensuring that the products and services meet minimum requirements for various aspects of operation and use. Products and services that have been reviewed by international standards boards and organizations are assured to be more reliable than other similar products or services that do not adhere to these standards. Because of the extensive coverage of various subjects, these standards are seen as valuable quality assurance tools internationally.

There are various standards boards and organizations that operate internationally, but the most renowned and respected are the International Organization for Standardization (ISO), American Society for Testing and Materials International (ASTM), and United States Pharmacopeial Convention (USP). ISO develops and promotes standards for industry and commercial purposes worldwide. ASTM develops technical standards and testing protocols for products, systems and services. USP provides standards for medicine, dietary supplements and vitamins, and ingredients used in food production.

Because the team is designing a new device, it is important to keep in mind the ISO, ASTM, and USP standards for device operation, protocols, and materials used in the construction of the device. From the initial client statement, we know that we will be working with threads (or sutures) constructed from biological material as well as stem cells, we know that the device could be sharing incubation space with other cell culture experiments, will mechanically or electrically stimulate the threads, and will be imaged using a microscope. Thus, we need to ensure that our device meets the minimum criteria for standards including sterilization,
biocompatibility, cytotoxicity, cell culturing, general safety, and mechanical testing. Thus, the following standards will be applicable to this project:

- ISO 11737-2:2009 - Sterilization of medical devices
- ISO 10993-7:2008 - Biological evaluation of medical devices - Part 7: Ethylene oxide sterilization residuals
- ASTM E1837 - 96(2014) - Standard Test Method to Determine Efficacy of Disinfection Processes to Reusable Medical Devices

ISO 11737-2:2009 specifies criteria for tests of sterility performed on medical devices [42]. ISO 10993-7:2008 refers to the allowable limits for residual Ethylene oxide on medical devices, as well as specifies the procedure for measuring the quantity of Ethylene oxide residue [43]. ASTM E1837 - 96(2014) refers to a procedure for testing the effectiveness of disinfection techniques on reusable devices [44]. These standards will be important for the team to consider, as our device will need to be sterilized between experiments. Thus, it is necessary that we follow the set standards for sterilizing devices and ensuring that any residual Ethylene oxide, a common sterilizing agent, falls below the allowable limits such that it does not interfere with our device’s performance. Additionally, should the team decide to incorporate reusable parts in the final design, it is necessary to follow the guidelines of ASTM E1837 - 96(2014) in order to ensure that the sterilization techniques that are being used are efficient and achieve the desired results.

- ASTM F813 - 07(2012) - Standard Practice for Direct Contact Cell Culture Evaluation of Materials for Medical Devices
- ASTM STP810 - Cell Culture Test Methods
- ASTM F2739 - 08 - Standard Guide for Quantitating Cell Viability within Biomaterial Scaffolds

ISO 10993-5:2009 specifies cytotoxicity tests for in vitro mammalian cellular applications, and includes assessments of cell damage by morphological means, measurements of cell damage, and measurements of cell growth. Additionally, it specifies the incubation of cultured cells in contact with a device [45]. ASTM F813 - 07(2012) similarly specifies standard practice for cultured cells that will be in direct contact with a device, and also specifies
evaluation techniques for materials that devices can be made out of [46]. ASTM STP810 specifies standard cell culture testing methods, and ASTM F2739 - 08 specifies protocol for cell counting and determining cell viability on scaffolds made of biomaterials [47]. Because this project involves cell culture, it is important to be able to determine cytotoxic effects of any materials that might come into contact with the cells, including the microthread material and the device material. Additionally, while we are cell culturing, we must ensure that we are adhering to the standards and protocols set forth by the ASTM. The last standard in this subject will be useful in determining both overall cell viability on the microthread and the distribution of viable and nonviable cells.

- ISO 10993-1:2009 - Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process
- ISO 4849:1981(en) - Personal eye-protectors - Specifications
- ISO 23907:2012(en) - Sharps injury protection - Requirements and test methods

ISO 10993-1:2009 sets the standard for performing safety evaluations of medical devices such that those using the medical devices can manage the amount of risk associated with the device. This also includes the biological safety of the device [48]. ISO 4849:1981(en) specifies the functional requirements for personal eye protection while working with potentially dangerous materials that could enter the eye [49]. ISO 23907:2012(en) specifies the requirements of single use sharps containers used in the laboratory setting such that risk is minimized for those using the sharps [50]. These standards all cover safety issues within the laboratory. This is important to consider for our project, as we will potentially be working with needles (sharps), will be wearing eye protection when working with the cells, and will need to ensure that the materials within our device (and the device itself) does not pose any risks to the user or the cells and threads that are being used with the device.

- ASTM STP1173 - Biomaterials’ Mechanical Properties

ASTM F2255 - 05(2015) specifies standards for tensile testing tissue adhesives, including sutures. This standard specifically gives a protocol on testing the strength of various adhesive materials used on soft tissues [51]. ASTM STP1173 specifies various mechanical properties of biomaterials [52]. These will be useful for this project, as one of the goals is to produce strain in
the threads through tensile loading. Having a standard for fibrin mechanical properties will help us in evaluating if our design is achieving the desired results.

3.4 Revised Client Statement

After constant discussion with the client throughout the course of this project, the team developed a revised client statement:

Design a device to assist in mechanical and/or electrical stimulation of stem cells on biopolymer threads to be used for myocardial regeneration post-infarction. To accomplish this goal, the ~2 cm seeded sutures will be subjected to stimulation and the device should allow for live imaging without contamination of cells/threads in order to evaluate the progress of ongoing experiments. Additionally, the device should be able to produce both an adjustable strain up to 10-15% and frequency of 0.5-3 Hz. The device should be safe and easy-to-use and must function at standard incubation environments, for a time period of up to 30 days.

When developing the revised client statement, the main question with the first sentence came from whether the stretching of the microthreads or the alignment of the cells would be the most important aspect of the design. After meetings with both the team’s advisor and client, the main goal of the project was determined to be for the device to cyclically stretch the microthreads. A tour of the laboratory where the device will be used also revealed that the client planned to use bundled microthreads that are approximately 2 cm in length, rather than individual threads developed by the laboratory. The team also included both mechanical and electrical stimulation in the revised client statement in order to ensure the team would not be limited during the design phase of the device.

Live imaging was then presented in the next sentence instead of the last sentence, as in the initial client statement. Based on meetings with both the advisor and the client, live imaging was seen as one of the more important functions of the device rather than an objective. The emphasis of adjustability was also made clear in the revised client statement. The team included the specifications in terms of cyclic strain and frequency and included the ability to adjust the strain and frequency in order to increase the number of experiments that can be performed with the
device. Finally, an emphasis was made on the objectives safety, and easy-to-use, as well as keeping the device in incubator-like conditions for 30 days. This last portion of the revised client statement was made so the team was, again, not limited in terms of the design process.

3.5 Project Management and Timeline

The success of this project depends on whether the device meets all of the functions, objectives, and constraints defined earlier in the chapter. In order to accomplish this, the group was given deadlines for the project by the end of each term. This section explains the expected accomplishments for each term and a detailed Gantt chart can be found in Appendix #.

By A-term, the group was expected to conduct research on basic subject material pertaining to the project and similar experiments performed by other laboratories, and create a revised client statement based on the initial client statement given at the beginning of the term. In addition, chapters 1, 2, and 3 of the report needed to be completed as well. A-term was primarily used to set up the project for the upcoming terms and have a greater understanding of what the client desires for their device.

B-term was designated to design considerations, purchasing materials, and prototyping. The group will develop a prototype that is believed to meet all of the functionalities set up for in A-term. Once the prototype is developed, initial testing will be performed so that it can safely hold the microthreads for testing, as well as apply a cyclic mechanical or electrical stimulus so that experiments can be performed the following term.

C-term was designated for performing tests on the functions of the device and determining if there will be any changes that need to be made to the design overall. Success of the experiment will be determined by whether there is adherence and alignment of the cells on the microthreads, if live imaging is possible on the device, and if the device is capable for running at the proper specifications for thirty days straight.

Finally, D-term was designated for completion of any additional components of the design, and determining what parts of the device can be improved in future research. In addition, a presentation will be made and presented to better explain the aspects of this project and the overall success of the device.
4 Design Process

4.1 Needs Analysis

After determining the importance of each objective in relation to the project, the next step in the project was to define the functions the device should accomplish. It was also important to distinguish between features and functions that the device must have and perform in order to be considered successful, and features and functions that the device must have and perform that, while beneficial, are not necessary for the success of the project as a whole. Table 3 below lists the primary objectives for the device with a rank indicating how necessary the objective is for the device’s success. A ranking system of 1-7 was used; a rank of 1 indicated that the particular objective or feature would be nice to have in the final product, but not a necessity, while a rank of 10 indicated that the particular objective was crucial for the design’s success.

<table>
<thead>
<tr>
<th>Objective</th>
<th>Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live Imaging</td>
<td>7</td>
</tr>
<tr>
<td>Measures Force, Frequency, Strain, and/or Voltage</td>
<td>5</td>
</tr>
<tr>
<td>Versatile</td>
<td>4</td>
</tr>
<tr>
<td>Marketable</td>
<td>3</td>
</tr>
<tr>
<td>Safe</td>
<td>2</td>
</tr>
<tr>
<td>Easy to Use</td>
<td>2</td>
</tr>
<tr>
<td>Sustainable</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 3: Numerically ranked, project objectives.

The general hierarchy for the functions and objectives was determined by completing the Pairwise Comparison Chart with the team and the clients. This process is outlined in the previous chapter, Project Strategy. This hierarchy was used once more to assist the team in assigning ranks to each function or objective. Values assigned to each objective within the Pairwise
Comparison Chart by the team, advisors, and clients were averaged to obtain their respective rank. The only alterations that were made were to the rank of safety and ease of use - originally, through this averaging method, safety would have a rank of 6, while ease of use would have a rank of 4. These were both changed to a rank of 2, as many members of the team had ranked safety and ease of use relatively high, while the advisors had ranked them relatively low. Additionally, this change is reflected in the order of the objectives taken from the Pairwise Comparison Chart, which was explained in greater detail in the previous chapter.

The ability for the final product to facilitate live imaging of cell-seeded fibrin sutures was given a rank of 7, as the original client statement was largely focused on the ability to live image the cells on the sutures in order to evaluate the cellular adhesion, proliferation, and contractile movement. The ability to measure various parameters, including the force and frequency of the contractile movement of the cells, and the strain experienced by the sutures and voltage output of the cells during contraction, was given a rank of 5. While the revised client statement does not explicitly state that the goal of the device is to stimulate human mesenchymal stem cells such that they exhibit contractile behavior similar to that of native myocardial cells, this is the broad aim for a device such as this. Additionally, the revised client statement states that the frequency and strain produced by the device must be adjustable and within a specified range, which indicates that there needs to be a means for measuring these parameters. Thus, the ability to measure the selected parameters is necessary for the success of this device in terms of both the client statement and the overall aim for devices of this kind.

The versatility of the device was given a rank of 4, as it was given a higher importance than the following objectives by nearly every member of the team, advisors and client included. Creating a device that is versatile such that it can be used with various sizes and types of sutures (fibrin, silk, or other biomaterials) and is adjustable to facilitate that use is crucial for the success of the device for applications outside of the scope of this project. While versatility was not necessary for our particular use of the device, it is important to consider should the device be used outside of the WPI Myocardial Regeneration Laboratory.

Marketability was given a rank of 3 by the team. Much like versatility, the team prioritized marketability over safety, ease of use, and sustainability in order to facilitate the transition of the device outside of the WPI Myocardial Regeneration Laboratory at the completion of this project. Marketability, however, was ranked lower than versatility due to the
inherent notion that marketable items should be aesthetically pleasing. While the team would like to have an aesthetically pleasing device, it was not necessary for the success of the device within the scope of this project and potentially within other labs at WPI. The team felt that aesthetics are not an issue so long as the device performs its intended functions. Thus, marketability was given a neutral rank of 5, as marketability is necessary for the device to succeed outside of WPI, but this project was primarily focused on its use at WPI.

Safety was given a rank of 2, as it was rated relatively low by the advisors and client in the Pairwise Comparison Chart. While the device should be inherently safe and adhere to safety standards set forth by ISO, ASTM, and other regulatory bodies, the team felt that adding features to further improve the device’s safety may restrict the device’s ability to perform all of the functions necessary for its success. The team recognized that safety is important, but believes that additional safety precautions beyond what is necessary would be nullified through proper training in the device’s use.

The device’s ease of use was given a rank of 2 by the team, for many of the same reasons that safety was ranked relatively low. The device should inherently be easy to use, as one of the objectives of the project was to design an alternative to the Flexcell device, which has a relatively steep learning curve associated with its use. The team assumed that most of the students (both graduate and undergraduate) and faculty that may be working in the WPI Myocardial Regeneration Laboratory have experience with either the Flexcell, basic fibrin microthread formation and manipulation, cell culture, or any combination of these techniques, which would facilitate the transition to the new device. Thus, it isn’t necessary that the final design of this device be any easier to use than the Flexcell, and this is reflected in its given rank.

Lastly, the device’s sustainability was given a rank of 1. While sustainability would drive down costs for the device and prevent large amounts of waste that could be harmful to the environment with the application of mostly reusable, sterilizable parts, the team did not think that sustainability was crucial for the design’s success. The team foresaw that the substitution of reusable parts within the design could create challenges during the design phase that could prevent the team from focusing on the initial goal presented in the revised client statement. Additionally, the team foresaw that the use of these parts could limit the designs for the device or inhibit the device’s function.
After ranking the initial objectives from the Pairwise Comparison Chart, the team determined four main functions to consider in the final design of the device: methods for suture loading and unloading, cyclic stretching methods, methods for live imaging the sutures and cells, and methods for loading and unloading media from the device. Sections 4.1.1. through 4.1.4. cover primary means, as well as secondary and tertiary means and functions for each primary function.

4.1.1 Methods for Suture Loading and Unloading

Appendix B shows a function means tree for the loading and unloading function identified by the team. The items in the square boxes represent functions, while the items in the trapezoidal boxes represent means to accomplish those functions. The first square, labeled “Suture Loading”, identifies the primary function, while the first set of trapezoidal boxes represent the primary means. The primary means to accomplish the suture loading function include a pin, a clamp, a ring, a second needle, a hole and stopper, a removable adhesive, and wrapping. Each of these means must provide similar functions, so the trapezoidal boxes are connected to the stem leading from the box labeled “Pin”, so as to not cause repetition. As shown in the second set of square boxes, the secondary functions for these means include holding the suture in place, being biocompatible, not causing any damage to the suture or the cells loaded onto the suture, minimizing the amount of parts, being easy to use, and being easy to transport. The secondary mean to accomplish the first secondary function is to provide a tight grip on the suture, or to provide enough compression between the suture and the anchoring point on the device such that it does not slip. The means to accomplish the biocompatibility function include the use of non-toxic metals that do not experience corrosion or ion-leaching when submerged in media, and plastics and other polymers. In order to not cause damage to the suture or the cells on the suture, the suture loading device must not have any sharp edges that could puncture the suture, fraying it or causing cell necrosis. Lastly, in order for the suture loading device to be easily transported, it must be able to be separated from the main device, and thus must be a separate system itself.
4.1.2 Cyclic Stretching Methods

Appendix B shows a function means tree for the cyclic stretching function identified by the team. As with the previous figure in Appendix B, items in square boxes represent functions, while items in trapezoidal boxes represent means to accomplish those functions. The first square identifies the primary function, cyclic stretching, while the first row of trapezoidal boxes shows the primary means that the team determined could accomplish that function. These means include a piston, a camshaft, an electromagnet, and a spool, and these means will be further explained in section 4.2. The next row of squares shows the secondary functions associated with each mean. For the sake of simplicity and space, only the secondary functions for the piston are shown, as many of the secondary functions for the primary means are similar. The secondary functions for the camshaft, electromagnet, and spool are connected to the secondary functions of the piston to show their relationship. These secondary functions include powering the piston (or cam, electromagnet, and spool in the case of the other primary means), adjusting the frequency, force, and strain of the device, and storing data. The fourth row indicates the secondary means used to accomplish the secondary functions. For cyclic stretching, the secondary means include a motor to power the piston (or other primary means design), and an Arduino to adjust the frequency, force, and strain, as well as store the data. To save room on the function means tree, the items that would appear in under “Store Data” and “Power the motor” direct the reader back to the box labeled “Arduino”, as these secondary and tertiary functions require an Arduino.

Under “Arduino” in the trapezoidal box, there are two tertiary functions: control and power the Arduino. The tertiary means include coding to control the Arduino and two means for powering the Arduino, including outlet/wall-based systems and battery-based systems. The square boxes below “Battery-based system” outline functions for the battery-based power system, while the trapezoidal boxes under “Control the Arduino” outline means for coding. Functions for the battery-based power system include the ability to control electrical current, store electricity, and convert chemical energy to electrical current. Means for controlling the Arduino include different coding languages, including Java, Python, and MATLAB.

4.1.3 Methods for Live Imaging

Appendix B shows a function means tree for the live imaging function identified by the team. As with the previous figures in Appendix B, items in square boxes represent functions,
while items in trapezoidal boxes represent means to accomplish those functions. The first square box represents the primary function, live imaging, while the three trapezoidal boxes below represent the primary means to accomplish the live imaging function. These primary means include using light microscopy of the whole stretching device, creating a separate imaging chamber for the sutures, and using a portable USB microscope to live image the device inside the incubator. The secondary functions are those items in the second set of square boxes that outline what those primary means must do in order to accomplish the primary function. The secondary functions for light microscopy include maintaining sterility, keeping the sutures flat on the imaging surface, having the ability to be marked easily such that progress can be easily viewed, be smaller than a standard incubator, and stay incubated while being imaged. The separate imaging chamber mean also shares these secondary functions with light microscopy, and the relationship can be seen by the line connecting the separate imaging chamber mean with the light microscopy mean.

Additionally, the separate imaging chamber must have the ability to apply a voltage to the fibrin sutures to simulate a heartbeat. In order to do this, one of the secondary means that the team developed was to use electrodes attached to a six-well plate. This secondary mean has two tertiary functions: to keep the sutures and electrodes in place, and the power the electrodes. For keeping the electrodes and sutures in place, the team came up with two tertiary means - silicone glue and PDMS molds. For powering the electrodes, the mean directs you to a tertiary function in the light microscopy section.

The secondary means for the functions of the light microscopy idea include designing the device to fit inside a T75 flask or a culture plate, which keeps the sutures flat on the surface of the device, maintains sterility, allows the device to be marked for imaging, allows for a smaller size such that it can fit in an incubator, and thus makes the device able to be incubated while imaging. Additionally, in order to stay incubated, the team determined that a heated stage or a separate incubator or cell humidifier would help with this secondary function. Tertiary functions to accomplish both the heated stage and separate incubator secondary means include applying heat to the device, generating electric current, converting heat to electricity, and protecting users from burns. In order to generate electric current, the team identified two tertiary means - a wall/outlet system, and a battery-based system, similar to what was done in 4.1.2. This battery-based system must have at least three quaternary functions, which include controlling electrical
current, storing electricity, and converting chemical energy to electrical current. For converting heat to electricity, the two, tertiary means include a heat pump and a resistive wire.

For the portable USB microscope, the team determined that the secondary functions of the microscope were to collect data in real time, as well as have some sort of external power. The means for externally powering the microscope and for collecting data in real time include using an external computer with a software that can both power the microscope and collect raw image data. The functions and means for that external software are similar to those of the heated stage and separate incubator, and thus the function directs the reader to the “Generate electric current” function under the light microscopy function.

4.1.4 Methods for Loading and Unloading Media

Appendix B shows the function means tree for the media loading and unloading function. As with the previous appendices described in this chapter, the square boxes represent the functions, while the trapezoidal boxes represent the means to accomplish those functions. The main function, media loading, has three primary means identified by the team: pipetting, stopcock valves, and a peristaltic pump. All three means have similar secondary functions that they must accomplish, which are outlined under pipetting only, and connected with lines to show the relationship, in order to save space on the tree. All three, primary means must minimize the amount of media usage, as well as minimize any spilling or waste that may occur, must be efficient, simple, and have high accuracy, and must minimize interference with the main device’s function. In order to minimize spilling and media waste, the team determined three secondary means: a smaller container for the device so that less media is used, a removable lid to allow for media loading and unloading while also preventing spillage, and a container with no seams to prevent leaking. In order to be efficient, simple, and have high accuracy, the team decided that using micropipettes would be a sufficient secondary mean for this function. In order to minimize interference with the rest of the device, the team also decided that a small device used to load the media would prevent interference with sutures and cells while still allowing for the replenishing of media.

The stopcock valve and peristaltic pump have more specific secondary functions and means, such as being able to remove all media from the device and being biocompatible. Because these two means are less accurate than pipetting, the team needs to ensure that all media
will be removed from the device to prevent contamination of the cells from old media. Thus, placing the valves lower in the container that holds the media is an acceptable secondary mean for this function. Additionally, there would be tubing involved, which would need to be biocompatible such that it does not allow for media to collect inside it. The secondary mean for this function would be to use a hydrophobic tubing, perhaps silicone, which would allow the safe passage of media through the device without creating buildup in the tubing.

Lastly, the peristaltic pump has specific functions, including a way to power the pump. Similar to the powering means for cyclic stretching, the pump would need to be powered by a motor, which would be powered by an Arduino. The Arduino would then be controlled by code, either Python, Java, or MATLAB, as identified by the team. The Arduino would be powered by either an outlet/wall system or a battery-based system, which would need to control electrical current, store electricity, and convert chemical energy to electrical current.

The team concluded that in order for the device to be successful, each conceptual design for the device must in some way address each function. These functions were then used to evaluate each conceptual design compared to a baseline to determine the best conceptual designs for that particular function. The conceptual designs that best addressed each function were combined to form preliminary designs that were evaluated based upon the ranked objectives.

4.2 Conceptual Designs

For each primary function, the group developed designs of the different means by which to perform said function. The following subsection is divided according to each of these main functions. This was done so that when the group began creating preliminary prototypes for testing, there would be a different set of tests the group could perform at once. These means are presented in either drawings or pictures to complete the function.

4.2.1 Suture Loading and Unloading

The team considered several different design ideas to complete the function of loading and unloading the fibrin sutures from the device. Knowing that the sutures themselves would be only 2 centimeters long, and that there would be a suture needle tied on at least one end, the team developed a series of methods for attaching the sutures into the device such that they could be cyclically stretched. Some of the limitations of the function as a whole were also kept in mind.
when ideating various means. For example, the sutures would most likely be constantly removed and placed back into the device, so the mechanism for loading and unloading the sutures had to be simple to perform. In addition, the sutures are delicate, especially when in a dry environment, and could fracture if not handled properly. The sutures could also contain seeded cells throughout the experiment, and any method of loading and unloading should not cause any damage to the cells.

Some of the means developed for loading a suture into the device included using a pin to hold the suture to a surface, a clamp to pinch the suture in place, tying the suture to a ring, attaching a second needle to the suture so it can be held in place using a needle on either end, a hole and stopper so the suture can be placed through a hole and pinched down using a larger object, a removable adhesive to glue the suture onto a surface, and simply wrapping the suture around a small peg or post. It is important to note that all of the means mentioned are meant for the end of the suture that does not contain the surgical needle, as it would be much simpler to attach the surgical needle to a rigid component of the device. Figure 1 below outlines each method side by side with one another. This subsection overviews the advantages and disadvantages of each mean displayed in Figure 1.

The pin mean of loading and unloading the sutures is a method by which the non-needle end of the suture is pinned to a flat surface while the end with the needle is held in place by a ring or a hook. Some advantages to this mean was that the process would be very quick to perform and could be easily repeated using the same pin. In addition, it is possible to obtain a pin
that is biocompatible with the sutures, cells, and media that it is held in, provided that it is made from a proper material. However, a disadvantage to this mean was that although a pin can be biocompatible and can be placed in the same environment as the suture, it is possible that constant pinning could damage or even fracture the sutures, limiting the number of times that live imaging could be performed. It was determined that it may also be difficult to keep the pin in place depending on the design of the device. Because a mean should minimize the effect it has on the rest of the functionality of the device, it was important that this mean, as well as the other means to load and unload the sutures, allow for the other functions to operate properly.

A clamp, either using paper clips or alligator clamps, were also considered as a mean to keep the sutures in place. With this mean, the non-needle end of the suture would be placed inside of the clamp. An advantage to using a clamp was that it is simple, helps minimize the amount of moving parts, and can be biocompatible to prevent any contamination of the sutures. A disadvantage this mean has, however, was that it could also damage the suture when it is repeatedly placed in and out of the clamp. The amount of damage that could occur depends on the suture being used. The sutures should not fracture while in the device, as this could cause a greater number of supplies needed in order to successfully stretch one suture. In addition, the clamp may be difficult to access depending on how the device is put together and can only be accessed by using sterilized surgical tools, which may be difficult to use.

Another method of loading the suture into the device was tying the suture to an appendage such as a ring or a hole where the non-needle end can pass through. An advantage to this mean was that it is possible to get a tight knot if tied correctly. This way, the suture would not become loose while it was being cyclically stretched. Although it depends on how the knot was tied, this mean could also lower the risk of fracturing of the sutures. A disadvantage to this mean was that tying the sutures is difficult to do. When dry, the sutures are very brittle and can snap if bent incorrectly. When wet, the sutures become sticky and can attach to themselves, making it difficult to straighten the sutures, let alone tie a knot. This process would also be taking place in sterile media, so the process of tying a knot with the suture would also have to be performed using surgical tools. Overall, the process would not be easy and the sutures would have to be unloaded as well, meaning the knot would need to be untied, which can provide even more difficulty.
Another mean of loading and unloading the sutures was by placing a second needle on the other end of the suture by either tying the suture around the suture needle or by using a clamping needle, where an end of the suture could be inserted into a hole at the end of the needle and then clamped shut. This way, there could be two rings on either side of the device that are capable of holding either end of the suture. An advantage to this mean is that it is quick to load and unload the sutures to and from the device after the needle is put in place. This makes it easier to handle the sutures so that they can be placed into the device and helps reduce any damage that may come from human handling of the sutures. A disadvantage is that this mean may not accurately represent the amount of strain applied on the suture. For example, if a suture was measured to be 2.5 cm instead of 2 cm, then it is possible that when it is loaded into the device, it would not be stretched as much as a standard 2 cm suture. Not only would a second suture needle be difficult to put on the suture before it is loaded, but it also would increase the cost for materials that the WPI Myocardial Regeneration Laboratory has to provide. In order for all of the fibrin sutures created in the laboratory to be loaded in the device, the laboratory would need to purchase more suture needles in order to meet this demand. The needles are also difficult to place onto the sutures due to the precision needed to properly clamp the suture into the needle, and the fraying of the suture at the ends makes it more challenging.

The hole and stopper mean to load and unload the sutures involves placing the end of a suture through a small hole where it can be pressed or held down by another larger object from the other side, and cannot slip back through the hole. Again, this mean is much simpler and would be easier to perform, especially when using surgical tools. It could also be adjusted to use any of the variations of sutures that may be greater or less than 2 centimeters by ensuring each section of the sutures held in tension are 2 centimeters in length. A disadvantage to this mean, however, is that it could be easier for the sutures to detach from the loading mean as opposed to the other means. The ability of the sutures to be cyclically stretched relies mostly on the ability of the object stopping the suture from exiting the hole, which may become problematic.

Using a removable adhesive, such as silicone, to load the sutures into the device is another mean the group explored. This involves placing a suture onto a surface that contains an adhesive, or placing a small amount of adhesive on the suture itself. The benefits of this mean are that it is much simpler to load the suture in a device, even if it needs to be performed using surgical tools. Silicone adhesive is also available in the Myocardial Regeneration Laboratory,
reducing the time needed to test and use it in the device. Some of the disadvantages to this mean are that it is unknown if the adhesive will have an undesirable effect on cells and cause any cytotoxicity. It also limits the sutures’ abilities to be unloaded from the device so they could be imaged. If that adhesive is strong enough to hold the sutures in tension over a period of thirty days, the sutures may have to be taken off the adhesive by either pulling the suture, which could damage it, or cutting a portion away, which decreases the size of the total suture.

The last initial mean the group developed for loading and unloading the suture was to wrap the suture tightly around a peg attached to a rigid post. A peg can be made from a multitude of materials and is relatively easy to produce through rapid prototyping. This is also a possible mean that would be easy to perform using surgical tools. The main disadvantage, however, is that this mean would be very ineffective at keeping the suture loaded for an extended period of time with cyclic stress. Once the suture is placed under cyclic stretching, it is very likely that the suture will become loose.

4.2.2 Cyclic Stretching of Sutures

Once the sutures are able to be successfully loaded into the device, the main function that determines the success of the device is the ability to cyclically stretch the sutures at 0.5 to 3 Hz with a strain of approximately 10%, without causing fracture of the sutures. Because of this, there were fewer means by which to accomplish this function. One of the means developed by the group included using a piston to stretch the suture attached to two bending posts as seen in Figure 2.
The suture would be attached to two rigid posts connected to a flexible surface. The piston would then force the surface to bend from beneath, stretching the two posts and the sutures loaded onto them. The process by which the bending posts bend was hypothesized to assist in measuring the force placed on the sutures. In addition, the method of having the piston underneath the device to cause stretching prevented any concern of having moving parts inside of the chamber where the sutures would be stored.

Some issues with this method, however, included the restriction on some of the parameters. While frequency could be readily adjustable, strain and force could not be changed unless a new piston with certain parameters was placed in the device instead. This would require additional maintenance for whomever is operating the device, and complex mathematics in order to create the piston with the correct dimensions.

The second means to create cyclic stretching of the sutures was using a camshaft connected to a motor. Similar to the piston method, the camshaft again comes from underneath...
to stretch a flexible surface with two rigid rods where the sutures are connected, as seen in Figure 3.

A motor would turn the camshaft, pushing the piston up and then back down without sudden movements that could damage the sutures. As with the piston means of performing cyclic stretching, it would be much simpler to calculate the amount of force applied on the sutures, especially if the two rigid posts are used in this design. It also possible to use a motor that only has to rotate the camshaft at a specific rate.

Some disadvantages to this means, however, also include complex mathematics. A camshaft has to be designed correctly in order to obtain a proper cam profile, pressure angle, base circle, and other parameters in order to have the correct piston displacement necessary for the means of this project. In addition, this means for cyclic stretching hinders the ability to change the strain rate placed on the sutures. In order to apply a different strain rate that the laboratory might want to investigate, an entirely new camshaft would need to be created and applied to the device. This introduces several moving parts that may become cumbersome for anyone operating the device to handle.

The team also investigated using a spool as a means of applying cyclic stretching of the sutures. Unlike the previous two means, the spool would not be located beneath the device. Instead it would be utilized around the sides of the device or above it. The sutures would be connected to the spool on one end, and to an immobile surface on the other end. The spool would
be connected to a motor where it was possible to rotate in both directions to allow for the sutures to be stretched and then returned to their relaxed state. Figure 4 below displays a preliminary sketch as to how the spool will look and function.

![Figure 4: Spool means of cyclic stretch.](image)

A benefit to using this mean as a way to cyclically stretch the sutures is that there are fewer moving parts that need to be considered, since only one end of the sutures would be stretched. In addition, the spool provides much more versatility, with the spool able to be placed in several locations around the device, and allowing for adjustable force, strain, and frequency parameters. In addition, it would be much simpler to construct to allow for easier rapid prototyping.

Some of the disadvantages include difficulties attaching the sutures to the spool. The spool would be rotated and is expected to be cylindrical to avoid any bends in the sutures that can cause fracture. This, unfortunately, also hinders the ability of the sutures to be loaded onto the spool. There is also a concern as to where the spool should be placed on the device. If placed inside the device where the sutures are loaded, it could cause media to leak out of the device. If placed outside of the device, there then needs to be an additional connector between the spool and the sutures. This could also be a problem with cytotoxicity and contamination, as any foreign object could potentially harm the cells seeded to the sutures.

Next, the team investigated using electromagnets in order to cyclically stretch the sutures. Similar to the piston and camshaft means, this mean requires that the sutures be attached to two rigid posts on either side. However, instead of stretching the sutures by imposing a force from below, this mean uses magnets that can have their polarities reversed as a method of moving one
of the rigid posts so the sutures stretch, and then return back to their original position. One magnet would be connected to an Arduino so that its polarity can be reversed cyclically, while another magnet is attached to one of the rigid posts where the sutures are attached. Figure 5 provides a drawing of an example electromagnet design concept that can be used.

![Electromagnet means for cyclic stretch.](image)

An advantage to this method includes having all of the materials inside of the device, rather than having some parts of the device on the outside that can cause contamination or media leakage. Instead, the Arduino, magnets, rigid posts, sutures, and media can be placed in the device at once. In addition, there are less moving parts, with only one of the posts being mobile in this design, as opposed to other motors and components moving along with stretching sutures. Although there will be more coding required, this is a great advantage because it reduces the complexity of movement of the sutures in the device.

Although the device can be sealed, cytotoxicity is still an issue, because it is unknown if the magnets will react with the media, sutures, cells, or other components of the device. This depends on the material that the magnets are made from, however a cytotoxicity test is still necessary in order to determine which material to use. This is a similar concern when considering the Arduino board and wires. These electrical components should not be placed in the media, not just to prevent cytotoxicity, but also to protect the integrity of the components. In addition, an electromagnet that turns on and off would not be sufficient enough to cause the rigid posts to move back to their original position. Therefore, the polarity of the magnet would need to be reversed or a spring would be needed in order to move the post back. These solutions, although they would be effective, may not cause a uniform cycle of stretching and could have portions of stretch that have higher or lower accelerations than other portions, which can damage the sutures.
4.2.3 Live Imaging of Sutures

Along with being the most important objective based on the pair-wise comparison chart, live imaging was also an important function to consider when designing the device. Live imaging of the sutures and cells is supposed to indicate the status of the experiment as the experiment is progressing. This helps in not only reducing the time to halt or stop a failed experiment entirely, but also in saving supplies that could be used in other areas in the laboratory. In order for this function and objective to be successful, the cells should be visible on the sutures when viewed under a microscope. In order for this to occur, the sutures and cells need to be placed flat on the surface they are being viewed under. To accomplish this objective, the group developed three different means by which to provide live imaging: design the device inside a container similar to a T75 flask or Petri dish that will allow for light microscopy, using a portable microscope capable of connecting to a USB port on a computer, and designing a separate container for the sutures to be placed for live imaging.

The first mean for accomplishing live imaging was through light microscopy. Because the Gaudette Laboratory has access to an inverted light microscope, it would be simple to design the device to cyclically stretch and maintain the sutures while being viewed under a microscope. In order to accomplish this, the sutures would need to be flat on the surface of the device in order to be visible. The team hypothesized that designing the device to be similar in design to a T75 flask or Petri dish would be the best way to allow for live imaging underneath a microscope. Advantages to this include that there would be fewer total parts needed in order to complete all of the objectives, the device could be marked easily in order to ensure that a user is looking at a suture in the same location consistently, and it would take up a smaller amount of space in an incubator, allowing for easier access for others who are also performing experiments.

One disadvantage to this mean, however, is that the device would need to be incubated while it is placed underneath a light microscope to keep the cells in a stable environment. This means that while there will be less parts to create for the device itself, the team would have to build an incubator capable of not only keeping the cells in a stable environment, but also not interfere with viewing the cells under the microscope. Another disadvantage includes the effect the T75 flask or Petri dish design will have on the other functions and objectives of the device. As mentioned above, in order for the sutures to be viewable underneath a microscope, they have
to remain flat on the surface of the device. Although this is possible to do, it would also hinder the ability of the device to cyclically stretch the sutures. If the sutures remain flat on the surface of the device while they are being cyclically stretched, the friction between the sutures and device could lyse a large number of cells seeded on the sutures. The device may also not provide enough room in order to accommodate the means to complete the other functions, such as cyclic stretching with either a piston, spool or electromagnet.

The second mean the group developed to perform live imaging was the use of a portable microscope that could be placed on top of the device in order to allow live imaging as the device was running. In particular, the group found a Zeiss Axiocam, a portable microscope capable of capturing color images at 47 frames per second, able to be connected to a computer via a USB cable, and is compact enough to fit inside of an incubator without taking too much space [53]. Figure 6 provides an example of how this portable microscope could be tested in order to determine if it is able to be used in the device.

![Portable microscope means for live imaging.](image)

Advantages to using this mean is the ability to keep the device in an incubator while also recording live data on the progress of the experiment. This prevents any issues with having to maintain the device in an incubator-like environment as it is taken out of the incubator. In
addition, it would also be simple to analyze data obtained from the microscope with the use of the USB cable, thus accomplishing both the function and the objective.

A disadvantage to this mean, however, is that, once again, the sutures would need to be placed flat at the bottom of the device in order for the microscope to focus on the sutures. If the sutures are placed on an elevated surface, then the device has the same issue as the FlexCell, in that it is not possible to focus on the sutures and the cells. The microscope is also expensive and would require a large amount of the budget allotted to the group in order to acquire it. With this in mind, and without knowing for sure if the device would successfully allow live imaging, using a portable microscope would be a risky mean to test and could prevent the group from testing other means.

The next mean for performing live imaging involved creating a separate device where the sutures can be removed from the device where they are being cyclically stretched and placed into a container where they can be live imaged. With this in mind, it would also be beneficial for the device to allow stimulation of the sutures and cells in order to observe how they would react while in in-vivo conditions. This could be performed by applying a voltage on the sutures with the use of electrodes while observing them under a light microscope. A graduate student in the Gaudette Laboratory developed a 6-well cell culture plate with these electrodes attached (Figure 7) that the group believed would provide a good simulation of in-vivo conditions while also allowing for live imaging.

Figure 7: 6-well plate with electrodes for cell stimulation.
The group made slight changes to this design so that it would better suit the needs of the project. For example, the group did not want the sutures to be exposed to adhesives or glues that would potentially damage the sutures or cells in the culture plate. The group also did not want to have the suture fold in on itself when current flows through the culture media, which could damage the cells and cause the sutures to stick to each other, making them difficult to unfold and place back into the device to be cyclically stretched. Figure 8 outlines some of the changes the group developed to the original concept of the electrodes in the culture plate.

![Thread holder for live Imaging]

Figure 8: Separate live imaging system.

The group hypothesized that two clamps made from PDMS would prevent the sutures from folding in on themselves while also allowing the sutures to be visible for viewing and electrode stimulation. This way, the sutures could be easily placed in and removed without any adhesives or glues.

An advantage to this mean includes keeping the cells and sutures in a viable environment that will not damage either of them. This mean would theoretically keep the sutures anchored
down while also allowing in-vivo tests and live imaging during said tests. This mean is also able to fit within the budget of the project, since less expensive materials are required to create the device, such as a silicone adhesive, stainless steel, titanium, or tungsten, which are also readily available within the Gaudette Laboratory. This is also a much less expensive alternative to platinum wires. The main advantage to this mean is that it has little to no hindrance on the other functions. This is because live imaging of the sutures is performed in a separate container than the cyclic stretching of the sutures.

A disadvantage to this mean, however, is that it is riskier to move the sutures from one device to another. It is possible that one or more sutures could be dropped outside of either device while transferring from one device to another, or that the sutures could be picked up where there are cells located, damaging the cells. In addition, this mean requires that additional testing and prototyping be performed, which can be more time consuming to test both device functionalities and their compatibility with one another.

4.2.4 Media Loading and Unloading

Inserting cell culture media into the device and then replenishing the sutures with new media was another major function that had to be considered. Because the sutures would eventually have cells seeded onto them, it is clear that those cells need nourishment throughout the experiment in order to maintain viability of the device. One major obstacle the group considered included the amount of spillage that would come from the device while it is running. This could not only damage the cells on the sutures, but could also be an inconvenience to other experiments contained in the same incubator. Another major obstacle included the usage of media in this experiment. Although the device would be small in order to accommodate the small sizes of the sutures, the group had to consider using as little media as possible in order to minimize costs for the laboratory.

The means developed for loading and unloading media in the device included pipetting media into and out of the device. This is both the simplest way of loading and unloading media and is one of the more efficient methods of performing this function in cell culture. The method is highly accurate, especially when using micropipettes that can be set to insert a specific volume of media. This also ensures that all old media is removed from the container so new media can be inserted.
Disadvantages to this means include possible interference from the micropipette tips in the device, and increased costs from using micropipette tips. It is important that there is little to no contact with the sutures that could kill any cells. Although this is a common practice performed in aseptic cell culture techniques and micropipette tips can be sterilized, it would also mean that a foreign object that could interfere with cell growth is placed inside of the device. The lab has a good supply of micropipette tips; however, the group also wants to minimize the cost to operate the device so as not to add any unneeded expenses on the Gaudette Laboratory.

The second means the team developed by which to load and unload media was to have two stopcock valves attached to the device, one where media can enter into the device and one where it could exit. This method helps prevent constantly opening and closing the device to remove media that could cause any contamination to the cells or damage the device. Instead, old media could be exported from the device through one valve and then new media could be inserted through another valve. This also helps reduce any price increases that come from constantly using micropipette tips and can be a very simple process that anyone can perform. Figure 9 provides a drawing for this means of media loading and unloading.
Some disadvantages of this method, however, include difficulty in removing the full amount of media from the device. Spare media that is not removed from the device could cause cytotoxicity issues for cells. In order to remove all of the media, the stopcock valve must be located at the bottom of the device so that the media flows down. This may not be possible when considering the space in the incubator allotted to the group to test the device. In addition, if the device is placed in a communal incubator that used by other individuals at Gateway Park, it may not be possible or would be very difficult to place the media container in a location that allows for easy flow of media into the device.

The last means developed by the team to allow for media loading and unloading is to use a peristaltic pump to move media into and out of the device. Figure 10 provides a drawing for this means below.

Figure 10: Peristaltic pump system for media loading.

This means for media loading and unloading is similar to the stopcock valve means in that it tries to prevent any contamination to the sutures in the device by having the media be
inserted through tubing, rather than having it be manually inserted. In this case, the peristaltic pump moves media in and out of the device with more control and precision. This is also used to help reduce the amount of media used by the device so that the Gaudette Laboratory can lower costs for obtaining fresh media.

The disadvantages to this mean are also very similar to the stopcock valves in that it may not remove all of the used media from the device unless the two tubes leading to a waste container are placed at the very bottom of the device. The amount of materials needed inside of the incubator would also be a hindrance to others who may need to use the incubator.

4.3 Alternative Designs

The following section contains combined design ideas based on the conceptual designs for each function mentioned in the previous section. This includes the benefits of each design, along with any constraints and concerns where a design was not expected to perform as well as other designs.

4.3.1 Piston/Camshaft Design

The first preliminary design devised by the team was centered on a piston and camshaft mean for cyclic stretching, as seen in Figure 11.

![Figure 11: Drawings of piston/camshaft design.](image)

The sutures would be secured with clamps between two rigid posts fastened to a flexible, rectangular base made from PDMS. A firm block would connect the bottom of the PDMS platform to the piston. Beneath the piston, a DC motor-driven camshaft would power the pistons such that the sutures would be cyclically stretched at 0.5 - 3 Hz to 10% strain.
This design was attractive due to its simplicity. An Arduino board could easily be programmed to automate the DC motor control to turn the camshaft at a rate of 0.5 to 3 Hz. With each rotation of the camshaft, the piston would drive up the solid platform, which in turn would bend the PDMS block. As the PDMS block bends, the fixed posts bend outward causing the suture to stretch. As the piston and posts returned to their starting positions, the suture relaxes in preparation for the next cycle. Additionally, since the DC motor would only have to turn one direction to cyclically drive the camshaft, it would allow a high degree of control. The code could easily be written such that the stretch part of the cycle could occupy a specified portion of the stretch period, the suture could be held in the stretched position for a set period of time, and the suture could relax for a specified portion of the stretch period.

Despite the piston/camshaft design’s advantages, it presented a number of potential problems. The primary problem with the piston/camshaft design was the lack of flexibility with suture length. Since suture lengths can vary within a range, the device must allow for adjustment of the stretch displacement. Longer sutures require a larger displacement to achieve 10% strain, while shorter sutures require less displacement strain the suture by an equal percentage. In the camshaft/piston design, the suture displacement would vary as a function of the size of the cam, meaning the device would have to include different size cams for each possible suture length. The user would also require the user to swap cams each time sutures of a different length are loaded. Similarly, since the stretch cycle would be driven by one cam and one piston, all sutures would be displaced by the same amount. This would require that all sutures loaded simultaneously be the same length to avoid shorter sutures experiencing greater strain and longer sutures experiencing less strain.

4.3.2 Upright Spool/Carousel Design

The second, preliminary design proposed by the group was centered on an upright spool. Figure 12 illustrates the design.
The spool is housed within a hollow cylinder. Sutures would be loaded with the needle-end attached to the cylinder with clamps and the opposite end attached to the spool. The volume between the cylinder and the spool would be filled with adequate media to cover the loaded sutures. A servo motor would be positioned below (or alternatively above) and fixed to the spool. An Arduino microcontroller, housed in a separate container, would control the stretch cycles driven by the servo motor. Once the sutures were loaded, the motor would rotate the spool a specified number of degrees until the loaded sutures had undergone 10% strain. The spool would then rotate the opposite direction, returning the sutures to their resting length in preparation for the next cycle.

This design presents a number of significant advantages. The most valuable advantage is the ease of automation. A servo motor could easily be automated to cyclically stretch the sutures, and the user to modify the cycle by adjusting a few parameter values. This design would afford the user a wider range of modeling possibilities. In addition to the ease of automation, the carousel design maximizes the number of sutures that could be loaded at once. The upright spool opens the entire spool’s circumference for loading, and sutures could be loaded in levels depending on the height of the spool. Figure 13 below illustrates the loading capabilities of the carousel design.
Despite its advantages, the carousel design has two major flaws. Primarily, since the device is built around a spool which sits within a hollow cylinder, the device cannot be adjusted to ensure that sutures of varying lengths all receive 10% strain. A fixed distance exists between the spool and the inside wall of the cylinder meaning that all sutures will experience the same displacement during a stretch cycle. Since there is a degree of variation in suture length, this will result in the shorter sutures experiencing more than 10% strain and the longer sutures experiencing less than 10% strain. The only way around this problem would be to limit the device to accept sutures with lengths very close to the standard length such that they would be strained within an acceptable range of error.

The second problem presented by this device is the loading mechanism. The cylinder would have a radius of approximately 5 cm in order to accommodate the spool and the sutures. It would be quite difficult for a user to load layers of sutures into a device of this scale all while working in a biosafety cabinet under sterile conditions.

4.3.3 Horizontal Spool Design

The third preliminary design proposed by the team sought to draw from the advantages of the carousel design while accounting for varying suture lengths and minimizing loading.
difficulties. Two configurations of this design were proposed — the illustrations of which can be seen in Figures 14 and 15 below.

Figure 14: Spool design with diagonal stretch.

Figure 15: Spool design with aerial stretch.

These spool designs both take advantage of the carousel design’s easy automation. Both operate by turning a spool which stretches the attached sutures by an amount proportional to the spool’s angle of rotation. The design also allows for the introduction of attachments which account for the length of each individual suture to ensure all sutures experience the desired strain percentage. Additionally, neither design includes the hollow cylinder surrounding the spool which significantly facilitates the loading and unloading.
These advantages come at a cost however. The horizontal orientation of the spool prevents the use of the entire spool circumference significantly reducing the number sutures that could be loaded in comparison to the carousel design. Also of concern, with each stretch a segment of the connectors which join the spool and the sutures would be pulled outside the sterile box and then return into the sterile box during the relaxing part of the cycle. The repeated crossing of the sterile boundary could easily contaminate the inside of the sterile box and compromise the whole experiment.

4.3.4 Electromagnet Design

To solve the sterility issue of the spool design, the team developed a novel idea based on electromagnetism. This design proposal features an electromagnet which would be automated to control the cyclic stretch cycle. Figure 16, below, illustrates the design.

![Figure 16: Electromagnet design.](image)

As seen in Figure 16 above, this device consists of two boxes. Box 1 is a non-sterile environment designed to house an Arduino and a precision electromagnet. Box 2 is designed as a sterile housing for the sutures. The stretch system consists of one fixed post and one sliding post positioned parallel to each other. The sliding post contains a core of magnetic core which can be attracted or repelled by the electromagnet depending on the polarity. Sutures are loaded and fasted with one end to each post. The Arduino code controls the current sent to the magnet such
that the magnet attracts the moving post and causes the sutures to stretch before repelling the post which returns the sutures to the relaxed state.

This preliminary design offers two major advantages. Most importantly, the use of separate compartments and the absence of any components crossing boundaries during the stretch cycle eliminates the possibility of contamination. Secondly, the sutures could be loaded and unloaded with ease compared to the spool designs. The user has adequate room to maneuver, and would not need to remove any components to access the sutures.

As with the other designs, the electromagnet design lacks in certain areas. The most significant issue relates to the automation of the electromagnet. This device would be especially difficult to automate. The current supplied to the electromagnet would require extremely specific control to ensure the resulting magnetic field was an appropriate strength during all phases of the stretch cycle. The first difficulty in controlling the magnetic field arises in changing the polarity of the electromagnet. Figure 17, below, illustrates the stretch cycle phases with their accompanying polarities.

![Figure 17: Electromagnetic phase diagram.](image)

Changing the polarity of the electromagnet is done simply by switching the direction of the current. However, the reversed magnetic field does not instantaneously follow the current reversal. Also, the strength of a magnetic field varies with the inverse cube of the distance. This would make the current control very difficult to automate. During the stretch phase, the distance between the magnetic post and the electromagnet would decrease. The strength of the magnetic field would increase dramatically as the magnetic post approached the electromagnet. The current supplied to the magnet would have to be specifically reduced to slow the stretch phase to a gradual stop instead of accelerating it until it contacted the electromagnet. When the
electromagnet polarity was reversed to return the sutures to their resting phase, the current would have to be specifically increased as a function of the distance between the electromagnet and the magnetic post so that the motion would be controlled. These factors greatly increase the difficulty of automating a stretch cycle that would adequately model a cardiac waveform. In addition to the difficult automation, the electromagnet would generate a large amount of heat. This heat could potentially damage or even kill the seeded cells.

4.4 Final Design Selection

After ranking the objectives in chapter 4.1, the next step was to compare the designs outlined in 4.3 to the baseline device, the FlexCell. The FlexCell was chosen as the baseline device because it is currently the only device employed by the WPI Myocardial Regeneration Laboratory that cyclically stretches cells on fibrin sutures. For this comparison, each design was given a score for each objective based on how it compares to the baseline device. A design that outperforms the baseline on a particular objective was given an objective score of +1, while a design that underperforms compared to the baseline device was given an objective score of -1. Designs that neither outperform nor underperform compared to the baseline device were given scores of 0 for that objective. Table 4 below shows each design compared to the baseline for each objective.
Table 4: Pugh method concept selection. This table shows the comparison between each design and the baseline for each objective. It also includes each objective’s rank in order to obtain a total score for each design.

This method is known as the Pugh method of concept selection, and is widely used in the medical technology field [54]. The Pugh method is particularly useful for final concept selection because it connects design objectives with predetermined concepts, and is relatively simple to perform and understand. This method also forced the team to review the design objectives once more in order to understand how each conceptual design addresses each objective, and provided a quantitative, objective method for evaluating conceptual designs [54].

Table 4 also includes each objective’s rank (in parentheses) next to each design’s objective score. In order to obtain a total score for each design, the rank and score were multiplied for each objective and each design, and the columns were summed. For example, the
electromagnet was given an objective score of +1, and that objective is weighted with a rank of 7, so that cell receives a 7. The same method was used in each cell, and the cells for the electromagnet were summed to get a total score of 18.

All designs received a +1 objective score for live imaging, as all designs intend to incorporate the separate live imaging system. Because live imaging with the FlexCell is impossible due to the opacity of the plates and the inability to remove the sutures from the device without terminating the experiment, any device that allows for live imaging in any capacity is inherently more ideal for that objective than the FlexCell. Similarly, all designs received a +1 objective score for measuring force, frequency, strain, and voltage, as those measurements will be incorporated into the separate live imaging system, while the FlexCell has no such way of measuring these parameters.

For versatility, the electromagnet and the piston/camshaft designs were given an objective score of 0, as the team believed that they neither outperformed nor underperformed compared to the FlexCell in versatility. Both the electromagnet and the piston/camshaft design had a few inherent issues in terms of versatility - the piston/camshaft design was limited by the size and shape of the cams rotating to drive the piston. While one could machine any size necessary and easily mount it in place, the calculations involved in determining which size and shape would produce a specified amount of strain were tedious and did not offer much room for variation in strain within the same experiment. The team faced similar problems with the electromagnet - in order to produce a particular strain, the electromagnet would have to produce a specific magnetic field shape with a specific strength. Should the user want to modify this strength or field in any way, there would be tedious mathematics and additional coding, which would come at a high cost to the user in terms of ease of use. While the team attempted to look at ways to eliminate some of these potential issues through easy to understand coding programs, the project timeline simply does not allow for the work necessary to complete those tasks, especially because they do not directly impact our two most important objectives. The two spool designs were given versatility scores of +1 because they have easily removable and replaceable parts, and the strain can be adjusted easily by the user through the use of a simple program to adjust the speed and degree of rotation of the spool.

The electromagnet and spool designs were given marketability scores of +1 because they are all novel designs that can be easily manufactured and have the potential to be aesthetically
pleasing. Additionally, they show promise for use with other materials; only slight modifications need to be made to each of the designs in order for them to be useful to laboratories that may be focusing on a different material, such as a patch. In this case, only the clamping mechanism need be changed. Additionally, these designs allow for the mechanical stimulation of multiple sutures at once, and have the potential to be scaled up. The piston/camshaft design was given a marketability score of 0 due to its limited use. Additionally, it does not pose many advantages over the FlexCell in this regard, as some form of mechanical grease to keep the piston lubricated would be necessary, which is one of the issues that those working in the WPI Myocardial Regeneration Laboratory have with the FlexCell.

All four designs were given a score of 0 for safety. None of the designs are inherently unsafe, but they are also not any safer than the FlexCell. Each design was created with the intention of adhering to safety standards, but none of the designs’ main focus was safety. The electromagnet, the horizontal spool, and the piston/camshaft were all given scores of +1 for ease of use. All three of these designs offer relatively easy suture placement and removal for imaging, easy sterilization, and easy access to parts should they need replacement. The carousel spool design, however, was given a score of 0 for ease of use, as the team felt that the circular design would make suture loading and unloading much more difficult for the user, especially at such a small scale.

Lastly, all four designs were given a score of +1 in sustainability due to their numerous reusable parts. While portions of the FlexCell are sustainable, the FlexCell uses 6-well plates that cannot be reused after an experiment is completed. Additionally, the system uses large amounts of grease to keep it lubricated, which is neither economically nor environment ally friendly. Each of the proposed designs have 100% reusable parts, with the exception of any cell culture media, cells, and sutures themselves. All of these designs can fit entirely inside of an incubator as well, which eliminates the need for an external cabinet for a pump or any other large equipment that cannot enter the incubator.

Using these numerical values, the team determined that the final design for the remainder of this project was the horizontal spool design. This design met or surpassed the FlexCell in all objectives, including the three most important objectives, making it an ideal choice for the team to move forward with. A more detailed explanation of this design, with slight modifications, is included in Chapter 4.4.1.
4.4.1 Description of the Final Design

The team chose to begin testing a modified version of the aerial horizontal spool design. This design, seen in the figure below, featured an overhead, horizontal spool just as the previously described aerial spool design. Figure 18, below, illustrates the revised device.

![Figure 18: a) Computer aided design (CAD) model of the revised, spool design. b) CAD model with exploded components.](image)

First, this section will describe the device as a whole before breaking it down for a closer look at each of the critical components. The proposed device is based upon the concept of a rotating spool driving the cyclic stretch function. An acrylic box serves as the bioreactor chamber and houses all moving parts. To address the sterility concerns of the previous spool design, the new design contains all moving parts within the box. It features no parts actively crossing the sterile/nonsterile boundary. A servo motor connected to the spool will rotate the spool, and an Arduino will serve as the motor control.

The largest component of the device is the bioreactor box chamber. This box will be constructed of acrylic to provide a cell-friendly environment. Figure 19 illustrates the box and its dimensions.
The box will be 6 cm long, 3.9 cm wide, and 7.0 cm tall with a wall thickness of 0.50 cm. As shown in the cross-sectional view seen in Figure 20 (c), the box design includes a small protrusion of 1.50 cm x 1.0 cm from its otherwise rectangular shape which allows for easy insertion of a pipette for adding or removing media. The described dimensions give the box a final internal volume (not including wall volume) of 105.3 ml. Also, as seen in Figure 20 (b), and each side of the box has a hole with a diameter of 6.0 mm located 5 cm from the outer wall of the box’s bottom.

Next on the list of components is the spool. The shaft of the spool rests at a height of 4.5 cm above the interior bottom wall of the box. The spool assembly (seen below in Figure 20) consists of the spool, a rotating shaft, a pin, a nut, and six pegs.
The shaft is inserted into the spool and pinned in place. The assembly is then mounted in the box by sliding both of the rods into the holes in the side of the polystyrene box. The end of the removable shaft is connected to the servo motor and a bold is applied to the fixed rod on the opposite side of the spool to hold it in place.

The spool itself will be 3D-printed using MED610 plastic. A diagram of the spool and specific dimensions are shown in Figure 21 below.
The spool dimension specifics are as follows: total length of 4.50 cm (including fixed projection of 1.50 cm in length), width of 2.0 cm, and max height of 1.5 cm. A square tunnel will be bored 2.5 cm into the spool to allow for insertion of the motor’s shaft. Along the bottom of the spool, a series of six holes of 1.1 mm diameter and spaced 0.5 cm apart will be drilled 3.0 mm into the spool. These holes allow for the insertion of the needle attachment pegs.

The needle attachment pegs are small cylinders with a diameter of 1.0 mm and a height of 2.0 mm and are 3D-printed using ABS plastic. Figure 22 displays the CAD diagram of a peg.

![Diagram of a peg with 3D design and cross-sections](image)

Figure 22: Dimensions of peg design. a) 3D peg design. b) Vertical cross-section of peg. c) Circular cross-section of peg.

The suture needle will be hooked through the hole in the peg, and the peg will be pushed into the receiving holes on the bottom of the spool.
Once the pegs have all been firmly inserted into the spool, the motor shaft will be attached by sticking the square end of the shaft deeply into the square tunnel running along the spool’s axis and placing the pin to secure the shaft in place. Figure 23, below, illustrates the shaft design.

![Figure 23: Dimensions of shaft design. a) Shaft dimensions. b) 3D shaft diagram. c) Cross-section shaft diagram.](image)

The shaft crafted from stainless steel is divided into two sections: the round motor attachment and the square spool attachment end. The round end is inserted through the hole in the side of polystyrene box and vacuum grease is used to create a seal around the shaft and reduce friction. The square end of the shaft, designed to prevent slip during the stretch phase, is inserted into the spool and pinned in place.

The sutures also require an attachment point at the bottom of the box. A rectangular post accomplishes this goal. Figure 24 illustrates the bottom, attachment post.
This post consists of a block 0.5 cm x 3.0 cm x 1.20 cm, as seen in Figure 24. Six holes spaced by 0.5 cm will be bored through the magnet to allow for the attachment of the suture needle on the end of the suture near the bottom of the tank. The post will be secured to the box using a strong, external, magnetic strip. The post will be placed at the bottom of the box directly underneath the spool. A thin magnetic strip will be applied to the external of the box’s bottom wall. The resulting force will hold the post in place.

The box’s cover is designed to fit loosely over the top of the box to allow airflow. Figure 25 displays the CAD diagrams of the box cover.
In addition to the cyclic stretch function, the revised spool design provides easy live imaging. To image the sutures, the external magnet is removed from the bottom of the box freeing the fixed post. The pin is removed from the spool and shaft, and the shaft is removed. The sutures can be removed simultaneously and still attached to the spool and the bottom post. The spool and post with the sutures between them can then be laid in a special imaging plate for imaging. After imaging, the assembly can be returned to the device and the cyclic stretch experiment resumed. Figure 26, below, illustrates the imaging apparatus.
Figure 26: CAD suture imaging apparatus design.
5. Design Verification

The following chapter outlines the results for the experiments performed in order to confirm the correct functionality of the group’s final design. During the performance of the experiments, the group made edits to the design in order to improve upon the functionality. These tests include observing if the motor operates correctly and can operate in incubator-like conditions, the individual design components such as the pegs used to hold the needles and the fixed post, the live imaging apparatus at taking images of the threads while loaded in the device, the strain placed on the threads tested through high-density mapping, and the potential for cytotoxicity based on the materials the device is constructed from.

5.1 Component Testing

In order to ensure that the device would work properly once constructed, we tested different components of the device individually. Individual component testing is important in order to validate the success of each component prior to testing the full assembly. In this way, it would be easy to identify the potential problems with our device on the component level and could be adjusted accordingly. In this fashion, we could then increase the chance that our full assembly would be successful. Additionally, single component testing identifies the weak links of our device, which can be carefully monitored once the device is fully constructed. In this section, we include single component testing for the motor, pegs inserted into the spool, the fixed post guide at the bottom of the device, and the live imaging apparatus.

5.1.1 Tower Pro SG90 Micro Servo Test

Since the device must operate within an incubated environment to ensure cell viability, it was important to test the motor’s functions under incubator conditions. To determine whether the motor would maintain its function, the TowerPro SG90 micro servo motor was set for a cyclic, 11° rotation and was run for an observational reference. It was then placed in an incubator at 37°C, 5% CO2, and 95% relative humidity and was left to run without interruption. After 24 hours of continuous rotation, the motor’s function was observed. No observable changes existed.

After establishing the motor’s ability to operate under incubator conditions, a preliminary motor longevity test was conducted. This experiment was aimed to determine whether or not the motor was capable of turning the spool and rod configuration within the device for an extended
period of time without any functional deficits. To perform this test, the device was assembled as shown below on the bench top.

![Image of motor longevity test configuration](image)

Figure 27: Motor Longevity Test Configuration. (Left): Arduino running the spool rotation program. (Top): Motor sandwiched between two glass containers to hold motor in place. (Bottom right): Full system, which includes the rod, spool, and acrylic box.

The spool and rod were connected within the device. The 3D-printed adaptor was used to connect the motor shaft to the end of the rod protruding from the box, and the motor was firmly clamped between two glass weights such that it remained completely stationary. It was decided to test the motor over a 72 hour period and observe function over time. This was to ensure that the motor would not experience fatigue from constantly rotating the spool. A reference point was etched on the spool to assist in measuring the degree of rotation. Every 12 hours, a protractor was used to measure the angle of rotation. Motor rotation was verified at every 12 hours over a 72 hour period. Over that time span, the same 10-degree rotation range was observed. The following data was collected.
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</table>

Table 5: Motor rotation angle over 72 hours.

As seen in the data, the motor did not experience any loss of rotational ability over the entire test period. Although the motor was set to rotate by 11°, difficulty in performing accurate measurements with the protractor likely accounted for the 1° difference. However, the motor consistently turned the spool by 10°, and no functional changes were observed. Another motor longevity test was performed inside of the incubator in order to confirm the full components tested above would not only operate in incubator conditions, but also rotate threads with the appropriate amount of force. This test is discussed later in this chapter.

5.1.2 Peg Test

Loading of the microthreads into the device was an integral component in the device. Therefore, in order to make the process of loading the threads easy to use while also keeping the threads in place, we designed three different types of pegs as outlined in the figure below:
All three peg designs are cylinders with a diameter of 0.25 cm and a height of 0.5 cm. The difference in each peg comes from the holes in each, which are designed to hold the bored needle attached to the microthread. The hole in Peg 1 has a diameter of 0.1 cm with an extrusion on the inside of the hole that decreases the diameter to 0.08 cm inside the hole. Peg 2 has an overall diameter of 0.08 cm. Both Pegs 1 and 2 are designed for the suture needle to enter through the hole and then be held in place. Peg 3, on the other hand, acts similar to a clip with the hole having a diameter of 0.1 cm.

To determine which peg would be most efficient at holding the microthread needles, the team performed two tests. The first test involved taping the spool along a wall with the pegs inserted and then inserting the needles into the pegs. This is illustrated in Figure 29 below.
From left to right, the types of pegs are 1, 2, 3, 1, 2, and 3. Because we had used two
types of bored needles when creating the microthreads, we decided to test using two of each peg
with the first three using the 0.75 cm diameter needle and the second three using the 0.12 cm
diameter needle. The spool was left to the wall for a total of 24 hours in order to see if the pegs
could hold the needles by themselves. Only one needle fell out during the total 24 hours, the 0.12
cm diameter needle in Peg 1.

While the other needles stayed in, we were also curious as to how users would react to
using the different types of pegs and with the different types of needles. To do this, we asked
three individuals who work in the Gaudette Laboratory to insert both a 0.12 cm diameter needle
and a 0.075 cm diameter needle into the three types of pegs using only hemostats. We timed the
amount of time needed to complete this task and also asked for the individual’s preference for
which needle they liked best. The table below illustrates these results.

<table>
<thead>
<tr>
<th>Individual</th>
<th>0.075 cm Diameter Needle Loading (s)</th>
<th>0.12 cm Diameter Needle Loading (s)</th>
<th>Preference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peg 1</td>
<td>Peg 2</td>
<td>Peg 3</td>
</tr>
<tr>
<td>Subject 1</td>
<td>4.8 s</td>
<td>5.9 s</td>
<td>5.4 s</td>
</tr>
<tr>
<td>Subject 2</td>
<td>8.2 s</td>
<td>10.3 s</td>
<td>12.7 s</td>
</tr>
<tr>
<td>Subject 3</td>
<td>10.2 s</td>
<td>8.9 s</td>
<td>7.6 s</td>
</tr>
<tr>
<td>Average</td>
<td>7.7 s</td>
<td>8.4 s</td>
<td>8.8 s</td>
</tr>
</tbody>
</table>

Table 6: Microthread-spool loading experiment times and preference. DNF (did not finish) indicates that the users
were unable to load the sutures into the pegs, and N/A (not applicable) indicates that there was no data available.

The results from this test demonstrated that the 0.12 cm diameter needles would be
insufficient to use for the device. Overall, the preferences of peg that the individuals preferred
varied. All three individuals had different opinions as to which peg worked the best. Since our
peg comparison test was inconclusive, we decided to perform additional testing to observe the
functionality of the pegs with additional pieces of the full design.
5.1.3 Suture Loading Test

Similar to the test performed above, the group asked two of the three volunteers from the previous section to test loading the microthreads into both the spool and fixed post to mimic the loading process before the spool and fixed post are inserted into the box together. The test consisted of using dry twelve thread bundles with 0.075 cm diameter bored needles on either end. The larger 0.12 cm diameter bored needles were not used in this experiment based on the data gathered from the previous experiment in that the needles would be unable to fit into the pegs or fixed post. The individuals were only allowed to use hemostats for inserting the needles into the pegs and fixed post to simulate the microthread loading inside of a culture hood.

The measurements taken included the time needed to load the microthread bundles, their preference of peg for the loading of the threads, a rating of the overall experience on a scale of 1 to 5 (1 being difficult/frustrating and 5 being easy/pleasant to use), and any additional notes or comments on the process. The table below lists the data taken from the experiment.

<table>
<thead>
<tr>
<th>Individual</th>
<th>0.075 cm Diameter Needle Loading (s)</th>
<th>Rating of experience (1-5)</th>
<th>Preference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peg 1</td>
<td>Peg 2</td>
<td>Peg 3</td>
</tr>
<tr>
<td>Subject 1</td>
<td>15.9 s</td>
<td>16.0 s</td>
<td>16.7 s</td>
</tr>
<tr>
<td>Subject 2</td>
<td>14.5 s</td>
<td>34.6 s</td>
<td>18.6 s</td>
</tr>
<tr>
<td>Average</td>
<td>15.2 s</td>
<td>25.3 s</td>
<td>17.7 s</td>
</tr>
</tbody>
</table>

Table 7: Microthread loading into spool and fixed post times, rating, and preference.

As expected, the overall time needed to load the microthreads into the fixed post and spool took longer, with the longest time being performed by Subject 2 on Peg 2. The purpose of the rating of experience measurement was to ensure that loading of the microthreads does not make the loading of the threads unnecessarily difficult to use. In addition to the ratings given by both Subjects 1 and 2, both had additional notes on how to improve the process so that the overall rating can be made higher. Subject 1 suggested reorienting the bored needles so that the ends of the needles face in opposite directions as shown on Figure 30 below.
Subject 2 also suggested moisturizing the threads below loading to make the threads more flexible. This also comes from the fact that while trying to first load the threads into Peg 1, Subject 2 broke two microthreads. If both suggestions were taken into consideration, the two Subjects stated that they would increase their overall ratings to be 4 and 5 respectively.

5.1.4 Live Imaging Apparatus Test

One of the objectives for this device was for it to allow for live imaging. To test the device’s capabilities of performing this task, we set up the spool and fixed post guide connected together in the live imaging apparatus as seen in Figure 31.

Figure 31: Device setup for live imaging test. The fixed post/guide is connected to the spool. Threads are attached to the spool and the guide is laid down flat so the threads can be easily imaged. The system is in a petri dish that is set up with two electrodes.
Testing for this was done under the Leica DFC420C Inverted Fluorescence microscope and performed with three microthreads inserted into the device at once. The microthread farthest from the fixed post end of the spool was held by Peg 1, while the microthread to its immediate left was held by Peg 2, and the microthread held on the far left was kept by Peg 3. A microthread not visible in the Figure above was also placed into flat onto the surface of the apparatus and was used as a control. Using the imaging software Las X and ImageJ, we imaged the microthreads under dry conditions and observed the visibility of the threads at 5X, 10X, and 20X magnification under the microscope. The Figure 32 displays the images taken with scale bars for measurement.

![Figure 32](image)

Figure 32: Results of live imaging test on unseeded microthreads in the prototype design. N/A (not applicable) indicates that we were unable to obtain data for that particular specimen.
The only thread in the construct that was unable to be imaged was attached to Peg 1 at 20X magnification. The reason could be a result of both the location of the peg on the spool, and the orientation of the thread as it was loaded into the prototype. The control experiment was a microthread with bored needles on either end lying flat on the Petri dish where the spool and fixed post combination were located.

The main concern of the device before testing began was the potential difficulty for the microscope to focus on the sutures. This was due to depth concerns as the threads did not lie completely flat on the Petri dish. This experiment showed that it was possible to focus on the threads as they were loaded into the device and to get clear images of the threads. In addition, the experiment proved that the pegs caused no difference in functionality for the design. Because of this, we decided to use Peg 3 for the remainder of the experiments since we believed it did a better job at holding the threads taught and prevented any unwanted movement that might interfere with the strain rate affecting the threads.

5.2 Full Assembly Test

Once all of the components of our device were evaluated individually, our team ran a series of tests that determined the devices capabilities as a whole. In this section, we first tested how easy our device was to assemble and load with sutures in aseptic conditions. Next, we evaluated the performance of the motor in an incubator over an extended period of time with a load. Third, we determined the sterility of our device in order to confirm there was no risk for contamination from the materials that make up the device. Finally, we analyzed the actual amount of strain our device was applying to the fibrin sutures using high-density mapping (HDM). From these tests, we determined the functionality of our device as well as future considerations that can improve upon its present state.

5.2.1 Ease of Use

The purpose of this test was to validate whether or not our design was easy to use. To test this, the group asked two of the graduate students in the Gaudette Laboratory attach fibrin sutures to the device within a cell culture hood. They were timed to get an idea of how long it would take for a new user to attach three threads to our three different peg models, attach the three threads to their respective holes in the fixed post, and to attach the guide to the side of the
side of the acrylic box. Upon completion of this experiment, each fibrin thread was evaluated for damages and the users were asked to rate the difficulty level of the device. Users were also timed and the group requested feedback on transferring the threads from the device to a petri dish to be imaged. On February, 28th we completed our first test en route to the final step. Our graduate advisor, Josh Gershlak, was given our device components which were as follows: acrylic box, spool with attached pegs, guide/fixed post, metal rod along with three fibrin sutures. All of these components were sterilized and placed in the hood with a pair of hemostats. With these materials, Josh was asked to attached three threads to both the guide and place it within the box. Josh completed the task in approximately 4 minutes and 30 seconds. While the objective was completed successfully, there several issues with the device that made it more complicated to use than anticipated. First, Josh was unsure as to where to attach the needle to the peg. During this experiment, he ended up attached the needle closer to the tip, which resulted in the box obstructing the spool from properly rotating. Josh also commented on the uncertainty of what direction to place the spool in such that the structures would always be at the same strain with every experiment. We then asked Josh to rate his experience with our device on a 1-5 scale (1 being very difficult and 5 being very easy to use) he rated the device as a 3, commenting that the assembly was somewhat unclear and could lead to inconsistencies between experiments. To improve upon our design based on the following feedback, the spool and the guide were etched into so that the user could line up both etch marks, ensuring the spool starts in the same location with every experiment. To aid the user in proper need attachment to the pegs, a simple assembly diagram was created to provide clear visuals on the proper placement of the needles. Through our own testing, we found our initial fixed post design only loosely held the needles in place and altered the holes to implement the same clamp mechanism that we used for our pegs.

5.2.2 Motor-Driven Strain Consistency

The overall objective of the test was to determine whether or not the motor could successfully strain threads for a time period of 30 days. Since a 30-day experiment was too long given our limited amount of time, we reduced the experiment duration to 5 days with accelerated testing. By using accelerated testing, we increased the stress placed on components of the design that were of most concern and observed if those components could maintain function during the decreased time period. In this experiment, the motor and pegs were of the most concern for
motor longevity because the motor must be able to strain a number of threads placed in the device, and the pegs must be able to hold the threads taught for an extended period of time. To place extra stress on these components, we took bored needles attached to nylon string that had an aluminum foil weight attached to it. In total, two of these weights were put together, one having a mass of 0.91 grams and the second having a mass of 0.87 grams. The force placed on the motor by attaching these weights to the pegs on the spool was calculated to be 0.017 Newtons, about 14.81% greater than if six sutures were attached to the spool. The written calculations for the above can be found in the Motor Longevity Appendix. The experiment began by first sterilizing all of the device components 24 hours prior to the experiment. The components required for this experiment were: the acrylic box, rod, spool, guide/fixed post, 4 pegs, sutures with the aluminum weights, motor/rod connector, the motor, and extension wires to allow for connection to the Arduino, which was located outside of the box. The device was fully assembled in the culture hood, followed by the attachment of the weights to the spool pegs. Next, the device was moved into the incubator, plugged in, and periodically checked on. Figure 33 below illustrates the set-up inside of the incubator.

![Figure 33: Aluminum weights loaded to device in motor longevity in incubator experiment.](image)

After 5 days, we determined that our motor could successfully perform its function for an extended period of time without fatigue. The experiment also provided further insight on the function of the device that can help improve its performance in future experiments. First, the motor-rod connector losses grip of the motor over time. During periodic checks of the device, which occurred approximately every 12 hours, we found that the connector was almost falling on
the motor side and had to be pushed back in place to prevent it from falling off. On a couple occasions the connector would slip to a point where it could no longer move the motor. Every time the motor was pushed back into place, however, the motor would continue to rotate the spool the desired 10 degrees. Due to slippage, our team redesigned the motor-rod connector piece that has resolved this issue.

Secondly, we observed rapid wear of the pegs during the course of this experiment. As mentioned before, the pegs are made with a biocompatible material known as MED 610. While the material has lots of advantages, including being readily available at WPI, we discovered its inherent softness was a limitation. When the needles were initially clamped into the pegs, the needles were secured. The dynamic nature of the device led to small micro movements of the needles in the pegs. These micro movements subsequently lead to the needles scraping against the inside of the peg, which widened the hole. The widened hole made it increasingly difficult to keep the needles secure in the pegs. We concluded that while MED 610 ultimately was a suitable material for short term experiments, however, other biocompatible plastics or metals that could be investigated for longer experiments. Through this discovery, we decided that a more suitable material for the pegs would be medical grade stainless steel. Stainless steel is biocompatible, strong, and can be additive manufactured. While WPI does not have a metal 3D printer on site, there are companies that can do it for relatively cheap. Stainless steel pegs will also last much longer and would be more sustainable than the current model.

Lastly, we discovered that acrylic cracks when exposed to ethanol. Ethanol is the main sterilization solution used in the Gaudette Laboratory for cell culture. Ethanol is an important solution for reducing contamination when cell plates and culturing equipment is moved in and out of the culture hood. Typical protocol requires the user to spray down anything before it enters either the culture hood or the incubator. This led to the acrylic box to form a large crack on the side. The crack did not impact our test in anyway, yet needs to be made aware to those who will use it in the future. Ideally, the box will be made of polystyrene in the future, which is the most common material used for cell culture plates. Until we can find a cheap, affordable way to produce a custom polystyrene box, however, the acrylic’s exposure to ethanol should be keep extremely minimal.
5.2.3 Preliminary Sterility Test

To determine the device’s sterility, a three-day media test was performed to confirm a preliminary sterility test. First, all the individual components of the device were sterilized overnight. Next, the device was assembled in the culture hood, and filled with media. 50 mL of media was added, which filled up to the level of the spool pegs to simulate the level during a live experiment. The device was placed into the incubator, as shown in Figure 34 below, for a time of 3 days.

![Figure 34: Device containing media inside incubator for preliminary sterility experiment](image)

Upon completion of the period, two 5 mL samples were drawn from the box and placed in a 6-well culture plate. Similarly, two 5 mL samples from the control media used in the experiment were drawn and placed in a 6-well culture plate. After the 3-day period, there was no noticeable change in the media’s color, suggesting no bacteria had metabolized the media, and that no contamination occurred through the materials coming from the device.

While the media was sterile, the media from the system appeared to contain small particles when observed under the Rolle Lab microscope. The particles were clear and appeared to be some form of debris from the device itself. We determined that the debris most likely came from the silicone glue that was used to seal up the crack that formed on our box or MED 610 debris from the pegs, spool or fixed post. While silicone glue is bio-inert, we placed the media samples into the incubator for an additional 48 hour period to ensure that no bacteria growth
would occur on the debris particles. Once again bacteria growth was confirmed negative, and further confirmed the preliminary sterility of our device.

5.2.4 Strain Analysis Test

To verify strain consistency on the threads, a series of videos were taken using the CMOS camera used by the Gaudette Laboratory. The camera is primarily for High Density Mapping (HDM); a procedure the Gaudette Laboratory has previously used for mapping the strain concentrations of both the Flexcell and accessing regional mechanic function of the heart [6]. HDM requires the use of a CMOS camera that runs at 250 frames/s with 8-bit depth [6]. These images are then implemented into a MATLAB program that calculates the strain on the object along with a heat map of the image that indicates where the highest concentration of strain is located on the object. HDM was attempted to evaluate the strain on the fibrin sutures and determined a strain of 3% at a range of approximately 1 Hz, seen in Figure 35.

![Percent Strain vs. Time](image.png)

Figure 35: Percent strain vs. time data obtained from HDM experiment.

The strain, however, was determined inconclusive as the suture was too thin to properly evaluate the thread thickness. Because of this, images from the videos were evaluated with ImageJ. For each video, a screenshot of the thread relaxed and strained were placed into ImageJ, two of which are found in Figure 36 below.
Next, the images were scaled based on the approximate size of the pegs that were presented in the video. Once scaled, a line was drawn tracing the suture to determine the distances between two needles. The line drawn on the unstrained suture image resulted in an initial suture length value while the line drawn on the strained suture image resulted in a final suture length value. With these values, strain was calculated. The average strain from three analyzed videos was $24 \pm 2.83\%$ strain. While this strain is not 10-15%, the strain rate was consistent throughout the experiment. Additionally, the sutures were buckled in their initial state, which means the strain recorded is most likely lower than 25%. Alterations to the strain can also be easily accomplished by changing the spool rotation.
6. Final Design and Validation

This chapter explains the success of our device in being able to cyclically apply 10% strain to microthreads over an extended period of time while also allowing for the device to be stopped and started during an experiment so that the threads could be live imaged. This includes how well the device met the group’s objectives, how the device met the standards set in Chapter 3 of this report, and understanding the device’s impact on society.

6.1 Objectives

The objectives were defined as both the needs and wants of the client that would improve the functions of the device and make it preferable over the current gold standard. The objectives developed by the team in Chapter 3 included the device being easy to use, sustainable, versatile, safe for all stakeholders, and be able to measure parameters of the device. In each subsection, the objectives are explained in further detail.

6.1.1 Ease of Use

In order for a device to be easy to use, it must require minimal amount of training, have an understandable and responsive interface, and allow for viewing of results and data without confusion. If the device is not easy to use, then it is possible that the client will not want to use the device, and potentially opt for a different device. The client anticipates that the individuals who may be working with the device include graduate student workers and undergraduate student volunteers. The members of the Gaudette Laboratory were asked to assemble our device and gauge the device’s simplicity. The feedback gathered from these members verified that the device was easy-to-use and could be a valuable piece of equipment in future research.

6.1.2 Sustainability

Because of the high volume of waste developed by most laboratories, it is important to consider what materials are going to be used in the manufacturing of the device. A device that contains interchangeable parts, can be reused multiple times, and is made from recyclable material can reduce the amount of waste a laboratory produces and have a more positive impact on the environment.
6.1.3 Versatility

Versatility is defined as a device’s ability to perform under different parameters. This could be with testing different types of cells on the fibrin microthreads, extending the period of time the device is operational, and allowing the device to be exposed to different environments. The device also allows for easy live imaging, which adds flexibility to perform tests for research beyond the scope of fibrin suture research.

6.1.4 Safety

In order for a device to be safe to use, it should not pose a threat to the health or well-being of any stakeholders while using said device. This is important because if a device cannot guarantee one’s safety, then a client will most likely choose to use an alternative device. Methods for maintaining a safe environment come mainly from the stakeholder’s perspective. This includes wearing appropriate gloves when handling the device and the fibrin microthreads inside, wearing eye protection when necessary, and working and preparing the device in a sterile environment, such as a fume or cell-culture hood. The device was designed with its own safety parameters, including biocompatibility, sterility, and minimizing basic hazards of the device such as sharp edges and stability. Through testing, our device presented no harm to any of the users, which further confirmed its safety.

6.1.5 Strain and Frequency

For this project, parameter measurements is defined as actively measuring certain aspects of the device during an experiment. Parameter measurements are useful because it allows for an individual to more easily monitor the progress of an experiment, and to adjust the parameters when needed for a different experiment. Depending on whether mechanical or electrical stimulation of the fibrin microthreads is performed, some of the parameters that can be measured include the strain applied to the microthreads, the force applied by the device on the microthreads, the frequency of the cyclic stress applied, and the voltage applied through the device. The two measurements that were tested for in our device were both strain and frequency, which were the parameters our client was most interested in. Based on our testing, our device
applied a strain between 3 to 25% at a frequency of 0.5 to 3 Hz. Therefore, our device met the primary objectives required of it.

6.2 Engineering, Industry, and Manufacturing Standards

The design standards set in Chapter 3 include ISO, ASTM, and USP standards for device operation, protocols, and materials used in the construction of the device. Throughout the design, construction, and testing phases of this project, the team was careful to keep these standards in mind. The standards for sterilization (ISO 11737-2:2009, ISO 10993-7:2008, and ASTM E1837 - 96(2014)) including the sterilization of medical devices, allowable limits of ethylene oxide gas on medical devices, and the effectiveness of the sterilization techniques were applied during all three phases, but primarily during the construction and testing of the device. During the design phase, these standards were kept in mind when brainstorming materials for use in the device and how they would need to be sterilized. During the construction and testing phase, these standards were put into practice when the team prepared the components of the device for sterile testing.

Standards for cell culture (ISO 10993-5:2009, ASTM F813 - 07(2012), ASTM STP810, and ASTM F2739 - 08), specifically tests for in vitro cytotoxicity, direct contact cell culture protocols and test methods, and protocols for quantitating cell viability, were used during the testing phase of this project. Cytotoxicity testing standards, as well as direct contact cell culture protocols were used while testing the cytotoxicity of magnets made from different metals during the alternative design testing phase. Protocols for quantifying cell viability were used in the alternative design testing phase, as well as the full device testing.

Standards for safe laboratory practices, such as those outlining the use of personal eye protection equipment and sharps injury protection methods (ISO 4849:1981(en) and ISO 23907:2012(en)) were utilized while performing any laboratory testing. All users of the device during testing wore some form of personal eye protection, and all sharps during testing, including needles, were handled and disposed of in a way that would prevent injury or harm to a user.

Standards for biomechanical properties such as those outlining test methods for strength properties of tissue adhesives and biomaterials’ mechanical properties (ASTM F2255 - 05(2015) and ASTM STP1173) were used in testing the overall function of the device with the fibrin threads, as well as during the evaluation phase of the project. ASTM STP1173 was used to help
in evaluating if the device achieved the desired results for mechanical stimulation of the fibrin threads.

6.3 Economics

Although this device was intended to be used primarily by the Myocardial Regeneration Laboratory at WPI at the time of this project, one of the main goals for this design was to be used as an alternative to the FlexCell. According to graduate students working in the laboratory, the device has several problems that accompany its benefits. These include the inability to pause the device in order to monitor the progress of an experiment, the disposal of specialized well plates that are only good for one use, and the overall cost of a unit and software to operate the device. In total, the device itself requires a preliminary investment of approximately $10,000. This doesn’t include the additional pricing for special cell culture plate (untreated BioFlex plates cost approximately $17.50 per plate), vacuum grease, or oil to help the machine run properly [11].

The group designed our device so that it is cheap to produce, is made from materials that are readily accessible to the laboratory, could pause an experiment in order to monitor its progress, and is reusable. In total, this would benefit the laboratory by providing them with a device that could perform the same task as a $10-15K FlexCell unit. It could also be an opportunity for other laboratories that also work with specialized sutures or who work with cardiovascular tissues, especially those who are interested in the functionality of heart muscle and heart tissue.

6.4 Environmental Impact

Sustainability was one of our main objectives when constructing our device. To achieve this goal, our all of the components of our device, excluding the pegs, are reusable. Thus, our device is less expensive and produces less waste than the Flexcell. Because the entirety of the device is intended to be reusable, it helps reduce any waste that may be caused. The device would need to be periodically washed and sterilized as most common laboratory supplies are. However, the users of the device would likely not need to dispose of a copy of the device and require another one in order to perform another experiment. The main environmental concerns that come from using this device include the reusability of the live imaging component of the device, which may only be able to be used a set number of times due to issues of sterility, and the amount of cell culture media needed to sustain hMSC seeded microthreads. In total, the device is
capable of holding between 65 and 70 mL of cell culture media while a 6-well cell culture plate only requires approximately 2 mL of cell culture media. Future design considerations include reorienting the device so that a lesser amount of cell culture media is required to sustain cells.

The three main materials that are used in our design our MED610, Stainless Steel, and Acrylic. The stainless steel was used to produce the rod, and is the most durable piece in the system. Stainless steel is durable and recyclable, while also being cheap and inexpensive, making it an optimal material. Acrylic was used to construct the box in which our device runs in. Acrylic is strong, however not recyclable. The acrylic box, however, is intended to last a long time and was chosen because it is a cheap and accessible material. Lastly, MED610 is used in most of the individual components of the device including: the spool, pegs, guide/fixed post, and the guide-box fasteners. MED610 is the only biocompatible plastic that can be 3D printed on campus and was chosen for that reason. MED610 is not recyclable and pegs must be printed for each experiment as the pegs loosen over time. Future considerations include switching the peg material to something more durable as to last as long as the rest of the individual components in the device.

6.5 Societal Influence

The target demographic for our device are those who have already experienced at least one MI and have sustained significant tissue damage such that their heart does not function properly. Because the risk of heart disease and subsequent MIs increases significantly after approximately 50 years of age, we expect that our device would be mainly servicing patients who are middle-aged or elderly [55]. The currently proposed procedure to deliver fibrin sutures to ischemic regions of a heart involve relatively invasive surgical procedures, during which the chest cavity is open and the heart is exposed. These types of surgical techniques pose serious risks with patients in advanced age, with some studies citing a 31.5% complication rate in patients 80 years and older [56]. Additionally, elderly patients require longer hospital stays following cardiac surgical procedures, require postoperative medications for the remainder of their lives, and can often experience symptoms of insomnia, mood swings and depression, muscle pain and tightness, and loss of appetite [56]. While the risks of complications for open heart surgeries are relatively high in older patients, studies have shown that risk-adjusted
mortality rates are as low as 3.2% in patients over 85, suggesting that cardiac surgery in elderly patients does have consistent successful outcomes [56].

6.6 Political Ramifications

When considering the expenses of cardiovascular disease, medications rank second below hospital visits, including surgical procedures. In 2012, direct costs of medications intended to treat cardiovascular disease amounted to over 60 billion dollars, and is projected to reach 100 billion dollars by 2022 [57]. While our device does not eliminate the need for an invasive surgical procedure, it does have the potential to prevent the need for lifelong medications post-MI. By addressing the problem at the infarcted region of the heart itself, rather than addressing the symptoms brought on by the infarcted region, our device prevents the symptoms that would make these medications necessary, reducing the overall cost of treatment for cardiovascular diseases.

Because the main product of our device involves surgical insertion into cardiac tissue, the FDA would classify our device as a Class III medical device. Class III medical devices pose an increased risk to the patient due to their invasive nature, and thus are subject to much stricter levels of regulatory control.

6.7 Ethical Concerns

The Belmont Report outlines the ethical principles and guidelines for the protection of human subjects of biomedical research. As this project is a biomedical engineering project, the design, construction, testing, and use of our device must adhere to these guidelines and principles. The Belmont Report states that there must be a respect for persons, meaning that any subject involved in the testing or use of the device must be able to maintain their autonomy. Additionally, the Belmont Report states that all subjects must be treated ethically through respect of their autonomy as well as protection from harm and maintenance of their well-being. The third principle outlined by the Belmont Report states that there must be justice, or fairness, during the research process [58].

Our client statement references the use of stem cells in conjunction with fibrin microthreads to assist in regeneration of cardiac muscle. While stem cells show promise for tissue engineering and regeneration, human stem cell research is riddled with ethical concerns
and controversies. Many stem cells come from oocytes and embryos, and the use of these ESCs sparks debates about human personhood and autonomy [59]. While our project focuses on using hMSCs to model the way that stem cells will align and proliferate on the fibrin threads, the ideal cell type for use with our device is induced pluripotent stem cells (iPSCs). iPSCs are adult somatic cells that are reprogrammed to be pluripotent stem cells using adenovirus vectors, and thus avoid the ethical concerns brought about by ESC research.

6.8 Health and Safety Issues

As of the writing of this report, the Food and Drug Administration (FDA) has not approved stem cell derived treatments for use in the public other than blood forming stem cells [60]. Many stem cell treatments today are only offered through clinics, and even then, despite successful trials for certain types of treatments, the safety of patients who receive stem cell treatments cannot be guaranteed [61]. However, although there are concerns with how hMSCs seeded to fibrin microthreads may react in the human body, several studies performed by the Gaudette Laboratory indicate that the microthreads were successful in their engraftment onto the infarct area of the heart. It is important to perform additional testing after the stem cells and microthreads were placed and cyclically strained in the device. This can be performed by first examining the effects of striation and alignment of the cells, and then performing additional animal testing to observe if mechanical function of the infarcted region of the heart increases.

6.9 Manufacturability

Construction of the device used the following materials: extruded acrylic, MED 610, an Arduino board with the necessary wires and power supply, and a stepper motor. The stepper motor and extruded acrylic were purchased on Amazon and Home Depot respectively; and the acrylic sheet was further cut using the laser cutter available at the machine shop in Washburn Shops at WPI. The MED 610 is able to be 3D printed using the Objet rapid prototyping machine available at Higgins Laboratory at WPI. The purpose of using materials that could be easily purchased online or at local areas was to not only for ease in the construction of the device but also to help reduce the overall cost of the different manufactured parts. This also helps when students and volunteers would like to manufacture new parts to either construct a second device
or to replace broken parts. It is possible to be trained to use the laser cutter in Washburn Shops, and SolidWorks and Arduino program training are available in the WPI curriculum.

It is also important to consider our product’s ability to be mass produced. Since our product is made up with a series of plastics and acrylic, mass production could be easily accomplished. All the pieces that were 3D printed for our device could be injection molded; a process that injects liquid plastic into a preset mold. For the purposes of our project, we laser cut pieces of acrylic and glued them together to form a box. If our device was commonly manufactured, a box mold could be created, which would simplify our overall device as a box would not have to be assembled and run the risk for leaking of leaking. Additionally, injection molding the box would allow us to switch to polystyrene instead of acrylic. Polystyrene is the material use for the majority of cell culture plates and petri dishes. Polystyrene is cheaper than acrylic as well as biocompatible. Polystyrene also doesn’t run the risk of cracking when exposed to ethanol, a fluid which is commonly used for sterilization when working in a culture hood.

6.10 Value Creation

Presently, the value that our device creates is an alternative device for cyclically testing fibrin sutures instead of the Flexcell, which is the current device being used. The group that obtains the most value from our product is the Gaudette Laboratory, whom is our client. Our device offers several advantages over the Flexcell such as, live imaging, cost efficient, easier to use and more sustainable. With these factors in mind, our client will benefit significantly from our device as they will be able to evaluate how cells behave on fibrin sutures that are cyclically stretching. Before now, the Gaudette Laboratory has been limited to a machine that fails to meet their desired specifications for their experiment.

Beyond the Gaudette Laboratory, our device has created value in several other ways as well. The pegs that we designed for our device are versatile and can be used to not only hold threads in our system, but potentially other systems as well. From surgeons to researchers, working with multiple sutures can present a challenge. Furthermore, between the pegs and the fixed post/guide, our team has developed an innovative way to transport sutures from one location to the next more conveniently. The transport component can also be live imaged and fits easily into a petri dish, which adds to its versatility. With the demonstrated success of the mechanical strain on the sutures, and the potential addition of electrical stimulation in the live
imaging apparatus, this device may prove useful for other laboratories looking to prepare cells prior to implantation. In this way, our system has potentially created value for anyone who works with sutures and/or is looking for a more suitable solution for handling and preparing sutures and cells.
7. Discussion

Fibrin microthreads have shown success in delivering higher percentages of stem cells to their target areas in order to regenerate scar tissue in the heart to restore their respective function. However, the maturity of the cells on the threads themselves remains a challenge, as the mechanical function of the stem cells on the threads are naive so that their implantation in the heart causes problems in the overall mechanical function of the heart. Research has shown that placing these stem cells under cyclic stress can help the cells become more mature and obtain greater mechanical function. However, the methods for placing the threads in a cyclic stress environment using the FlexCell have limitations such as inability to view an ongoing experiment underneath a microscope, the overall cost of the device as a whole, and the limited amount of space in the device’s location.

In this project, we have created a prototype device to address these issues of the FlexCell device while allowing for placing the microthreads under cyclic stress environment. The objectives for the project included: 1) allowing the threads to be imaged during an experiment; 2) placing the threads under a cyclic strain rate of approximately 10% at a frequency of 1 to 3 Hz; 3) having the device be simple for the user to understand; 4) having the device be sustainable, 5) allowing the device to be versatile, 6) having the device be marketable, and 7) allowing the device to be safe for the user. Through testing the individual components and observing the device to operate as a whole, we believe our device has successfully achieved the objectives.

This chapter covers the discussion of the results from each of the individual components testing, and the full assembly. This includes the assumptions made during the experiments, the comparison of the results to those found elsewhere in literature, and the limitations observed when conducting the experiments.

7.1 Motor Longevity Test

When the motor was originally set up to rotate the device with no weights or threads, the purpose was to show that the device could operate consistently over a short period of time. The device ran for 72 hours and after measuring the angle of rotation approximately every 12 hours, we noticed that there was little to no change in the angle of rotation. This did not mean that the motor was completely operable as the device would need to run inside of an incubator in order for the cell seeded sutures to be sustained.
In order to confirm that the device can operate over a longer period of time than what was currently available to the group, we loaded nylon sutures that had aluminum weights attached to the spool only so that the motor would experience an approximate 14% higher force in rotating the spool than it would if six threads were loaded into both the spool and fixed post. Some of the assumptions made in these calculations were that gravity would have a negligible effect on the motor stretching the threads. This is because the threads would be attached to the spool and the fixed post at both ends, reducing the effect that gravitational forces have on the thread when it is loaded into the device. In contrast, the weights were not held at the fixed post end, leaving gravity to put an extra force for the motor to hold up. Another assumption made is that the incubator where the device is held will be opened and closed gently. The group noticed that the connection of the weights into the pegs on the spool were delicate and that shaking the device, such as that caused by opening and closing the incubator door, too much would cause the weights to fall loose. The group noticed that this was most prevalent with experiments that had pegs that were used in previous experiments and because of this, the group also confirmed that the pegs would have to be a disposable component of the device to prevent threads from falling out of the device.

Limitations from this experiment included a convoluted set-up when connecting the Arduino leads into the motor to cause it to turn. The Arduino board was taped to the side of the incubator with the leads entering inside the incubator to attach to the motor. The Arduino board could easily be knocked away and therefore, it is important for the Arduino to have a safer location in the set-up of the device, either by creating a separate container where the Arduino can be placed inside the incubator or on the outside in a more stable location and position. Other limitations included the ability of the motor and motor-rod connector to remain attached to one another. The group noticed that, similar to the pegs, the more often the motor-rod connector was used, the looser the connection between the motor and connector would be, causing the connector to fall out often during the experiment. It is important to note, however, that the motor continued to operate after it had fallen out of the connector so some possible remedies to combat this issue include having the connectors also be a disposable component of the device or to change the material of the connector so that there is less deformation that may cause the connectors to be unusable.
7.2 Individual Component Tests

The testing of the individual components was performed in order to ensure that each component would work properly on its own before it was placed into the device to interact with the other components. The main components that were tested included the pegs, and then a combination of the spool and fixed post in ease of use analyses. Eventually, we also tested the feasibility of the device to be constructed in a sterile environment under a culture hood. The results from these tests confirmed that our device can be assembled with relative ease, as shown by the loading of the threads into the device by other graduate students in the Gaudette Laboratory who have not been as involved in this project. This is also where we decided to use Peg 3 as the main peg design for future experiments based on comments made that it did a fair job at holding the needle securely with little rotation or unnecessary movement.

Some of the assumptions made for this experiment include that those working on the device would have a good knowledge base for how the device should be assembled and put together. Instructions for assembling and loading threads into the device is provided in the Device Assembly Protocol Appendix for use by individuals who are unfamiliar with this project, however, it is still assumed that the graduate students and undergraduate volunteers who work with the device have had the proper training on how to use such a device and are familiar with the protocol. This also becomes a limitation because although the device may be easy to use according to the graduate students, an undergraduate student may have more difficulty assembling the device due to their increased unfamiliarity with the project. The protocol created by the group is intended to alleviate some of the difficulties that come with assembling the device, however, further studies could incorporate ease of use experiments for undergraduate volunteers and their ability to assemble the device.

7.3 Live Imaging Test

The most important objective of the project was to allow the device for live imaging of the threads during an experiment without interruption. The experiment performed during this project gives promise to the idea that our device can culture cells seeded onto the threads and then imaged using the same set-up. All pegs, with the exception of Peg 1 under 20X magnification, were able to get similar quality images to that of a microthread placed flat on a Petri dish. The reasoning for the inability to get an image under 20X magnification with Peg 1
could be the location of the thread on the far end of the device, attached to the peg that was farthest from the fixed post and had noticeably poorer orientation to be placed flat on the Petri dish. Reorienting the thread in that peg could have a more positive effect to allow for more proper imaging.

One of the assumptions made during this test come from the fact that the threads were imaged on a dry Petri dish in order to get an image. Some resulting concerns include the drying of the cells on the threads, which can have a negative impact on the health of the cells. Therefore, we assume that the imaging process for this device will be relatively short to prevent any death of cells while they are being imaged. In addition, we also assume that if the imaging apparatus were to contain PBS to help sustain the cells, that the PBS will not have a negative impact on the imaging process and that the threads will still be able to be live imaged when submerged in PBS. Limitations for the device include the dryness of the imaging apparatus as that could cause problems for cells if the device is imaged for too long a time. Because of this, if the device is to remain dry while imaging is occurring, the process must be quick so that the cells can be placed back into the cell culture media inside of the device.

7.4 Strain Analysis

One of the specifications for the project was to have the device be capable of stretching the microthreads at 10-15% strain, which, based on our background research, was sufficient to mimic *in-vivo* myocardial environment. Our device is capable of adjusting the rotation of the spool in order to change the strain rate that the threads will theoretically receive. Upon performing HDM on the threads, the group observed how the device could be improved, as well as the assumptions and limitations by the two sets of calculations made on the stretching of the thread. Overall, the device is capable of stretching threads at a strain rate of 10-15%, however, there are methods for improving the orientation of the threads and rotation of the spool to ensure that the threads themselves experience the proper strain rate.

The main assumption was that the orientation of the spool would not change over time. Unfortunately, the motor longevity experiments were not able to perform, as it was not feasible to observe how the spool would react after stretching microthreads for an extended period of time. Upon performing the HDM experiment, the group observed that based on the distance between the bored needle in the spool and the bored needle in the fixed post, as long as the
microthread remained taught throughout the experiment, the thread should experience strain of approximately 24%, much higher than the goal set forward in the examples. Upon analysis of the video file during the experiment, however, we also observed that the thread was not held completely taught throughout the experiment. Therefore, it is possible that the strain experienced by the thread is much less than the analysis by measuring the distances between the needles.

The limitations that come with this experiment are that the spool needs to be oriented properly in order to have threads experience the proper strain rate. This can become a potential issue when a graduate student or an undergraduate volunteer attempts to assemble the device and load microthreads for experimentation. There are methods to alleviating this limitation, such as adjusting the spool so that the thread is held taught, reloading the thread onto the spool and fixed post to get better orientation, and reprogramming the Arduino to have a smaller rotation than the 11 degrees rotation set in the experiments the group performed. Limitations for the HDM recorded strain rates also had their limitations. Due to the inability for the code to distinguish between different colors of pixels, only one video was able to obtain data in terms of strain rate. This also leads to the idea that the 3% strain rate recorded may not be entirely accurate. Future experiments involving changing the lighting and orientation of the camera is recommended for future testing involving HDM in order to obtain more accurate and relevant data.

7.5 Sterility Test

Although our device was manufactured using both biocompatible plastics and other materials previously used in the Gaudette Laboratory for sterile experiments, it was important to confirm that our device would not allow for any contamination that could disrupt the growth of cells on the threads. Fortunately, our results confirmed that the device was produced from sterile materials and should not have any adverse effects on the cells.

Some of the assumptions made with the experiment were that connecting the motor and rod to power would not have any effects on the sterility of the device. The device was constructed as it would be during a full assembly in the same orientation as that of the motor longevity experiments. However, the motor was not rotating, allowing the cell culture media to sit in the device without any effects coming from a rotating spool or the threads themselves. It was assumed that having the culture media move around will have any adverse effects on cell viability, however, in order to confirm this, future experiments involving cell-seeded fibrin
sutures should be performed. In addition, another assumption was that the dots observed in the
cell culture media in the device under the microscope was residue from either the silicone
adhesive to hold the box together. Silicone adhesive is a biocompatible material, since the
material is constantly used in the Gaudette Laboratory and because the group observed no
distinct changes in media color or microbial growth when the samples were placed back in the
incubator for another day.

Some limitations with these experiments, however, came from not using cells to confirm
the viability of cells inside of the device. This was due to time constraints and, unfortunately, the
group was unable to prepare cells for seeding by the end of the project. We looked for general
microbial growth in cell culture media as an alternative because the cells would be subject to the
media once placed inside the device and it’s important to see if microbial growth of bacteria or
fungus would also be allowed under sterile conditions. In order to confirm the viability of this
experiment, future experiments should run cytotoxicity experiments using cells prepared by the
Gaudette Laboratory and seeded onto threads as they would be oriented in a typical experiment.
8. Conclusion and Recommendations

To conclude this project, the group has developed a device capable of placing fibrin microthreads under cyclic strain. The device is a fraction of the size of the current device used for placing cells and threads. It is made with common materials that are easily accessible to WPI with some of the parts able to be reused. It has the ability to pause experiments as they are running, and can allow for imaging of the threads without interfering with them when loaded into the device. In addition, we tested the ability of this device to be used in the Gaudette Laboratory by performing ease of use analyses with graduate student workers in the laboratory and can confirm that our device can be learned without significant arduous training. In total, the device meets the objectives defined at the beginning of this project listed in Chapter 3 of this report.

8.1 Recommendations

The following subsections contain the recommendations the team makes for future research concerning our device. This includes recommendations in ways the design can be improved for future experiments that can further test the validity of the experiment as a whole.

8.1.1 Design Recommendations

Over the course of the project, we have developed several iterations of different components of the design and have come up with a list of recommendations to incorporate when working with this device. To begin, we would advise researching into using different materials for the plastic components of the device, particularly, the parts made from MED610. While this material was readily available in the rapid prototyping machines on campus, we noticed that the material itself is soft and can be easily carved into or deformed. This was problematic especially with the pegs and motor-rod connector, which would deform after constant use making it impossible to load threads into the device securely or successfully connect the motor to rotate the spool. A stainless-steel motor-rod connector was manufactured at the conclusion of the project to allow for better connection but this was acceptable only because the part would not be exposed to cell culture media or the threads themselves. We recommend investigating other biocompatible plastics such as polypropylene or medical grade PVC.

In terms of the strain placed on the threads, we recommend investigating other methods to increase the strain placed on threads when they are loaded into the device. Although high-
density mapping contained issues in terms of recording accurate data and that the strain placed is less than what was originally anticipated, we have shown that it is possible for our device to place the threads under cyclic strain and it is possible to increase this strain to meet 10-15%. One method is to reorient the needles of the sutures so that the threads are held more taught in the device. In addition, reorienting the spool and the motor could allow for a greater amount of tension placed on the threads. This was observed to be possible during the high-density mapping experiment but, unfortunately, wasn’t able to collect data due to the focusing issues from the camera. Finally, another method for increasing strain on the threads include either editing the Arduino code to allow for greater rotation of the motor or obtaining a stronger motor that can better strain the threads. Another thing to keep in mind is to observe the effect gravity will have when the threads face vertical to their supporting surface rather than horizontal.

Changing the motor itself is another recommendation the team notes, based on the results from the motor longevity experiments. While the TowerPro SG90 Micro Servo motors fit in the project budget and were able to rotate the spool efficiently while on the benchtop of the laboratory, the team noticed that some problems occurred with the connection of the motor to the rest of the device and the ability of the motor to rotate the weighted threads. These could be caused by many issues, however, with the motor-rod connector and peg material, as noted previously, being deformed after constant reuse. Operating with a stronger motor, however, could also assist in making sure the device operates properly and could add a hand in fixing many of the observations noted in the experiments. Considerations for choosing a stronger motor include its ability to connect and recognize code from an Arduino, its operability in incubator-like conditions, and how easy it is for graduate student workers and undergraduate student volunteers to operate.

8.1.2 Experimental Recommendations

In addition to recommendations to the design considerations chosen for this project, we also recommend performing experiments in order to obtain information on the operability of the device. To begin, we recommend future testing to include the use of hMSCs seeded onto the fibrin sutures. One of the main functions for our device was to allow for future testing of the effects of cyclic strain placed on cells and to observe how their morphology and maturity changes. Therefore, it is important to observe if the cyclic strain applied by our device can
produce the same observations found in the literature review. The goal is to observe hMSCs and iPSCs becoming more mature and behaving more like heart tissue, however, if this is not observed, then there may be additional design considerations added to the device.

Finally, we also recommend comparing the strain of our device to that given by the FlexCell. Because the FlexCell is the device currently in use to place cells in a cyclic straining environment, it is important for our device to be equivalent to the FlexCell at applying cyclic strain over a period of time. There are already a number of advantages our device presents, such as less space required, the ability to temporarily pause experiments, and the ability to view the threads underneath a microscope. If the device can also provide a constant 10-15% strain on threads for an extended period of time, then it is possible that our device can become more marketable.
References


[18] American Heart Association. Types of Heart Failure. Available: http://www.heart.org/HEARTORG/Conditions/HeartFailure/AboutHeartFailure/Types-of-Heart-Failure_UCM_306323_Article.jsp#.V9Bu05grKCg.


Appendix A–Pairwise Comparison Charts

Below are the definitions given in order for the project advisors, client, and team members to each complete a Pairwise Comparison Chart (PCC). The first PCC seen is an example of a blank chart, while the following charts are those that were filled in by the advisors, client, and team members.

Device Objective Definitions:

- Easy to use
  - Undergraduates with minimal training can use it
  - Not overly complicated
  - Automation
- Sustainable
  - Device has reusable components
- Marketable
  - Device cost should be reasonable compared to similar devices, preferably inexpensive
  - Device should be replicable
- Versatile
  - Device should be able to be used for HMSCs, IPS cells (these are the target cells), or others
  - Device should be able to be adjustable such that it can accommodate one or multiple threads, as well as different sizes of threads
- Safe
  - Device should be stable
  - Should not fail or break under normal conditions
  - Should not expose user to harmful substances or components that could cause injury
  - Should not interfere with other experiments should it be stored in a shared incubation space
- Live imaging
- Plates must be able to be marked so that consistency can be maintained during imaging
- Device must facilitate live imaging such that the experiment should not need to be terminated to image histology

- **Measures force**
  - Device should measure the force that is output from the cells during contraction/stimulation

- **Measures frequency**
  - Device should measure the frequency at which the threads are being stimulated

- **Measures strain**
  - Device should measure the strain that the threads/cells are experiencing during stimulation

- **Measures voltage**
  - Device should measure voltage output by cells during/after stimulation (in the event that the cells can be electrically stimulated)
## Blank Pairwise Comparison Chart

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Appendix B – Function Means Trees

Microthread Loading and Unloading Function Means Tree

Cyclic Stretching Function Means Tree
Media Loading and Unloading Function Means Tree
Appendix C–Arduino Code

Below is the commented code which was uploaded to the Arduino and used to control the Tower Pro SG90 Micro Servo. The code is a modification of a sample code from an Arduino-servo motor tutorial provided by Adafruit.

```c
// access servo library
#include <Servo.h>

// define pin used to control servo
int servoPin = 9;

// defines new variable of type 'Servo' from accessed servo library
Servo servo;

// initialize servo starting position
int angle = 0;  // servo position in degrees
// attach servo to controller pin
void setup()
{
    servo.attach(servoPin);
}

void loop()
{
    // scan from 0 to 11 degrees
    for(angle = 0; angle < 11; angle++)
    {
        servo.write(angle);  // update position to specified parameter
        delay(15);
    }

    // now scan back from 11 to 0 degrees
    for(angle = 11; angle > 0; angle--)
    {
        servo.write(angle);
        delay(15);
    }
}
```

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Appendix D–Motor Longevity Test

Comparing two weights to that of 6 threads strained by 10%

Fibrin
\(\varepsilon = 0.10\)
Average mass of fibrin thread = 0.07 g
Fibrin modulus (E) = 1.104 MPa
Avg dry diameter = 0.083 mm = 0.083 x 10^{-3} m
Avg wet diameter = 0.169 x 10^{-3} m
Area of wet diameter = 2.24 x 10^{-8} m^2

\[E \cdot \varepsilon = \sigma\]
\[\sigma = \text{Force} \times \text{Area}\]

\[(1,104,000 \text{ Pa})(0.10) = \text{Force} \times (2.24 \times 10^{-8} \text{ m}^2)\]
**Force = 0.014857 N**

Aluminum Weights
Average mass of aluminum weight = 0.89 g
2 weights (total mass): 2(0.89 g) = 1.78 g = 0.00178 kg

\[F = m \times a\]
\[F = (0.00178 \text{ kg})(9.8 \text{ m/s}^2)\]
**F = 0.017444 N**

Percentage of Difference
\[\frac{[(0.017444 N - 0.014857 N) / (0.017444 N)]}{0.1481} \times 100 = 14.81\%\]

**There is a 14.81\% increase in force in using the two aluminum weights than straining 6 microthreads attached to the device by 10\% strain.**
Appendix E–Device Assembly Protocol

Construction of Uniaxial Tension System

Materials Needed:

- 12 pegs
- 1 spool
- 1 fixed post
- 2 square pegs
- 1 steel rod
- Box made from acrylic
- 1 motor-rod connector
- 1 TowerPro SG90 Micro Servo motor
- 3 wires
- 1 Arduino
Step 1:
Place the pegs into the fixed post and the spool as shown. 6 of the pegs should go into the fixed post while the remaining 6 belong in the spool. Ensure that the pegs are oriented such that the needle of the suture will enter the peg and will be parallel with the guide on the fixed post. Next, place the rod end of the spool into the hole of the fixed post. Note the picture below for proper construction.

![Image of pegs and spool](image)

Step 2:
Using a pair of hemostats, insert the double needle suture into the pegs of the spool and fixed post, ensuring that the pegs snap into place. **NOTE: In order to ensure the sterility of this experiment, this part onward is typically performed inside of a culture hood or in aseptic environments.**
**Step 3:**

Place the combination of spool and fixed post into the box such that the rod end of the spool can enter the smaller hole on one side of the inside of the box. The bottom left image gives an example of proper construction. Note that this side of the box also has square holes located near the surface of the box. Take the two square pegs and place them in these holes, ensuring they also enter the holes at the top of the fixed post, as seen below in the bottom right image.

![Image of step 3](image)

**Step 4:**

Take the metal rod and with the longer square sided end, insert it through the larger hole on the opposite side of the box and slide it into the square hole of the spool, as shown below. The rod should slide into the spool until it comes to a stop; this indicates it’s connected.

![Image of step 4](image)
Step 5:

Connect the motor-rod connector into the TowerPro SG90 Micro Servo motor using the circular hole to place into the motor, as shown in the bottom left image. The square hole, then enters the steel rod via the smaller square end on the opposite side of the rod. The bottom right image illustrates proper orientation.

Step 6:

Take the Arduino board and three wires and orient them as shown below. In the case of the image shown below, the yellow wire is connected to the 5V input, the green wire is connected to the GND input, and the white wire is connected to the analog pin 9 input. These
same colors will be mentioned in the next step to assist with properly orienting the wires into the motor.

**Step 7:**

Place the wires into the motor inputs as oriented below. The 5V input on the Arduino (yellow wire) should be connected to the red wire on the motor, the GND input on the Arduino (green wire) should be connected to the brown wire on the motor, and the analog pin 9 input on the Arduino (white wire) should be connected to the orange wire on the motor.
Step 8:

The uniaxial tension system is now fully constructed. Place the device in the incubator with the Arduino on the outside of the incubator and the box itself inside the incubator. Plug in the Arduino and check to make sure the motor and spool are rotating.