

May 2014

Device for Evaluating the Contraction of Cardiac Cell-Seeded Fibers

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MQP GRG-1405

Device for Evaluating the Contraction of Cardiac Cell- Seeded Fibers

A Major Qualifying Project Report
Submitted to the Faculty
of the
WORCESTER POLYTECHNIC INSTITUTE
in partial fulfillment of the requirements for the
Degree of Bachelor of Science
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Date: May 1, 2014

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1. Drug screening
2. Cardiotoxicity
3. Fibrin Microthreads

Abstract

Drug-induced cardiotoxicity remains a primary reason why new pharmaceutical compounds are withdrawn from clinical trials and the consumer market. Many current cardiotoxicity screening devices utilize two-dimensional monolayers of cells, which are not a representative model of the heart, resulting in poor detection of cardiotoxic compounds. Current methods are also time-consuming and expensive. We have developed a novel cardiotoxicity screening device that utilizes cardiomyocytes seeded on fibrin microthreads to form a three-dimensional model for cardiac tissue. Our device can be used with standard laboratory equipment to analyze the contractile properties of these seeded threads. Upon the addition of pharmaceutical agents, the device has the potential to detect changes in contraction and thus identify cardiotoxic compounds. The device is also easy to use, inexpensive, and biocompatible. In the future, this prototype can improve the detection of cardiotoxic drugs, saving time and money in the drug development process to ultimately increase the safety of drugs on the market for consumers.

Acknowledgements

Throughout this project, we had countless assistance from many people and sources. Without their help, we would not have been able to complete our project successfully and on-time.

Our biggest source of assistance came from Professor Glenn Gaudette. In whatever stage of the project we were in, he was always supporting us and helping us along the way. He also was always willing to explain technologies in the lab that we needed and didn't understand. The biggest thing Glenn did was his continued encouragement for us to follow an engineering design process and be creative. We certainly couldn't have done this project without him as our advisor.

A lot of the work that the group did was focused in the biomedical engineering MQP laboratory in Goddard Hall. The lab manager, Lisa Wall, always kept the lab stocked for student use and she was always just upstairs if we needed assistance. She also assisted us in ordering products that we needed to complete our design.

Another pivotal group in this project were the Higgins Labs Machine Shop Managers, Matt DiPinto and Kevin Arruda. They provided the team with technical advice on how to proceed with our project and also machined a number of key components in order to get our device fully functioning. They helped us craft our polycarbonate inserts and machine our molds for our PDMS anchor points and cantilever beams.

A huge amount of assistance came from the Gaudette Lab. We would like to especially thank Katrina Hansen, Robert Orr, Kyra Burnett, and Emily Abbate. These students assisted with our cell testing, acquired fibrin microthreads for us, helped us acquire any equipment we needed, and always answered any questions we had. Without them, we certainly wouldn't have been able to finish all of our testing, and our device would not be effective.

Authorship Page

All four project members contributed equally to both the project work and writing of the report.

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Chapter 1: Introduction

Drug-induced cardiotoxicity is one of the main reasons newly developed drugs are eliminated from pre-clinical and full clinical trials. Over the last thirty years, twenty-eight percent of all drug withdrawals in the United States have been due to adverse cardiac effects, putting medication consumers at risk and posing serious financial and legal consequences for pharmaceutical companies. Almost every class of therapeutic drugs has caused harmful side effects on the heart, including powerful anti-cancer agents as well as everyday over the counter antihistamines and nonsteroidal anti-inflammatory drugs (NSAIDs) (Crivellente, 2011). Cardiotoxic drugs cause weakness in cardiomyocyte activity to produce a variety of heart diseases, ranging from reversible arrhythmias to permanent cardiomyopathy. Because of the high occurrence of drug failure due to harmful cardiac side effects, there is both a market and consumer need for innovative *in vitro* assays that enable earlier, more accurate detection of cardiotoxic compounds. Pharmaceutical companies require faster and more reliable screening systems to save time and money in the drug development process whereas patients who require all types of medication are in need of safer drugs as well as more drug options that better suit their unique medical and personal needs to improve their quality of life.

Engineers and scientists have developed several methods to screen for drug-induced changes in cardiac tissue properties and functions. These methods have demonstrated the ability to detect changes in cardiac cell contraction strength, contraction frequency, and relative tissue death. However, these technologies do not fully meet the needs of pharmaceutical companies because they exhibit limitations in terms of accuracy, data acquisition time, biocompatibility, and cost. Specifically, most drug-screening systems are based on 2-D monolayer cell culture, which is an inaccurate model that does not completely mimic the complex cell-cell and cell-matrix

interactions of the 3-D *in vivo* cardiac tissue environment (Moya et al, 2013). While other drug screening platforms may incorporate a more realistic 3-D model, they are otherwise limited in their efficiency. For example, some systems use the technique of high-content screening (HCS), which detects minute intracellular changes through the use of fluorescence microscopy and multi-parameter software analysis (Vandenburgh, et al., 2008). Although HCS is ideal for producing reliable results, it is not a practical option to meet the high demand of new and improved drugs because it requires special, expensive equipment and takes a very long time to acquire data. Additionally, current screening protocols may exhibit biocompatibility issues as their assembly methods often negatively impact cell health and proliferation, which can affect cell contraction strength and other properties (Eschenhagen and Zimmerman, 2005). To improve upon current drug screening technologies, there is a need for inexpensive, less complex systems based on three-dimensional tissue constructs that better mimic the cell-to-cell interactions and functionality of native cardiac tissue to serve as a more accurate model when measuring contractile strength of cells and screening for the effects of pharmaceutical agents.

Regarding a more accurate model system for *in vitro* cardiac assessment, cell-seeded biological sutures used for cell therapy also show great promise in serving as 3-D tissue models. This project utilizes fibrin microthreads, a newly developed cell therapy technology used to treat infarcted heart tissue. Researchers at Worcester Polytechnic Institute (WPI) have demonstrated that human mesenchymal stem cells (hMSCs) can be seeded on fibrin microthread sutures for reliable cell delivery to areas of cardiac infarction. Delivered stem cells have the capacity to differentiate into new cardiac cells and organize into muscle fibers and connective tissue, restoring heart function (Dominko, et al. 2011). These threads can also be loaded with growth factors to promote cell outgrowth and proliferation (Cornwell and Pins, 2010). Fibrin

microthreads have demonstrated the ability to greatly improve the alignment of cardiac fibroblasts along the suture axis, suggesting that the suture scaffold promotes the development of a three-dimensional tissue structure that can serve as a more accurate model for *in vivo* cardiac tissue than current two-dimensional models (Guyette, et al. 2013).

Our goal was to develop a drug-screening system utilizing fibrin microthreads as a three-dimensional model to better mimic cardiac tissue *in vivo*. The microthreads should allow for the seeding of cardiac myocytes and/or stem cells which contract upon electrical stimulation. Our device should be able to measure and collect this contractile data to allow for the high throughput screening of various added pharmaceutical compounds. The final system should generate accurate and reproducible results and be biocompatible and sterilizable. It should also be easy to use in terms of adding and removing the microthread bundles and pharmaceutical agents. Finally, the device should be safe to use and cost effective.

To meet the goals of our project, we utilized inexpensive, reusable, readily available materials to develop a non-complex device that could be used in conjunction with common laboratory equipment. We developed an attachment system that reliably held the cell-seeded fibrin bundles in place and also allowed for the application of electrical stimulation. We developed a unique design that allowed for the capture of high quality images in order to easily evaluate the contractile behavior of the fibers. Several tests were performed to confirm the biocompatibility and reliability of the final device.

In the following chapters, substantial background is provided for topics related to our project including most importantly cardiac biology, cardiotoxicity, and microthread technology. An overview of our strategy and various approaches for the development of our device is also given. A description of our alternative conceptual and preliminary designs is provided, followed

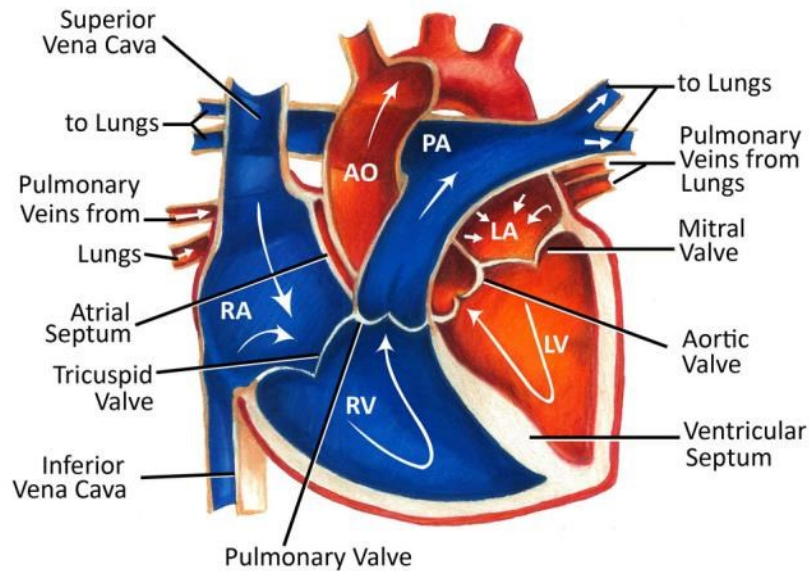
by detailed explanations and illustrations of our current design. We also provide the various verification and validation tests conducted to confirm the success of our device as well as a discussion of our results. Finally, conclusions are made about our project followed by recommendations to improve upon our design in the future.

Chapter 2: Background

Before we begin to generate design alternatives, it is first critically important to fully understand the problem our project addresses. In the following sections, we outline the contractile biology of the heart as a whole organ as well as at the cellular level, and then describe the various pharmaceutical agents and consequences associated with cardiotoxicity. Following this technical background, we summarize some current screening methods and their limitations. Finally, we discuss the fibrin microthread model system and cell types we will be implementing in our design, and how specific agents affect the contractile mechanisms of these cells.

2.1 *In vitro* Cardiac Function

The heart provides constant circulation of blood in order to ensure a continuous supply of nutrients and removal of waste from the body. This organ is divided into left and right halves that serve as transport pumps that contract to circulate blood throughout the body. Each half consists of an atrium and a ventricle. The right atrium receives deoxygenated blood from the body, which flows through the tricuspid valve and into the right ventricle. Here, the blood is pumped into the pulmonary arteries, which carry blood through the pulmonary circuit to the lungs, where it is oxygenated. This newly oxygen enriched blood is then received by the left atrium and passed to the left ventricle, where it is again pumped out of the heart, this time delivered through the systemic circuit to the rest of the body, completing the cycle (Figure 2.1). In this manner, the heart transports oxygen and nutrients throughout the body and also helps to remove waste (Marieb and Hoehn, 2010).

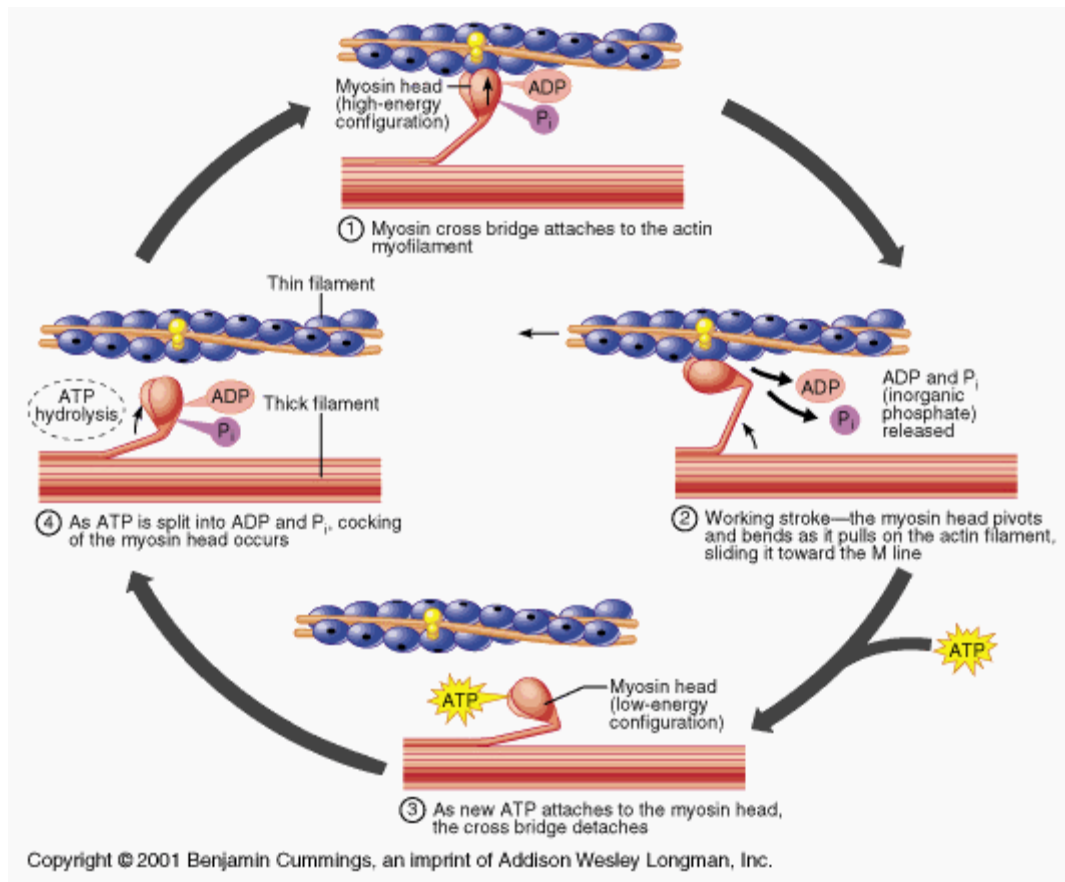


Cardiac Blood Flow. Feb 24 2013. <<http://forgottenphysiology.com/2013/02/24/the-zen-of-cardiac-blood-flow/>>.

Figure 2.1: Diagram of blood flow through heart

The heart wall is composed of three layers: the epicardium, myocardium, and endocardium. The epicardium, the outermost layer of the heart wall, is the visceral layer of the pericardium, which is the double-wall sac that serves as an enclosure for the heart. The myocardium is the layer responsible for contraction and is composed primarily of cardiac muscle, forming the majority of the heart itself. The third layer is the endocardium, which is located on the inner myocardial surface and lines the heart chambers (Marieb and Hoehn, 2010).

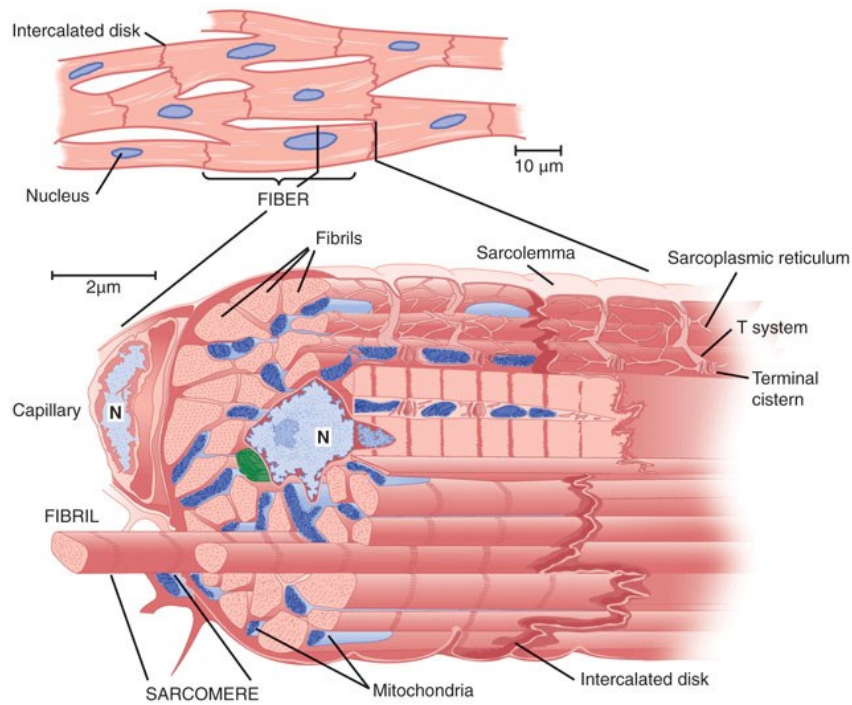
Cardiac muscle fibers, that make-up the myocardium, work to perform the contraction necessary for blood to be pumped throughout the body. Cardiac muscle is striated and contracts by the sliding filament mechanism, a method very similar to that of skeletal muscle (Figure 2).



Cummings, Benjamin. Stages of a Muscle Contraction. Addison Wesley Longman, Inc. <<http://legacy.owensboro.kctcs.edu/gcaplan/anat/notes/api%20notes%20j%20%20muscle%20contraction.htm>>.

Figure 2.2: Sliding Filament Mechanism for muscle contraction

Cardiac cells are short, thick, and interconnected. Each cell has one or more nuclei, while a connective tissue matrix called the endomysium fills intercellular spaces (Figure 3). This matrix is connected to the fibrous cardiac skeleton, a network of collagen and elastin, which reinforces the myocardium and anchors cardiac muscle fibers. This skeleton provides a structure for these fibers to pull against and exert their force (Marieb and Hoehn, 2010).



Cardiac Muscle Cell Structure. 2014. <<http://www.paradoja7.com/cardiac-muscle-cell-structure/>>.

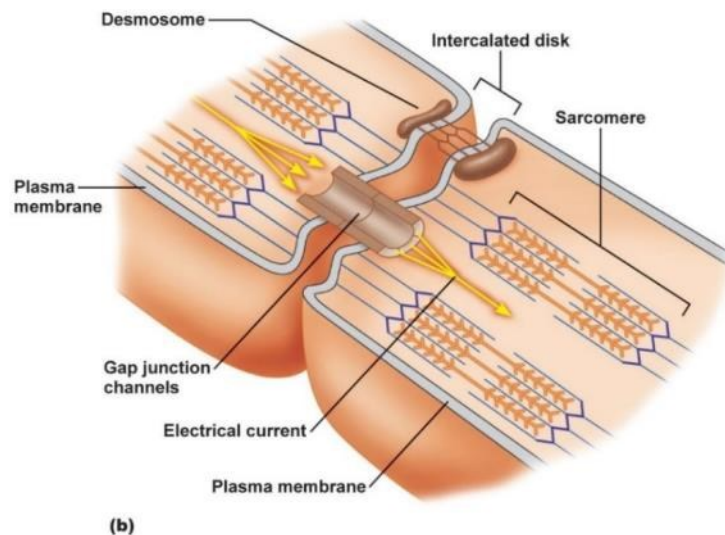
Figure 2.3: Structure of typical heart muscle

The contraction of cardiac muscle by the sliding filament mechanism is triggered by action potentials traveling across the cell membranes. Roughly one percent of cardiac fibers have the ability to depolarize spontaneously. It is this autorhythmic quality that gives these fibers the ability to pace the heart. The rest of the myocardium is composed of contractile muscle fibers that allow the heart to pump blood throughout the body. Contraction occurs due to a series of regulated steps. Depolarization first results in the influx of Na^+ ions through fast-gated Na^+ channels. This causes an increase in the action potential from -90 mV to about 30 mV . This depolarization wave is then transmitted down the T tubules, causing the release of Ca^{2+} into the sarcoplasm. This influx of Ca^{2+} then causes the sliding of myofilaments through cross bridge activation. The cells continue to contract as long as Ca^{2+} continues to enter the sarcoplasm. In order for repolarization to occur, Ca^{2+} channels are deactivated and Ca^{2+} re-enters the

sarcoplasmic reticulum. Simultaneously, K^+ channels are opened and there is a rapid loss of K^+ from the cells (Marieb and Hoehn, 2010). Because this mechanism stimulates contraction of cardiac muscles fibers, an increase in Ca^{2+} can cause a greater contraction.

2.2 Cardiac Cell Biology

The myocardium is able to function and contract as one unit because of the cell-to-cell adhesion found in cardiomyocytes, which is made possible by intercalated disks (ICDs). These disks are composed of three major complexes – desmosomes, fascia adherens, and gap junctions (Figure 4).



Conduction Fibers. 2011. Pearson Education, Inc.
<http://droualb.faculty.mjc.edu/Course%20Materials/Physiology%20101/Chapter%20Notes/Fall%202011/chapter_13%20Fall%202011.htm>.

Figure 2.4: Diagram of intercalated disk

Together, desmosomes and fascia adherens are responsible for mechanically reinforcing and coupling cardiomyocytes while gap junctions are utilized for proper electrical conduction between the cells (Sheikh, et al. 2009). These three structures work together to maintain the structural integrity and synchronized contraction of the myocardium, allowing it to behave as a single coordinated unit, known as a functional syncytium (Marieb and Hoehn, 2010).

Desmosomes serve to anchor the cardiac cellular membranes to the fibrous cardiac skeleton, which is particularly important in areas of constant stress, as caused by the rhythmic beating of the heart. Fascia adherens work in a similar manner, but instead anchor cells by linking the membrane with the actin cytoskeleton. Gap junctions form aqueous pores between cells, which allow for electrical and metabolic connections between two adjoining cells. Thus, gap junctions are responsible for the coordinated contraction of all cardiac muscle cells as a single contractile unit (Sheikh, et al. 2009). Each of these complexes is composed of several unique proteins that together allow the ICDs to perform their overall function. Across these complexes, these proteins have been known to exercise cellular “crosstalk”. This “crosstalk” occurs because of the “close spatial proximity of the junctional complexes to one another” and also because these proteins share common binding partners (Sheikh, et el. 2009).

Data supports the notion that changes in the mechanical junctions of ICDs have a negative impact on the electrical functionality of cardiac cells (Noorman, et al. 2009). For this reason, it is important that any *in vitro* model for cardiac tissue closely mimics the mechanical structure found in natural heart tissue. If this is not the case, electrical conductivity and thus contraction of cardiac cells will not occur as they do *in vivo*. Therefore, three-dimensional models for cardiac *in vitro* testing should be highly preferred over two-dimensional models that do not mimic the mechanical junctions of the ICDs in the cardiomyocytes of the myocardium.

2.3 Cardiotoxicity

Cardiotoxicity is the event of heart damage caused by medication. The list of possible drug-related heart complications is extensive, including rapid heart rate, arrhythmia, myocarditis, coronary thrombosis, myocardial infarction, cardiomyopathy, and heart failure. There are two types of cardiotoxicities. Type I is the most serious and causes permanent damage to the

myocardium, while type II results in cardiac damage that is usually reversible. Cardiotoxic effects caused by type I agents are related to the cumulative dose of a drug and cause irreversible injuries to the heart tissue such as vacuoles, necrosis, and sarcomere disruption. Conversely, heart injury caused by type II agents is not related to dose and does not lead to harmful alterations in cardiac structure. To prevent the presence of these agents on the market, improved methods are needed that detect cardiotoxicity as early as possible.

Several classes of therapeutic drugs have generated unanticipated adverse cardiac effects, including chemotherapy drugs, antihistamines, and NSAIDs. One of the most studied classes of drugs is anti-cancer agents, of which the most common harmful side effect is cardiotoxicity. For example, anthracyclines (e.g. Doxorubicin) are among the most effective chemotherapy drugs. However, they have been identified as very dangerous type I agents that cause permanent cardiomyopathy even at low doses. This irreversible cell apoptosis occurs primarily due to oxidative stress but may also occur as a result of DNA and protein damage (Ewer, 2010). Trastuzumab, another well-known cancer drug, did not show any immediate signs of cardiotoxicity in pre-clinical studies. However, later clinical trials identified it as a type II agent, associated with an unanticipated high incidence of left ventricle dysfunction (Crivellente, 2011).

In addition to causing major health problems for consumers, this high occurrence of cardiotoxicity results in serious setbacks for pharmaceutical companies. If a particular drug is found to be cardiotoxic during a clinical trial, the program is terminated and long-term investments are wasted. If the drug causes cardiotoxicity in consumers after it is launched into the market, it is recalled and companies may be sued for negligence. The most notorious example of the latter situation began in 1999 when the pharmaceutical company Merck marketed the FDA approved drug Vioxx (Rofecoxib), an NSAID used to treat rheumatoid arthritis. Vioxx

was prescribed to more than twenty million people, making it one of the most prescribed blockbuster drugs in history. On September 30th, 2004, after five years on the market, Merck voluntarily recalled Vioxx after the drug caused as many as 140,000 cases of serious coronary heart disease, of which almost half resulted in death (Prakash, 2007). Merck was accused for ignoring evidence of the cardiotoxic effects of Vioxx, resulting in roughly 50,000 lawsuits, costing the company at least 6.38 billion dollars in settlement funds (CBS News, 2011).

Another example was the withdrawal of the antihistamine Terfenadine (Seldane). Terfenadine was made in 1985 by the company Hoechst Marion Roussel (now Aventis) for the treatment of allergies without causing drowsiness. More than 100 million consumers used Terfenadine as of 1990 and in the year before it was recalled, Hoechst Marion Roussel made about \$440 million in drug sales worldwide (Campbell, 2010). In 1998, thirteen years after it was launched on the market, Terfenadine was recalled for causing several cases of cardiac arrhythmia. Although the company suffered from legal expenses and a steep loss of market share to other allergy drugs, it was able to mitigate its damages to some degree by launching Allegra, an antihistamine similar to Terfenadine but without the side effects.

2.4 Current Screening Methods

There are several existing methods that aim to detect cardiotoxicity through the collection of contractile data of cells *in vitro*. However, these technologies do not fully meet the needs of pharmaceutical companies because they exhibit limitations in terms of accuracy, data acquisition time, and cost. Specifically, most drug-screening systems are based on 2-D monolayer cell culture, which is an inaccurate model that does not completely mimic the complex cell-cell and cell-matrix interactions of the 3-D *in vivo* cardiac tissue environment (Moya et al, 2013). While other drug screening platforms may incorporate a more realistic 3-D model, they may be

otherwise limited in their efficiency. For example, some systems use the technique of high-content screening (HCS), which is a very detailed and complicated process incorporating fluorescence microscopy and multi-parameter image analysis. Although HCS is ideal for producing reliable results, it is not a practical option to meet the high demand of new and improved drugs because it requires special, expensive equipment and takes a long time to acquire data.

One type of *in vitro* method that screens for cardiotoxicity uses glass-tube mounting. This technique consists of culturing cardiac myocytes in collagen gels incorporating extracellular matrix from tumors (Eschenhagen and Zimmerman, 2005). This cell mixture is then mounted between two Velcro covered glass tubes and placed in a rectangular well. Mechanical force transducers are attached to these tubes to measure and record contractile force. While this system is effective at assembling cells for heart tissue development and the application of pharmaceutical agents is permitted, drawbacks of this method include the inability to observe the viability of specific cells as well as limited application for electrical stimulation.

Another method is micro-post mounting, which involves the use of tissue-engineered three-dimensional muscle (Vandenburgh, et al., 2008). The muscle is grown and attached to micro-posts where it is distributed into well plates. Electrical stimulation is applied to measure contraction and motion. Pharmaceutical agents can then be added to monitor how cell properties are affected. The process of high-content screening (HCS) is used to gather data on the muscle tissue. While this system is effective at producing data for cells in a three-dimensional structure, there are certain drawbacks. HCS screening produces very detailed, semi-accurate data, but the HCS process takes a very long time to produce data. In clinical applications, to produce results for a device, this method would not be effective because of this long wait time between trials.

Biowire technology attempts to mimic cardiac tissue by providing a three-dimensional scaffold for cells to organize. This system uses pluripotent stem cell derived cardiomyocytes, which are assembled onto three-dimensional cardiac tissue (Nunes, et al., 2013). The fibers are then distributed into well plates and electrical stimulation is applied to the fibers to monitor contraction. Calcium is then applied and data is gathered to monitor how pharmaceutical products affect cell properties. This system is very effective at gathering data on cell properties and their interactions with drugs; however, the mechanical strength of the Biowire is not adequate because the strands cannot handle proper electrical stimulation.

2.5 Microthread System

Cell-seeded biological sutures have shown great promise in the field of cell therapy. Researchers at Worcester Polytechnic Institute (WPI) have demonstrated that human mesenchymal stem cells (hMSCs) can be easily seeded on fibrin microthread sutures for efficient delivery to areas of cardiac infarction. Cells delivered by this method have been shown to differentiate into new cardiac cells and organize into properly functioning tissue constructs that contribute towards heart activity, thus restoring function lost by infarction (Singer, 2010).

Fibrin microthreads are formed through the combination of fibrinogen and thrombin in narrow polyethylene channels. This fibrinogen-thrombin blend is extruded slowly into a HEPES bath and then dried, forming individual microthreads. These threads are then combined and twisted together in batches of four to twenty-four, called a fibrin bundle. These bundles are threaded through a surgical needle and folded over, forming the suture used in surgeries. Sutures and bundles are typically seeded with hMSCs prior to surgery using a rotator at low rpm to ensure consistent seeding. For detailed microthread extrusion and seeding procedures, see Appendix A (Cornwell et. al, 2011).

Fibrin microthreads are typically cut into 4 cm lengths and have diameters ranging from 55 to 65 microns for individual threads. The ultimate tensile strength of a hydrated thread is roughly 4.5 MPa at a 50% strain rate, and crosslinking through UV light exposure doubles this figure and greatly increases thread stiffness. However, this exposure negatively impacts fibroblast proliferation on threads (Cornwell and Pins, 2007).

Cornwell and Pins characterized the cell-seeding capacity of UV cross-linked and non-UV crosslinked microthreads. In their study, bundles of ten fibrin microthreads were glued to glass coverslips. Bundles were then submerged in media (500,000 cells/mL), and attachment was measured through a live/dead assay. Qualitative results show that while initial seeding was similar between the two microthread test groups, after seven days proliferation of non-UV crosslinked threads was much higher (Cornwell and Pins, 2010). Similar quantitative seeding experiments on microthreads achieved seeding densities as high as 731 ± 101 cells per mm^2 and $5,903 \pm 1,966$ hMSCs per centimeter of suture length (Proulx et al., 2011)(Guyette et al., 2013). Fibrin microthreads have also been successfully loaded with fibroblast growth factor-2 (FGF-2) at concentrations up to 200ng/mL. While FGF-2 did not increase initial fibroblast attachment, it greatly boosted cell outgrowth and proliferation (Cornwell and Pins, 2010).

Cells seeded on fibrin bundles support fibroblast attachment, proliferation, and alignment along the thread axis (Cornwell and Pins, 2007). These microthreads have been used to successfully treat large-scale musculoskeletal wounds in mice, reducing fibroplasia-associated collagen deposition while promoting formation of skeletal muscle fibers, connective tissue, and PAX7-positive cells (Domkinko et al., 2011). hMSCs can be easily seeded onto fibrin microthreads, enhancing efficiency and localization of cell delivery to specific regions of the

beating heart. This efficiency ($63.6 \pm 10.6\%$) is much greater than injection of cells ($11.8 \pm 6.2\%$) (Guyette et al., 2013).

In short, fibrin microthreads have demonstrated an impressive ability to deliver seeded cells to areas of cardiac infarction and help restore function in the area. This ability is primarily attributed to these bundles' high cell seeding capacity and ability to organize these cells into functional tissue constructs that contribute to *in vivo* heart activity. It is likely that these attributes of fibrin microthreads can be applied to drug screening efforts and serve as a model system of *in vivo* cardiac activity.

2.6 hMSCs and Neonatal Rat Cardiomyocytes

Adult human mesenchymal stem cells (hMSCs) are self-renewing, multipotent precursor cells that can be isolated from a variety of locations in the body, most commonly the bone marrow stroma where stages of hematopoiesis are regulated. hMSCs can be grown *in vitro* to generate mesenchymal stromal cell cultures, which under certain conditions can differentiate into several cell types including myocytes (muscle cells), adipocytes (fat cells), chondrocytes (cartilage cells), and osteoblasts (bone cells) (Pittenger and Martin, 2004). Due to their differentiation capability, expansibility, and ease of isolation, the use of hMSCs as a tool for cellular therapy, tissue engineering, and regenerative medicine has been extensively studied for both research and clinical applications. Systemic infusion of hMSCs has proven to be beneficial in several pre-clinical models of tissue injury, including that of myocardial infarction. Since they easily proliferate, are easy to acquire, and maintain in the laboratory, hMSC cultures are advantageous to use in *in vitro* studies (Nombela-Arrieta, et. al., 2011). For example, they may be used in staining assays to give insight into cell density and viability achieved on scaffolds such as fibrin microthreads.

While hMSCs are a beneficial cell population to use for general cell viability and proliferation assays, they are not able to effectively serve as a cardiac model *in vitro* in their undifferentiated form because of their lack of cardiomyocyte-like properties, such as the ability to contract. Neonatal rat cardiomyocytes on the other hand provide an effective model for cardiac tissue, allowing for the study of morphological, biochemical, and electrophysiological characteristics of heart cells. The phenotype of neonatal rat cardiomyocytes is very stable, and, unlike adult cardiomyocytes, their contractile properties are similar to that of native heart tissue (Chlopčíková, et. al., 2001). Neonatal rat cardiomyocytes are able to spontaneously contract *in vitro* and are also able to be paced with the application of an electrical signal to achieve simultaneous contraction, mimicking the heart *in vivo*. However, these cells are not as readily available as hMSCs and are also extremely expensive so they are not an ideal cell choice for experiments not specifically related to assessing cardiac function.

2.7 Stimulation of Cardiac Cell-Seeded Fibers

One of the most important aspects of cardiac cell testing *in vitro* is the application of electrical stimulation. After creating a biological scaffold seeded with cardiomyocytes, it is necessary to pace the cells so that the structure may contract as one unit and behave mechanically as the heart does *in vivo*. Electrical stimulation of the cell-seeded scaffold may promote the cells to beat synchronously as well as increase the rate of cell contraction. Moreover, clinical trials suggest that longer duration of stimulation results in structures with greater tissue-like properties (Radisic, et al., 2004). Electrical stimulation may be achieved with a voltage generator or stimulator (a commonly used brand of stimulator is the Grass stimulator). Electrodes are placed on either side of the cell-seeded scaffold to send a current across the

structure to allow for simultaneous contraction, where the voltage may be altered using the electrodes, to gain the desired rate of contraction.

In addition to electrical stimulation, the application of biological compounds can alter the rate of cellular contraction. For example, intracellular calcium within the heart is responsible for contractile force (Eisner, et. al., 2000). Calcium is released from the sarcoplasmic reticulum of cardiac cells during contraction to cause the cardiomyocytes—and thus the whole heart—to relax, a process known as excitation-contraction coupling. Introducing calcium to cardiac myocytes in culture or on a scaffold alters the cells' contractile rate. Acetylcholine, a neurotransmitter, increases the outward movement of potassium ions in heart tissue, which increases the organ's resting potential to decrease contractile force (Reinking, 2002). In clinical applications, acetylcholine is injected to decrease heart rate. It can be observed that calcium and acetylcholine can serve as positive and negative controls to test the reliability of a device that screens for adverse effects of pharmaceutical agents on cardiac function.

Chapter 3: Project Strategy

To begin, we were provided with the following initial client statement from our advisor and client, Professor Glenn Gaudette:

Design a device/system to incubate cells on fibrin microthreads to produce a muscle fiber that can be used for high throughput screening. The device/system should be easy to use (including loading cells, recording data, feeding cells, etc.), provide a sterile environment and fit in a standard cell incubator. The design should also allow for the reproducible loading of cells on the microthreads. The device/system should hold at least six cardiac muscle fibers.

With information provided by Professor Gaudette and our background research, we took this initial client statement and developed a thorough list of objectives. From this list, we distinguished between primary and secondary objectives and then ranked our primary objectives using a pairwise comparison chart as seen in Appendix B.

The following is our final ranked list of primary objectives, with secondary objectives listed underneath:

1. Reliable
 - a. Reproducible
 - b. Accurate
2. Biocompatible
 - a. Optimize cell growth
 - b. Prevent byproducts
 - c. Minimize contamination
 - d. Easily sterilizable
3. Easy to use
 - a. Easy to acquire data
 - b. Seed cells
 - c. Change media
 - d. Add/remove microthreads
 - e. Add/remove pharmaceutical agents
4. Safe for user
5. Cost-effective

Our pairwise comparison analysis ranks reliability as the most important aspect of our design. A major drawback of current screening methods is their use of two-dimensional cellular systems to model cardiac tissue. Our screening device relies on three-dimensional fibrin microthread scaffolds to better depict the structure of natural heart tissue. Therefore, we focused on developing a screening device that can acquire data quickly and reliably. Device biocompatibility and ease of use are important in providing accurate data with minimal effort required from the operator. Safety, while important as a constraint, is not as vital as device users will be experienced and highly trained in laboratory safety. Cost effectiveness plays a minor role in our design, as a more expensive but higher quality device would be preferred.

The most and least important objectives were assigned unanimously among all group members. There was slight debate when ranking the importance of biocompatibility, ease of use, and safety, though these discrepancies were resolved through majority voting and collaboration with our advisor.

Along with these identified and ranked objectives, we also determined several design constraints for our device:

1. Fit in a standard cell incubator
2. Allow for gas exchange
3. Zero contamination
4. Sterilizable
5. House 6+ fibers

With these objectives and functions, and through feedback from Professor Gaudette, we revised our initial client statement as follows:

Design a device to incubate cardiac myocytes and/or stem cells on fibrin microthreads to produce “muscle fibers” that contract upon stimulation. The device should collect contractile data and allow for high throughput screening (HTS), defined as twelve fibers per hour. It should hold at least six cardiac muscle fibers at a time and allow for the addition and removal of various pharmaceutical

agents. It should be easy to use, provide a sterile environment, and fit in a standard cell incubator. The design should allow for the reproducible loading of cells on microthreads.

3.1 Technical Approach

With this revised client statement, we were able to develop our project approach. We first worked with fibrin microthreads produced by Vitathreads to establish hands-on knowledge of their mechanical properties. In addition to handling the threads, we performed extensive background research on their mechanical properties. This allowed us to better understand the physical properties of these microthreads and identify ways in which these threads can be used in future device designs.

We explored and practiced cell culture techniques necessary for growing and maintaining healthy hMSCs and rat cardiomyocytes, including feeding and passaging. This prepared us for future tests involving cell seeding and testing.

With a strong understanding of the properties of microthreads and the cells being seeded, we then developed several design alternatives. All designs were compared using a numerical evaluation matrix, and the top scoring designs were considered. We established mathematical models and performed theoretical calculations to ensure that our selected design was feasible before proceeding to revise and develop the design.

Once our final preliminary design was optimized, we drafted a Solidworks model to better visualize the design's components and plan how to best building the prototype. This model was further revised to emphasize our objectives. We then ordered the necessary materials and constructed the prototype. This prototype was tested to validate whether or not the design met our objectives and constraints.

3.2 Management Approach

To ensure we made constant progress throughout the year, we held weekly meetings with our client and advisor, Professor Glenn Gaudette. These meetings were used to update Professor Gaudette on our progress, review our work during the previous week, and set long and short-term goals for future weeks. In addition to these meetings, we developed a work breakdown structure (Appendix C). To accomplish our overall goal of designing a device for the evaluation of contraction on cell-seeded fibers, we developed eight different requirements. These included understanding client requirements, analyzing functional requirements, generation of design alternatives, evaluation of alternatives, selection of the best alternative, construction of a prototype, documentation of the process, and project management. The timeline to perform each of these tasks is included as a part of our quarterly Gantt charts (Appendix D). Following these tasks and set timeline ensured that we were able to successfully complete our project requirements in a timely manner.

3.3 Financial Approach

Material costs were the main source of spending. Our device was anticipated to require media, materials to hold the thread and media, fibrin bundles, and cells to seed onto bundles. Media came included in the \$100 cost to use equipment in the Goddard Lab. Fibrin bundles were provided to us by Vitathreads free of charge, and the Gaudette lab provided us with cells to use. We had our remaining budget available to spend on materials to make our device. The materials came at a low cost, far below our allotted budget. There was no cost associated for the labor required for producing our device prototype, as assistance was provided by the Higgins Labs Machine Shop free of charge. We expected the cost of materials and manufacturing of our

prototype to be far below our allowed budget, allowing for flexibility in the future for any necessary changes to our final design.

Chapter 4: Alternative Designs

Upon establishing our project strategy, the team proceeded to develop preliminary device designs, evaluate these designs, and select the design that best achieved the goals of the project.

4.1 Needs Analysis

Given our list of objectives identified in Chapter 3, we assigned weights to help quantify the relative importance of each objective. We decided to use a 1-10 scale, with one being a less important objective and ten being very important. These scores are displayed in Table 4.1.

Table 4.1: Weighted Objectives Table

Objective	Score
Reliable	10
Biocompatible	9
Ease of Use	7
Safe for User	4
Cost-effective	2

We previously identified reliability and biocompatibility as our most important objectives. Thus, we decided to set these objectives as the upper limit of our ten-point scale. Because we value reliable measurements above biocompatibility, we gave biocompatibility a slightly lower weight of nine. We proceeded to weight the remaining three objectives based on their relative importance to reliability. Ease of use is a fairly important objective that will be a key focus of our device development. An easier, simpler device is more appealing from a buyer and operator standpoint, and so we gave this objective an above average weight. The last two

objectives were not as critical to the design of the project and thus received below average scores.

By using a broader ten-point scale versus a typical linear scale, we are able to better specify the importance of each objective for a more accurate final evaluation of conceptual designs.

4.2 Functions and Specifications

Through discussions with our client and group research, we identified the following design functions and specifications corresponding to these functions, shown in Table 4.2.

Table 4.2: Functions and Specifications

Functions	Specifications
Facilitate seeding, growth, and contraction of hMSCs and rat cardiomyocytes	Seeding density must be comparable to 731 cells/mm ² (2,296 cells/cm)
	Contractile force comparable to theoretical calculation of 5.442 μN for a 1 cm thread
Electrically stimulate the cells to cause contraction	Provide voltages between (0 and 1V/cm)
Allow for addition/removal of threads, media, and pharmaceutical agents	Media volume must sustain cell health for two-three days before changing
Hold threads and media	Well size – accommodate threads 1 – 4 cm in length
Collect quantitative data for contraction rate and force	Screen a minimum of 12 fibers per hour
Allow for incubation of cells	Size smaller than 17”x20”x20”

First and foremost, our device must facilitate the seeding and growth of human mesenchymal stem cells as well as the seeding, growth, and contraction of neonatal rat cardiomyocytes on fibrin microthreads. Previous research on fibrin microthreads has shown seeding densities of 731 cells per square centimeter (Proulx, et al., 2011). Our device should yield seeding densities similar to or better than this to be considered successful. Based on this cell density, a typical microthread with a diameter of 0.1 mm will have enough cells to produce a

contractile force of 5.441 μN per centimeter of thread length (see calculations in Design Calculations). Threads housed in our device should achieve contractile forces comparable to this calculation.

Electrical stimulation enables the user to control the contraction rate of the microthread. Past research suggests that the maximum voltage necessary to fully stimulate hMSCs is 1 volt per centimeter (Sun, et al., 2007). Thus, our device should allow for the application of zero to one volts of electricity to each thread.

Our device must be able to house microthreads of varying lengths and keep them hydrated in media. Typical microthreads used in surgical applications vary between one and four centimeters in length, and therefore the compartment containing the thread must accommodate these thread sizes (Guyette, et al., 2013). Our design must also be tailored towards easy addition and removal of threads, media, and various pharmaceutical agents. The volume of media being added and removed depends on the well size. We need to ensure that the wells contain enough media to keep seeded cells healthy for at least two days at a time to reduce work required by the operator. However, minimizing the volume of media helps to save money and time required to prepare the media.

To promote a high throughput aspect of our device, we have established that our device must collect and interpret quantitative data for the contractions of twelve threads per hour. This is related to a second specification that our device holds at least six threads at a time. Our device must also fit in a standard cell incubator, measured to be 43x51x51 cm. This is the absolute size restriction. However, many incubators have removable shelves with adjustable heights. To allow for the sharing of incubator space with our device, we would prefer to minimize the height as much as possible.

4.3 Conceptual and Preliminary Designs

We established six conceptual designs that performed our functions and met our specifications outlined in the previous section. Each design incorporated different thread holding mechanisms and data collection methods. Sketches, descriptions, advantages and limitations of each of these designs are provided in the following sections.

4.3.1 Wheel and Camera

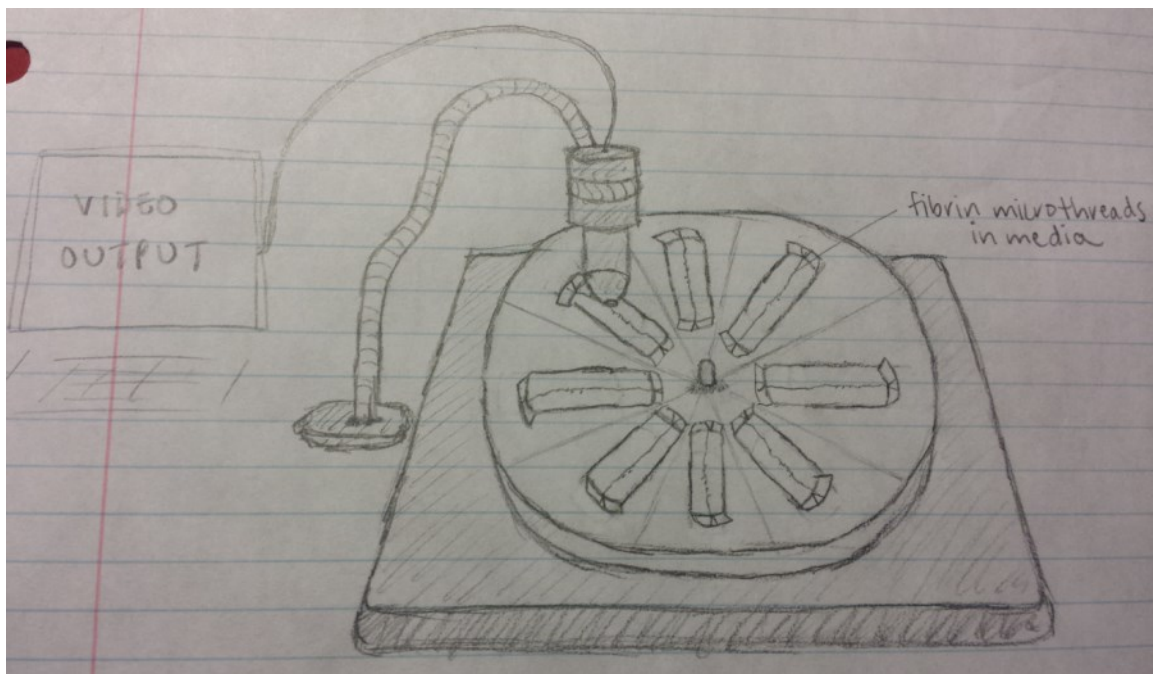


Figure 4.1: Wheel and Camera Sketch

The wheel and camera design uses an interchangeable rotating disk coupled with a handheld microscope for imaging and data collection (Figure 4.1). Cell-seeded microthreads are placed in media-containing wells arranged around the center of the disk. Threads are held in place at both ends by two small slits built into the wells. As various chemical agents are introduced into each well, a handheld microscope, held in place by a stand, records video of the

contraction of each thread. This video is displayed on a nearby computer in real time. The entire disk is rotated to image each of the wells while keeping the microscope stationary.

This design is very high-throughput. Each one of the interchangeable disks holds several threads at a time, allowing for the data collection of many threads. With interchangeable disks, one set of threads can be imaged or tested while another set undergoes seeding or growth. A stationary camera eliminates the need to readjust and refocus the camera between threads, making it easier for an operator to use. The well design promotes cell viability and thread integrity by allowing for minimal thread handling; the user loads the threads into the device a single time and never has to touch them again. However, this design may be limited by its inability to acquire quantitative contractile force data.

4.3.2 Spring and Camera

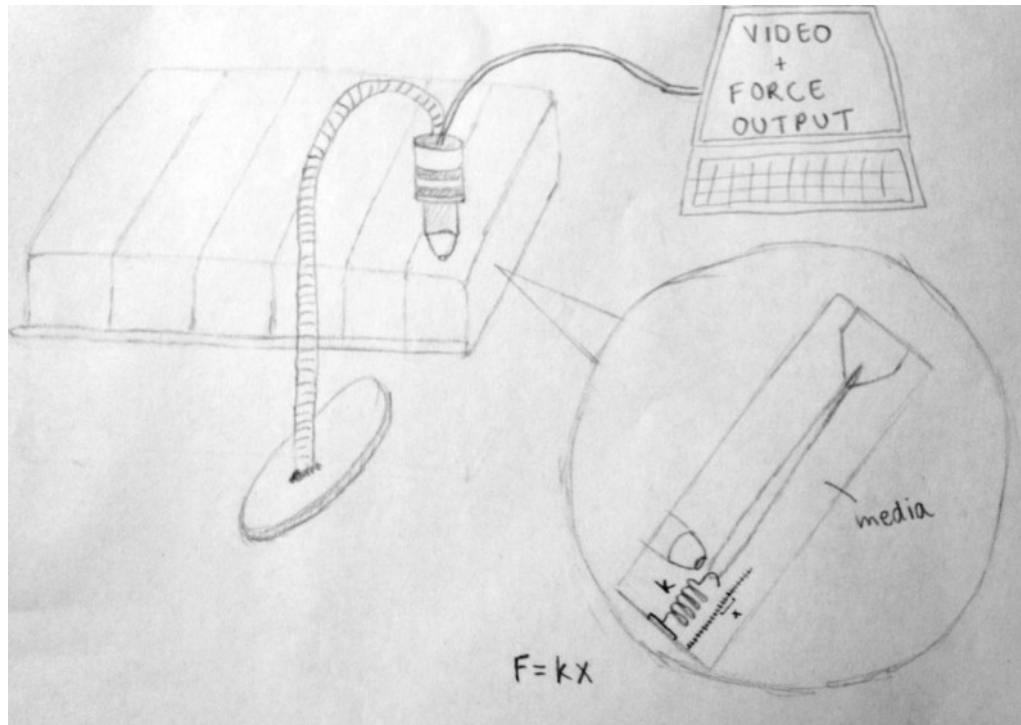


Figure 4.2: Spring and Camera Sketch

The spring and camera design, shown in Figure 4.2, uses a rectangular plate with six long, narrow wells in which the microthreads are held. In each well, the two ends of the microthread are clamped, and the thread is stretched taut and looped around a hook attached to a sensitive spring on the opposite end. A micro-scale ruler is etched beneath the spring, and a stationary handheld microscope video records the oscillations of the spring relative to this ruler, displayed on a computer monitor. With a known spring constant and a measured displacement, quantitative contractile force can be calculated through the equation $F = kx$, where k is the spring constant and x is displacement of the spring. This design is limited by the ability to purchase a spring sensitive enough to oscillate under lower forces provided by a cell-seeded microthread. In addition, it is difficult to measure data accurately, because the small-scale microruler may be difficult to read.

4.3.3 Limit Switch

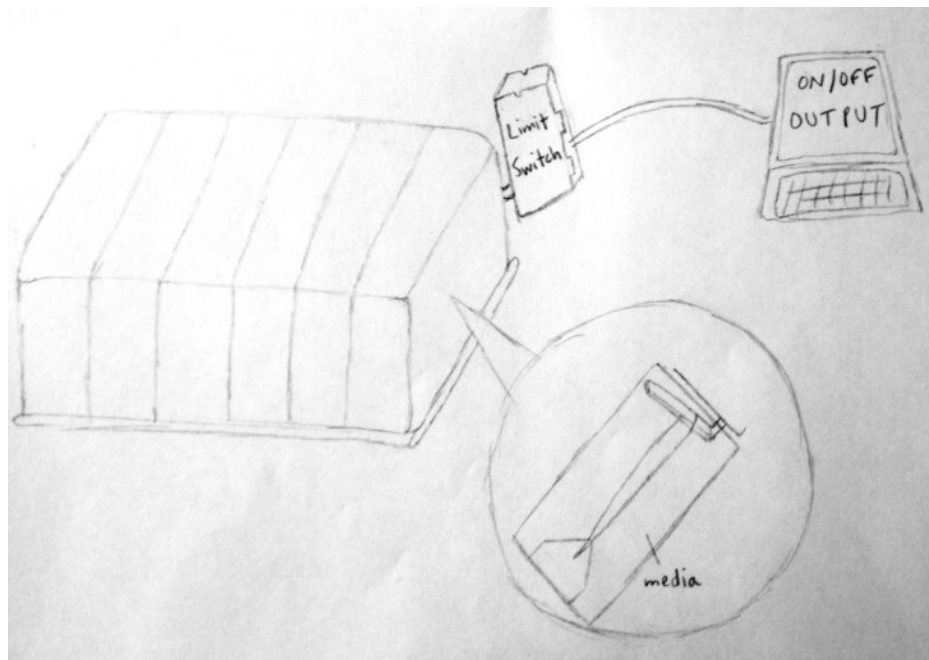


Figure 4.3: Limit Switch Sketch

The limit switch design uses a six-well plate with rectangular wells inside which microthreads are held in media (Figure 4.3). The two ends of the fiber are clamped at one end of the well and the thread is stretched taut and looped around the arm of a limit switch. As various chemical agents are introduced into each well, the limit switch is repeatedly tripped in response to the contractions of the thread. The limit switch is hooked up to a computer that displays an on/off output to determine whether the fiber is contracting or not. The binary nature of the data collected through this method is very easy to interpret. However, a limit switch design cannot calculate important multi-faceted contractile data such as force or rate. It is also possible that the limit switch will not have a high enough sensitivity to be triggered by a contracting microthread. In addition, a significant amount of thread handling is required to load each thread, which could potentially affect thread integrity.

4.3.4 Oscillating Weight and Transducer

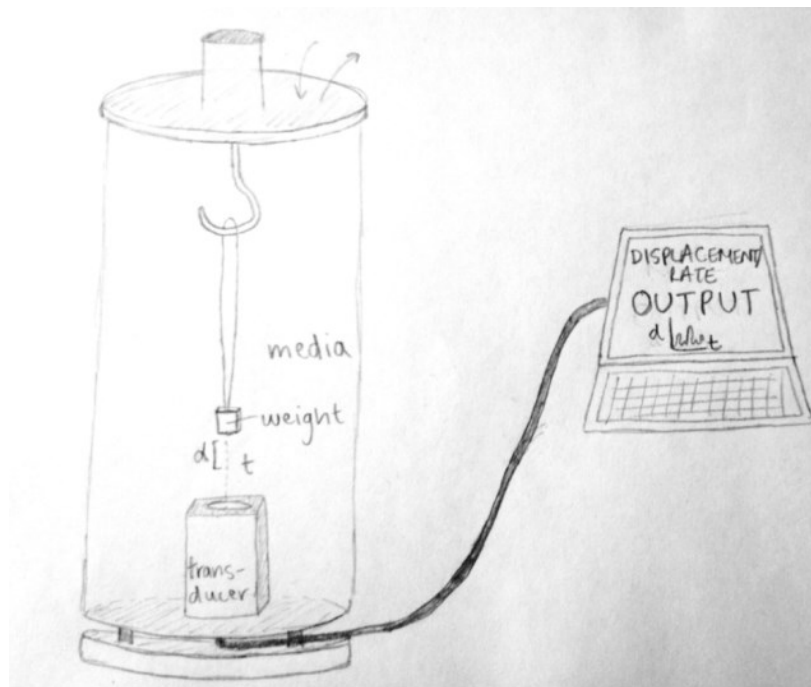


Figure 1.4: Oscillating Weight Sketch

The oscillating weight design, shown in Figure 4.4, incorporates a tall, cylindrical reservoir of media with a removable cover onto which a hook is attached. A small-scale weight is attached to the two ends of a microthread, and the thread is then looped onto the hook. The cover, with the thread attached, is lowered into the reservoir. As the fiber contracts, it will pull the weight upwards. Gravity will then lower the weight back to the original location. An ultrasound transducer, coupled with a piezoelectric crystal at the bottom of the tube, detects this oscillating weight through changes in the time interval of signals being transmitted and reflected off of the weight. The change in time interval can be used to calculate the force of contraction. The transducer is hooked up to a computer, which displays a software-generated graph of distance versus time to determine the rate and relative force of contraction. This design allows for easy interpretation of data, as it provides concrete quantitative distance values over time. Ultrasound transducers also have the sensitivity required to detect the small contractile movements of microthreads, which is a distinct advantage over many other designs. However, this design is not particularly user friendly. It requires long preparation time, because it can be difficult to attach the weight onto the thread and line it up with the transducer on a consistent basis.

4.3.5 Cantilever Beam and Transducer

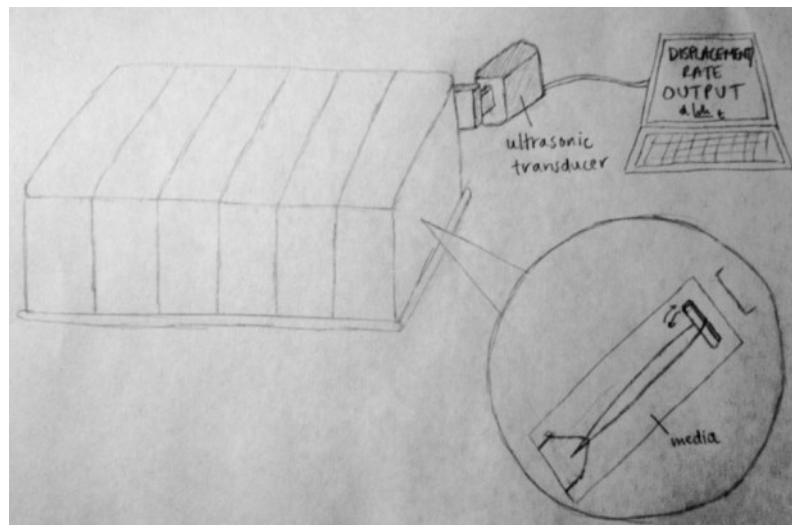


Figure 4.5: Cantilever Beam Sketch

This design uses a six-well plate with rectangular wells, inside which microthreads are held in media (Figure 4.5). The two ends of each thread are clamped at one side of the well, and the thread is stretched taut and looped around a sensitive cantilever beam. The beam deflects in response to the contraction of the thread. An external ultrasound transducer determines the displacement of the post, and this value can be used to calculate the force of contraction. A computer displays a software-generated graph of deflection versus time to determine the rate and relative force of contraction. This design allows for easy interpretation of data, as it provides concrete quantitative distance values over time. The ultrasonic transducer is sensitive enough to detect the small deflections of the cantilever beam. However, the design may not be easily reproducible, because all threads need to be anchored at the same height on the post for each test to ensure accurate measurements. There is also a high level of thread manipulation required.

4.3.6 Force Transducer and Spring

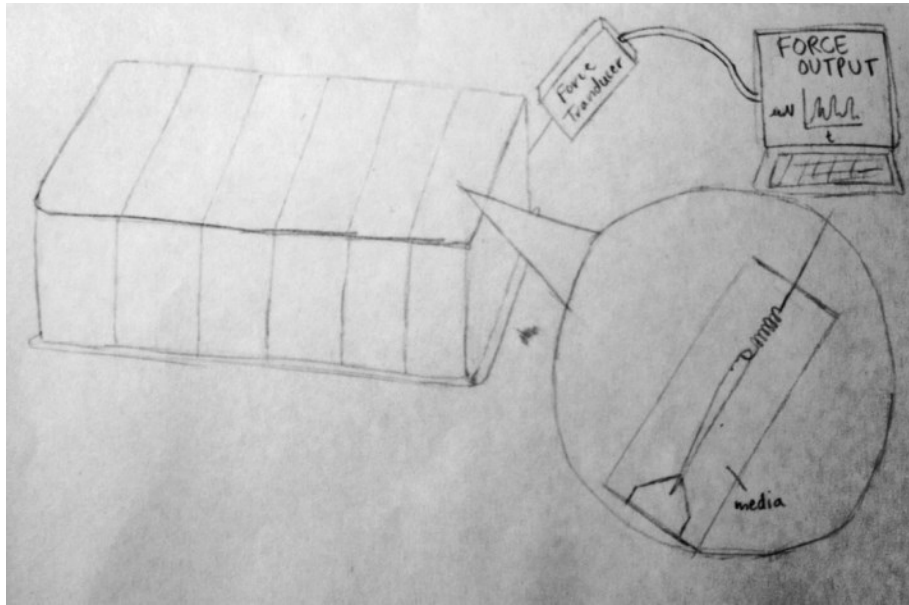


Figure 4.6: Force Transducer Sketch

This design uses a six-well plate with rectangular wells inside which threads are held in media (Figure 4.6). The two ends of each thread are clamped on one side of the well, and the thread is stretched taut and looped around a spring attached to a mechanical force transducer. The transducer will measure the force generated by the thread and send this data to a computer, which displays a force versus time graph. This design directly measures quantitative force data over time, and thus no calculations or data interpretation is required. However, finding a force transducer sensitive enough to detect micro/nano-scale forces may be difficult.

4.4 Design Evaluation Matrix

To determine how well each of our conceptual designs met our objectives, we evaluated each design using the weighted objectives table developed in section 4.1. The results are shown in Table 4.3.

Table 4.3: Design Evaluation Matrix

Objective & Weight	Wheel & Camera	Spring & Camera	Limit Switch	Weight & Transducer	Cantilever Beam & Transducer	Force Transducer & Spring
HTS (10)	8	4	5	3	6	4
Biocomp. (9)	9	5	5	3	7	4
Easy to Use (7)	8	6	7	4	6	5
Safe for User (4)	9	8	7	7	8	7
Cost-Effective (2)	7	3	4	4	6	4
Total	41	26	28	21	33	24
Weighted Total	267	165	180	121	209	147

We assigned a score between one and ten for how well we perceived each design to meet each objective. We then tabulated two scores for each design: raw and weighted. Raw scores were calculated by adding the five scores obtained from each objective. The weighted scores took each score and multiplied it by the relative importance of the objective (shown in parentheses). These weights were established in Chapter 3.

As determined by our evaluation matrix, the rotating disk design received both the highest raw total and highest weighted total, suggesting that overall, this design best achieves our objectives. Our cantilever beam and transducer design received the second highest scores. There were aspects of this design that we felt were very strong, particularly its ability to provide quantitative data that cannot be obtained through using just a camera. Thus, our team decided that it was best to incorporate aspects from both of these designs when establishing a preliminary design. By using a combination of a camera and cantilever beam, our device can collect both qualitative and quantitative contractile information.

4.5 Design Calculations

We assessed the feasibility of our cantilever beam/camera hybrid design through mathematical models to calculate theoretical cantilever beam deflections. We used the following model, shown in Figure 4.7 below.

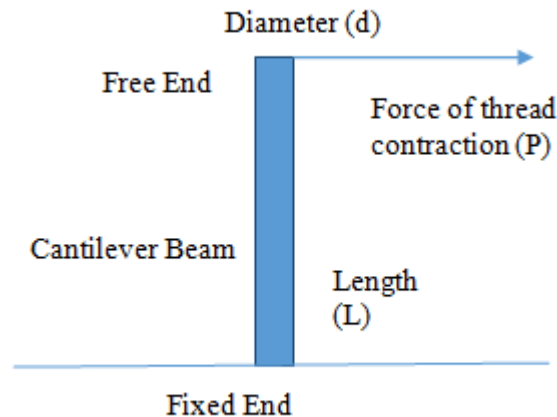


Figure 4.7: Cantilever Beam Model

We found that a single cardiomyocyte can produce a force of 2.37 nN (Nishimura, 2004). We used this figure along with previously mentioned data for the cell-seeding of microthreads to perform the following calculations that estimate the contractile force generated by a 1cm long fibrin bundle, where L is the length of the thread, D is the diameter, C is the circumference, and SA is the surface area.

FORCE PER CENTIMETER OF THREAD

$$\begin{aligned}L &= 1 \text{ cm} = 10 \text{ mm} \\D &= 100 \text{ micrometers} = 0.1 \text{ mm} \\C &= \pi * D = 0.31416 \text{ mm} \\SA &= C * L = 3.1416 \text{ mm}^2 \\ \text{Cell density} * SA &= 731 * 3.1416 = 2296 \text{ cells/cm} \\2296 * 2.37 \text{ nN} &= 5,442 \text{ nN} = \mathbf{5.442 \mu N}\end{aligned}$$

These calculations suggest that a contracting fibrin bundle seeded with a few thousands cells should produce forces on the scale of micronewtons. Therefore, we need a cantilever beam and camera setup that can detect forces on the micro-scale. Using a standard cantilever beam equation (Equation 4.1) along with previously identified dimensions of our cantilever beam, we can solve for a desired range of elastic modulus for our cantilever beam material. Alternatively, if a material has been selected, we can use its elastic modulus to solve for its optimal dimensions.

Equation 4.1: Deflection equation for end-loaded cantilever beams

$$P = \frac{3EI\delta}{l^3}$$

In equation 4.1, P is the force on the end of the beam, L is the length of the beam, E is the elastic modulus, and I is the moment of inertia of the beam's face. We generate an excel sheet of deflection values for two cantilever beam materials in Chapter 5, Section 1.

4.6 Preliminary Designs

Once our team decided on a camera-cantilever beam device, we worked towards developing several preliminary designs that incorporated these features. Considerations for design included cantilever beam orientation (standard or inverted), beam design, notch design, well size and number, well arrangement, and camera orientation. The following sections discuss alternative approaches to each of these aspects and explain our reasoning for selecting each feature for use in our final design.

4.6.1 Cantilever Orientation

The team considered three potential mounting orientations for the cantilever beams: upwards, downwards, and sideways. These orientations are illustrated in Figure 4.8. With the

cantilever beam mounted sideways, gravity becomes a potential problem. A long, narrow, flexible post may sag under its own weight and thus skew our measurements. Additionally, mounting beams to the side of each well is a more difficult process than is required of the other orientations. While the upwards and downwards posts can stand or hang respectively, a sideways post requires adhesives or overly complex anchoring mechanisms.

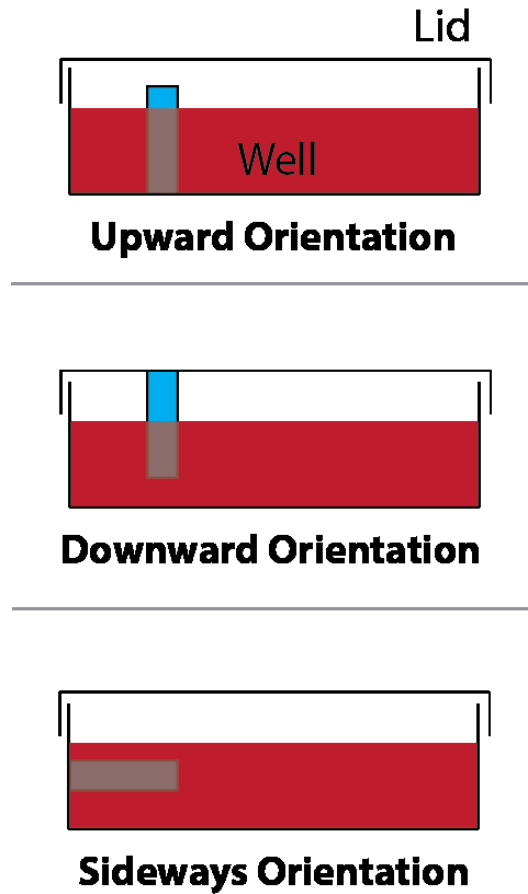


Figure 4.8: Cantilever Beam Orientations

There are several advantages to a downward-oriented cantilever beam system. To maximize cantilever displacement due to thread contraction, it is important to anchor the thread as close to the end of the cantilever as possible. In the upwards model, the thread is anchored towards the top of the well. This leads to reduced protection of the thread and an increase in media required (the entire well must be filled to keep the thread hydrated). The downwards

model solves these problems by encouraging the thread to be anchored towards the bottom of the well, increasing protection offered by the well and reducing the media requirement. Furthermore, with the upwards model, the cantilever beams are fixed to the well base. This restricts the user to loading each thread inside the small well volume. Loading by hand will be very difficult and require the assistance of tools. Attaching the cantilever beam to the lid in the downward model allows the user to remove the beam from the well and load the thread in free space. Based on the advantages provided by the downward orientation of cantilever beams, we have decided to use this feature in our final design.

4.6.2 Cantilever Beam and Notch Design

We considered both PDMS and 304 stainless steel wire as our cantilever beam material. PDMS is readily available in most labs, highly biocompatible, and has a low elastic modulus. However, producing these posts takes about a day and may be difficult due to the small diameter required. Stainless steel is also biocompatible and comes in dimensions as small as 0.005 inches. There is no production time associated with a stainless steel cantilever beam, however stainless steel is much stiffer than PDMS and may not deflect as much.

The user must be able to anchor seeded microthreads to our cantilever beams. PDMS is very rubbery, and a notch can be cut to serve as an anchor point for the microthread. We considered several notch designs that facilitated the reproducible attachment of threads on PDMS cantilever beams. The profiles of each notch are shown in Figure 4.9.

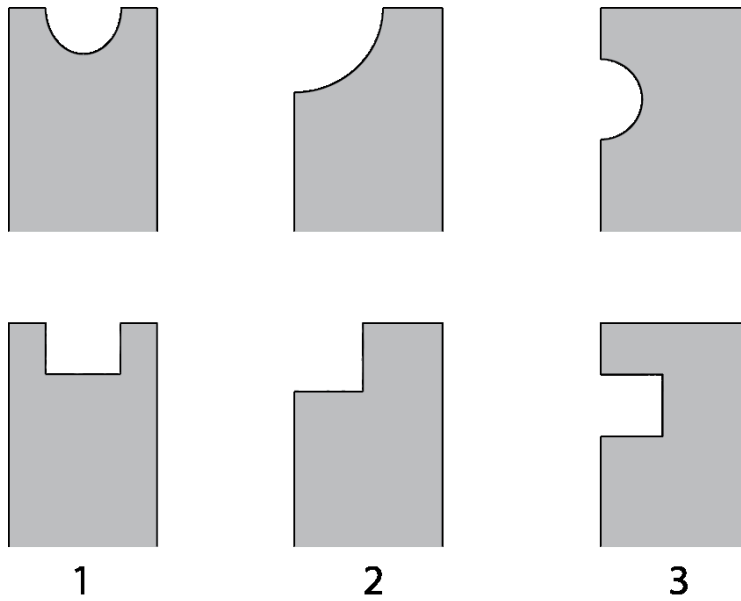


Figure 4.9: Notch Profiles

There were three basic designs: a centered notch, edge-justified notch, and a side notch. While all three designs are feasible with an upwards-oriented cantilever beam, only design three is compatible with our downwards-oriented system. This is because notches one and two risk thread slippage or detachment due to gravity.

A notch cannot be carved into a stainless steel cantilever beam. Instead, to anchor the microthread, a bend can be applied to the end of the beam that will prevent slipping of the thread (Figure 4.10).



Figure 4.10: Stainless Steel Post Profiles

4.6.3 Well Size & Number

When determining the size of each well, it is important to consider the desired length of threads to be tested. Based on our research, microthread sutures used for surgery typically range from one to four centimeters in length, with two centimeters being most common. For the greatest flexibility, our wells should accommodate for the testing of four centimeter-long threads. Since our device requires the user to wrap the thread around the cantilever beam and clamp it at the other end, the final length of the thread will be half of its original length. Therefore, the distance between our anchor points should be around two centimeters long. Wells must be wide enough to accommodate this length.

Regarding well number, our client requests that our device hold a minimum of six wells (see Revised Client Statement in Chapter 3). For design simplicity, we have decided that our device will have six wells. Our design can be scaled up to incorporate more wells in the future if necessary.

4.6.4 Camera Orientation

We have established that our device will use a camera/microscope in conjunction with flexible cantilever beams to image and determine the contractile strength of cell-seeded microthreads. There are four possible ways to orient the camera for imaging: top, bottom, sideways, and angled. These orientations are illustrated in Figure 4.11.

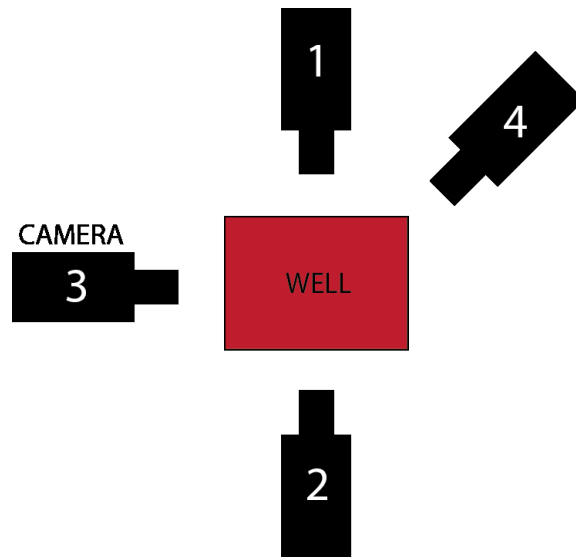


Figure 4.11: Potential Camera Orientations

An angled orientation (number 4) complicates the deflection measurements of the posts by distorting the image seen by the camera. Therefore, we ruled out having the camera at an angle. With a downwards-oriented cantilever beam system, a top-down camera (number 1) is not feasible. The camera would be too far away from the point of deflection and its view would be obstructed by components on the lid. Both the bottom-up and sideways camera orientations are valid options, and thus we considered well plate designs that incorporated the ability to be measure by either method.

4.6.5 Well Arrangement

The arrangement and orientation of the six wells is an important aspect that has a major impact on our design's final appearance. We considered three potential arrangements that minimized our device's footprint (Figure 4.12). We acknowledge the benefits of having a stationary camera during imaging. A stationary camera allows for more reproducible measurements, because the camera images the exact same location every time. It is also easier

for the user, as a stationary camera reduces the need to readjust and refocus the camera between wells. Therefore, we developed these well orientations with a stationary camera in mind.

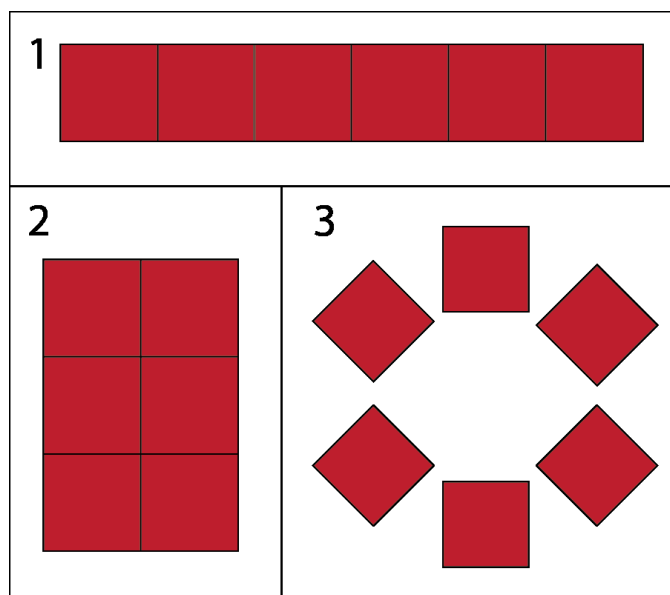


Figure 4.12: Possible Well Orientations

Designs 1 and 2 are more traditional rectangular arrangements. These designs are likely easier to manufacture, and well plates featuring these arrangements are very common. The one-by-six arrangement allows for a single camera to image all wells without needing to move. This can be accomplished by sliding the well plate along a track parallel to its long axis, past the imaging camera. However, a one-by-six arrangement creates a very long dimension that can be a burden in the lab. The two-by-three design has friendlier dimensions, but requires a more complex mechanism to image all of the wells with a single, stationary camera. Design 3 arranges the wells in a circular pattern that can be rotated around a stationary camera either in the center of the well plate or on the periphery. However, this design uses space inefficiently compared to the rectangular arrangements and is more complex to manufacture.

After discussion, our group decided on a two-by-three well plate, because its dimensions are the most versatile and plates like these are common and easily purchased.

4.6.6 Summary

A summary of our preliminary design analysis is shown in the table below.

Table 4.4: Summary of Results

Feature	Result
Beam Orientation	Downwards
Beam Material	PDMS or SS
Notch Design	Side Notch or Bend
Well Size and Number	~2cm, Six Wells
Camera Orientation	Sideways, Bottom-up
Well Arrangement	2x3 Rectangle

4.7 Final Design

Our team first considered a reusable well plate design made out of polycarbonate. Polycarbonate is a transparent polymer that can withstand temperatures and pressures of an autoclave, making it sterilizable between uses and a good candidate for a reusable plate material. Unfortunately, the polycarbonate slabs needed to machine our well plate were too expensive to consider further. We tried designs in which just the bottom panel, the one through which the microscope images, is polycarbonate. However, this led to unwanted complex design components.

We instead opted to integrate a preexisting disposable well plate into our design. We designed a reusable polycarbonate insert that is compatible with six-well plates from companies including Celltreat, Falcon, and Corning. This allowed us to maintain device reusability while minimizing manufacturing costs. Using a standard six well plate adheres to all of the design considerations discussed in the previous section. Wells are arranged in a 2x3 rectangle and have

a diameter of 3 cm and depth of 2 cm. They allow for imaging from the bottom and enable a downwards cantilever beam orientation. A typical six-well plate is shown in the figure below.



Figure 4.13: Standard Six-Well Plate

Figure 4.14 is a Solidworks model of our insert. Twelve rectangular holes are drilled into a thin (1/16") polycarbonate sheet. These holes accommodate removable PDMS anchor points illustrated in Figure 4.15. The insert is dimensioned and cut to fit into a standard six-well plate.

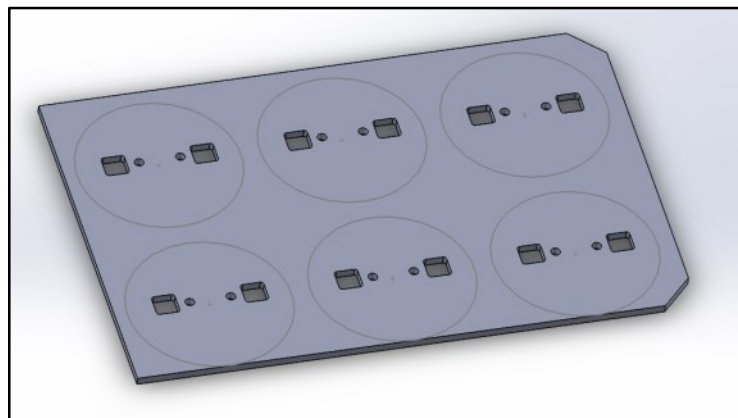


Figure 4.14: Polycarbonate plate insert

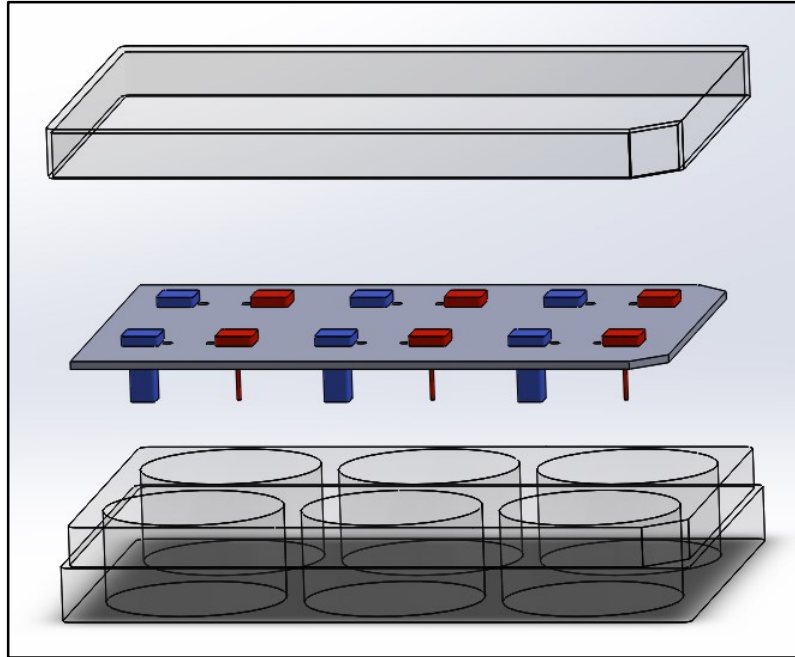


Figure 4.15: Full assembly of plate insert

First, the PDMS anchor points (shown in blue in Figure 4.15) are created by following the protocol in Appendix E. These anchor points are then gently pushed into the holes of the insert. PDMS or 304 stainless steel cantilever beams (shown in red in Figure 4.15) are prepared as described in the protocol in Appendix E and inserted into the plate adjacent to the anchor points. Using a razor blade, a slit is cut down the middle of the anchor point. A seeded microthread is wrapped around the cantilever beam, and the free ends of the thread are pinched inside the slit in the anchor point. This process is repeated five times, and the entire insert is transferred to a six-well plate containing fresh media. The entire assembly, including the plate, the insert, and the removable anchor points, is shown in Figure 4.15. The detailed protocol can be found in Appendix E. The fully assembled plate can be imaged under a microscope to record contractile data.

Chapter 5: Design Verifications

Our team conducted a series of tests to verify that our device satisfied our objectives. Our device had to be used effectively and collect results properly and reliably. These tests allowed the team to identify the effective elements of our design and the areas of improvement required in the future.

5.1 Mathematical Model Calculations

Before any testing occurred, we took the mathematical model developed in Chapter 4 and produced an excel sheet (Table 5.1) of theoretical deflection values of PDMS cantilever beams.

Table 5.1: PDMS cantilever beam deflection calculations

Length of Beam (mm)	Load (N)	Young's Modulus for SS(MPa)	Radius (mm)	moment of inertia (mm ⁴)	Displacement (mm)	Displacement (microns)
10	5.44E-06	0.36	0.5	0.049	0.103	102.651
15	5.44E-06	0.36	0.5	0.049	0.346	346.448
20	5.44E-06	0.36	0.5	0.049	0.821	821.211
25	5.44E-06	0.36	0.5	0.049	1.604	1603.928
30	5.44E-06	0.36	0.5	0.049	2.772	2771.588
10	5.44E-06	0.36	1	0.785	0.006	6.416
15	5.44E-06	0.36	1	0.785	0.022	21.653
20	5.44E-06	0.36	1	0.785	0.051	51.326
25	5.44E-06	0.36	1	0.785	0.100	100.246
30	5.44E-06	0.36	1	0.785	0.173	173.224
10	5.44E-06	0.36	1.5	3.976	0.001	1.267
15	5.44E-06	0.36	1.5	3.976	0.004	4.277
20	5.44E-06	0.36	1.5	3.976	0.010	10.138
25	5.44E-06	0.36	1.5	3.976	0.020	19.802
30	5.44E-06	0.36	1.5	3.976	0.034	34.217
10	5.44E-06	0.36	2	12.566	0.000	0.401
15	5.44E-06	0.36	2	12.566	0.001	1.353
20	5.44E-06	0.36	2	12.566	0.003	3.208
25	5.44E-06	0.36	2	12.566	0.006	6.265
30	5.44E-06	0.36	2	12.566	0.011	10.827
10	5.44E-06	0.36	2.5	30.680	0.000	0.164
15	5.44E-06	0.36	2.5	30.680	0.001	0.554
20	5.44E-06	0.36	2.5	30.680	0.001	1.314
25	5.44E-06	0.36	2.5	30.680	0.003	2.566
30	5.44E-06	0.36	2.5	30.680	0.004	4.435

Based on these calculations, a cylindrical PDMS cantilever beam with a diameter 0.5-2.5 mm and length of 10-30 mm will deflect a distance on the order of millimeters to microns. With the assistance of magnification from a microscope objective, this deflection should be easily detectable, suggesting that PDMS is a good cantilever beam material.

5.2 Imaging Capability

With the table of displacement values calculated in section 5.1, our cantilever beams would be displacing distances on the scale of millimeters. Thus, we needed an imaging system that could magnify this displacement to more easily distinguish between actual deflection and background movement. We designed our device to be used with a standard laboratory microscope. Since these microscopes can produce high quality images at magnifications ranging between 2X and 100X, these microscopes should greatly increase our device's detection sensitivity.

To test a microscope's ability to image fibrin bundles in our device, we loaded a bundle into our device, inserted our device into a six-well plate, and placed the assembly in a Zeiss PrimoVert microscope. Representative images can be seen in Figures 5.1 and 5.2 below.

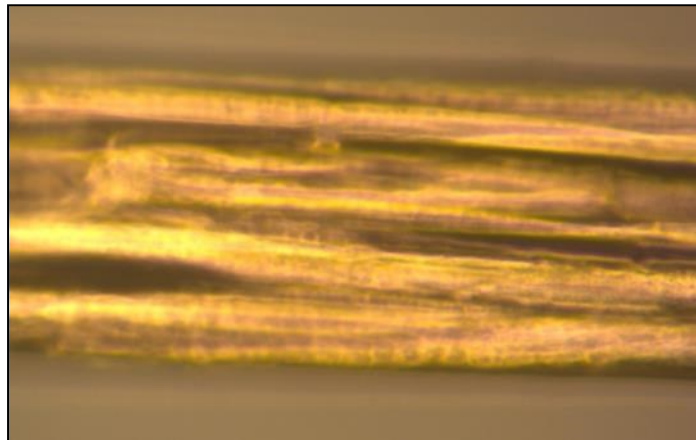


Figure 5.1: 100X image of fibrin bundle loaded in device

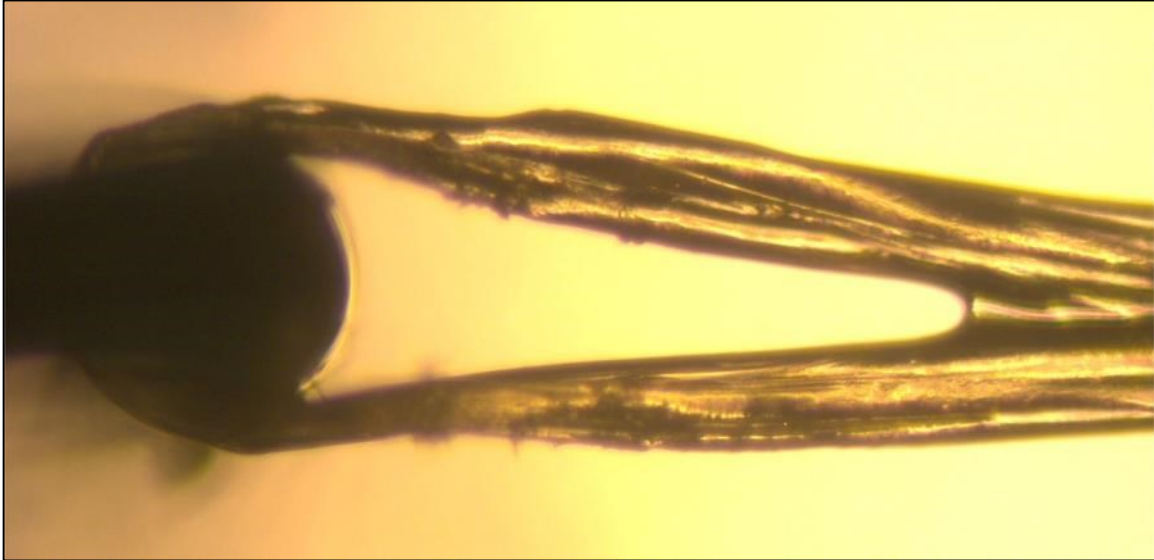


Figure 5.2: 40X image of fibrin bundle around cantilever beam

Our device allows for extremely high quality images at magnifications as high as 100X. This means that the initial millimeter deflection produced by a contracting thread should appear as a centimeter deflection on camera.

5.3 Microthread Loading and Fixation Testing

Once we identified PDMS as the material of choice for both the anchor point and cantilever beam, we conducted tests to quantify the anchor point's ability to hold a fibrin bundle in place without slipping. Our anchoring mechanism relies on a pinching mechanism, where a slit is cut into a piece of PDMS and the thread is flossed into the slit. We conducted a gravity-based testing rig to determine how much weight could be supported by this pinching of the fibrin bundle. Our polycarbonate plate insert was arranged perpendicular to the surface of tabletop, and both ends of a 12-thread fibrin bundle were loaded in the slit of a PDMS anchor point. A paperclip was hung on the loop created by the bundle, and weights of increasing mass were hung off the paperclip until the bundle slipped out of the anchor point. Figure 5.3 below illustrates the set-up of this experiment:

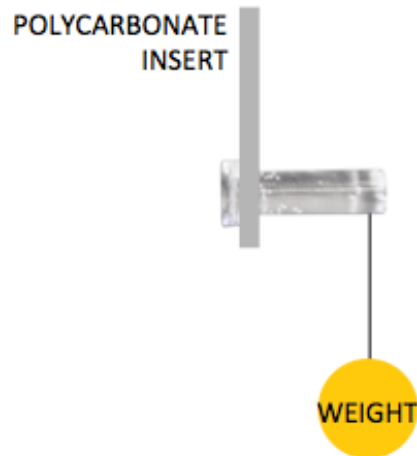


Figure 5.3: Diagram of Loads on PDMS Anchor Point Slit

Figure 5.4 shows an actual picture of our experiment. We started with 5 grams of weight and waited 10 seconds before adding an additional five grams. We continued this until the slit failed and the weighted suture slipped, occurring at approximately 15 grams. Using gravity and the known mass, we calculated the force with the equation $F=ma$. Since slipping occurred between 10 and 15 grams of weight, we can say that our anchor point holds loads between 0.1 to 0.15N. Since contracting microthreads were estimated to produce forces on the scale of micronewtons (see Chapter 4), no slipping should occur in our device due to the contraction of these threads.



Figure 5.4: Picture Taken From PDMS Anchor Point Loading Testing

5.4 Sterilization Testing

To make sure that our device could be reused, we tested whether or not our polycarbonate insert and PDMS anchor points and cantilever beams could be sterilized. The Gaudette lab uses ethylene oxide gas to sterilize the materials they use for experiments involving microthreads. Therefore, we tested the ability of our device's components to be sterilized by this method. Anchor points and cantilever beams were placed in an autoclave bag and gassed for twenty-four hours. We noticed slight expansion of the anchor points and cantilever beams after the gassing process, hindering the ease in which they could be inserted into the polycarbonate insert. This can be overcome by loading these features into the insert before the gassing process. The anchor points and cantilever beams are meant to be disposed after testing and do not require repeated sterilization.

The polycarbonate insert was placed inside a separate autoclave bag and gassed also gassed for twenty-four hours. The polycarbonate insert showed no changes in properties after the gassing process, suggesting that this insert can be sterilized multiple times for reuse. Polycarbonate should also withstand the temperatures and pressures of an autoclave, though these tests have not been conducted.

5.3 Cell Retention Testing

We conducted a series of cell staining procedures to assess our device's ability to maintain cell health and viability and qualify the presence of cells and seeding capacity of fibrin microthreads. Two fibrin microthreads were seeded with human mesenchymal stem cells following the seeding protocol in Appendix E. One thread was stained immediately upon completion of the seeding process with Phalloidin and Hoechst dyes (P/H protocol in Appendix E). The second thread was loaded in our device and incubated for three hours before being stained in the same way. Two representative Phalloidin/Hoechst staining images are shown side-by-side in Figure 5.5.

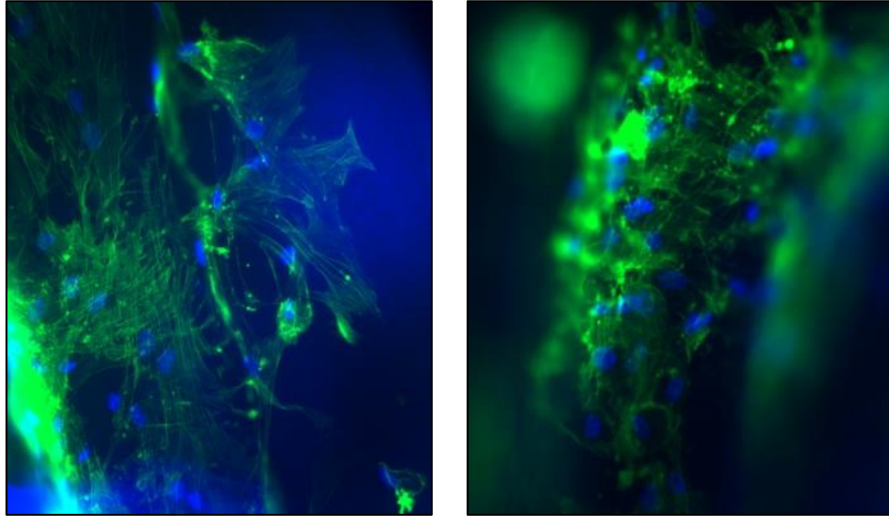
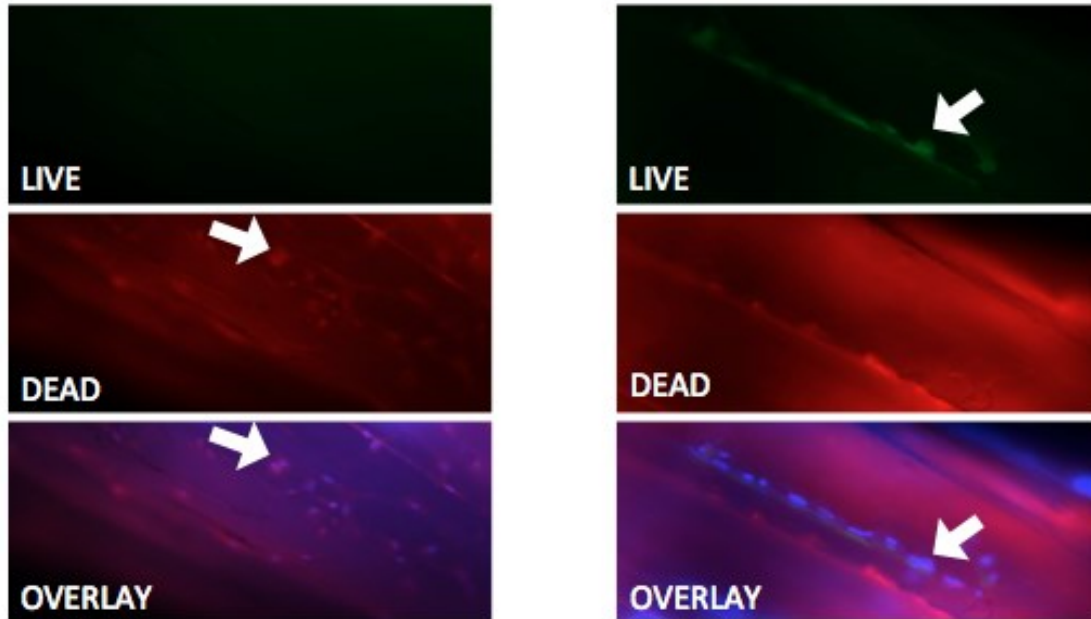


Figure 5.5: Images Taken During Phalloidin/Hoechst Staining:
Left Image: $t = 0$ thread (control); Right Image: $t = 3$ hours thread (experimental)

The green channel is Phalloidin, which stains the cytoplasm of cells. Hoechst is the blue channel and stains for cell nuclei. These two images, $t=0$ and $t=3$, show very similar cell density and morphology, suggesting that cells are not lost or altered during the loading process of our device.

We followed up with a live/dead assay to assess the viability and health of these cells. We produced two additional hMSC-seeded fibrin bundles. We loaded one of these bundles into our device for three hours before staining, while the other bundle was stained immediately. During staining, threads were cut into thirds. One third was used as a dead control, where cells were purposely killed with 70% ethyl alcohol. The other two sections were used as our experimental groups. Sections were stained as described on the protocol in Appendix E. Additional images of the Phalloidin/Hoechst stains are found in Appendix F.

Figure 5.6 shows Live/Dead images of time point $t=3$. The experimental image is shown on the right, with the corresponding dead control on the left.



**Figure 5.6: Images Taken During Live/Dead Staining (Live, Dead and Overlay Images):
Left Images – Dead Control (control); Right Images – Thread at t = 3 hours In Device (experimental)**

The cytoplasm of living cells are stained in green by calcein AM, while the nuclei of dead cells are marked red by ethidium homodimer-1. The Hoechst stain (blue) indicates where cells are present in the image.

Looking at the dead control on the left, there is no signal in the live channel. Meanwhile, there are many visible red dots in the dead (red) channel, indicating regions in which cells are dead. Looking at the overlay image on the bottom, these red dots line up with the Hoechst staining (shown by the white arrows), indicating that cells in our control are indeed dead.

Our experimental group on the right shows the presence of live staining. There is also an absence of red dots in the dead channel. Based on the overlay image at the bottom, the live (green) staining lines up with the Hoechst staining, suggesting that these cells are alive. The rest of our staining images can be found in Appendix F.

5.5 Contraction and Imaging Testing

The overall goal of this project was to load cardiomyocyte-seeded fibrin microthreads into our device and record their contraction using a laboratory microscope. We obtained four fibrin microthreads that were seeded with neonatal rat cardiomyocytes. These threads were incubated in media for three days as the group monitored them for spontaneous contraction. Although there was no noticeable detection of spontaneous contraction, the team successfully loaded the threads into our device. The threads were difficult to load because the three-day incubation period significantly weakened the mechanical properties of the threads. One thread was loaded successfully, though no contraction could be observed under a microscope with or without electrical stimulation provided by a Grass stimulator. This thread provided proof of principle that our threads could be loaded into our device. We were also able to obtain videos of the thread and the cantilever beam showing that we could acquire data if the threads were contracting.

Chapter 6: Discussion

6.1 Economics

Large-scale projects can have heavy impacts on the current market by revolutionizing the way an industry thinks and operates. Our project is still small scale, with our advisor being the main clientele. Since this device was developed for personal use in the Gaudette Lab, we do not foresee any direct economic impacts of our device. However, if this device is further developed and scaled-up in the future, it may influence the current market of drug screening methods.

6.2 Environmental Impact

Many projects for new products lead to environmental drawbacks based on materials and energy used. Our device was created to utilize materials and methods that are already present in labs to cut down on device cost and need for additional materials. For example, our device requires PDMS, a standard laboratory microscope, six-well plates, cell culture tools, cell media, and electrical stimulators, all of which are standard in most biological testing atmospheres. Additionally, the parts that our device requires for a lab to purchase are inexpensive and reusable, such as the molds for cantilever beams and anchor points, as well as the polycarbonate inserts. This way, few materials are wasted and our device can be used many times before additional purchasing is necessary. Overall, our device and testing procedures do not contribute to an adverse impact on the environment. If the philosophies applied by our project such as reusing already present laboratory methods and materials are applied to other testing systems, less harm can be done on the environment from creation of additional components, energy consumption, and unnecessary waste.

6.3 Social Influence

With the progression of our device out of the prototype stage and into full clinical application, this product has the capability of benefiting numerous people. While pharmaceutical companies will have a more effective method for analyzing the cardiotoxicity of a theoretical drug, reducing the likelihood of marketing a harmful drug, the most significant impact will be seen on patients. During procedures and patient monitoring, the specific application of a drug and its side effects will be completely understood to prevent patient harm. This device would lead to fewer instances of unnecessary harm, and hopefully lead to the saving of patient lives. As this screening method becomes more and more effective, companies and other countries would take notice and seek out this product.

6.4 Political Ramifications

In addition to the obvious health and environmental impacts of a new product, it is also important to consider the political and legal ramifications of this device. Our screening device has the potential to have a sizable impact on the legal protection of pharmaceutical companies. Specifically, because the device is designed to allow for the earlier and more accurate detection of cardiotoxic compounds in a pre-clinical environment, there is a reduced chance of these harmful drugs reaching the market. In turn, there is a reduced chance of consumers developing heart conditions due to cardiotoxic drugs; therefore pharmaceutical companies are less likely to be sued for negligence after the withdrawal of a marketed drug. This ultimately prevents drug companies from losing large sums of money, which is important to have for drug development research and clinical trials.

6.5 Ethical Concerns

With the creation of new testing methods, it is necessary to consider any ethical concerns that may provoke controversy surrounding the use of our device. After analysis of our device, the only possible ethical concern may be the use of stem cells. Throughout the years, with new biological testing methods and procedures, the controversy behind the use of stem cells has been in the forefront of the news. While our use of stem cells is solely for the use of modeling three-dimensional heart structure and preventing cardiotoxicity in pharmaceutical agents, it is possible for a person to argue that the use of stem cells is still wrong. We are aware of this concern, however we utilized methods that were readily available and we feel that these cells are the optimal representation of cells in this application.

6.6 Health and Safety

If our device proves reliable and becomes commonplace in industry to detect cardiotoxic effects of pharmaceuticals, we could significantly reduce the number of faulty drugs that make it to clinical trials and the market. This could potentially save many people from unknown negative side effects, thus contributing to the overall health and safety of the population. If *in vitro* cardiotoxicity screening becomes faster and more reliable, potential life-saving drugs could be brought to market more quickly, having a positive impact on human health.

6.7 Manufacturability

Manufacturability is important to the success of any device or product. Our device was carefully designed to promote easy manufacturing and reproduction of all components. Our polycarbonate insert is easily machined, and our posts can be quickly and reliably produced using provided molds. The insert and molds were produced with computer-aided manufacturing on CNC machines, allowing for easy scalability to mass production. Disposable six-well plates

are readily available and very inexpensive, and because we use a standard laboratory microscope to obtain images, no proprietary imaging equipment is required.

6.8 Sustainability

Our device serves as a model for sustainability. We have designed our device such that the polycarbonate insert is completely reusable. We utilize PDMS posts that are non-toxic and require little effort to produce. Therefore, the production of our device has minimal negative impact on the environment and serves as a very sustainable solution to a cardiotoxic drug-screening device.

6.9 Conclusions

The microthread loading and fixation testing was conducted to make sure that our device could successfully hold a contracting cell-seeded fiber. The PDMS slit was the main focus of this study because the seeded thread was wrapped around the cantilever beam. We discovered that the slit on the PDMS anchor point could hold up to 0.1 to 0.15 N of force before the slit would fail and the seeded thread would slip. From previous research and calculations, we expected that a single cardiomyocyte produced approximately 2.37 nN of force (Nishimura, 2004). Based on this value and the calculations presented in Chapter 4, Section 5, we determined that our device could successfully hold a cell-seeded fiber without slipping.

The second testing procedure focused on the sterilization of components of the device. It was necessary for the components and reusable materials to be successfully sterilized to confirm biocompatibility for the use with the seeded microthreads. The removable anchor points and cantilever beams were successfully sterilized using the ethylene oxide gassing process. These parts only needed to be sterilized once because they would be thrown away afterwards. Because the gassing caused only slight swelling that did not affect their ability to function or fit in the

polycarbonate insert, we were able to conclude that sterilization would not adversely harm these device components. The other component, the polycarbonate insert had its sterility capabilities tested using the ethylene oxide gassing process and through the use of an autoclave. Neither of these sterilizing procedures adversely affected the polycarbonate insert, even after repeated sterilizations. Therefore, we knew that our insert could be used repeatedly because it could be sterilized multiple times without changing its properties.

The presence and status of cells seeded on the fibrin microthreads in our device were analyzed through two cell staining assays. The first was a Phalloidin/Hoechst stain. This study compared a freshly seeded fiber to a cell-seeded fiber that had spent three hours in our device. Both threads showed similar cell density and morphology, indicating that they had similar properties. The successful comparison of these two time points allowed the group to conclude that the loading process of placing seeded threads in our device did not lead to significant cell loss. This also allowed the team to conclude that up to three hours in our device would not cause cell loss on the fibrin microthreads.

To analyze the health of the cells on the threads, a Live/Dead staining assay was conducted on a freshly cell-seeded microthread and a seeded microthread that had spent three hours in our device. The dead control showed numerous red dots for the dead panel indicating dead cells; while the overlay of the Hoechst stain confirmed that all of the present were indeed not alive. For the live experimental data, the live panel showed green color indicating that cells were alive. No red dots were seen in the dead panel, and in the overlay, the Hoechst stain matched up perfectly with the green color. This overlay indicated that all cells that were present were alive. These results showed that in comparison to the dead control, the thread that had spent three hours in our device had facilitated the cells residing on it and had not caused cell death.

Because of these cell-staining tests, we know that live cells can be maintained on the fibrin microthreads as they are suspended for periods of time in our device. In the future, we would like to conduct additional staining assays, in order to confirm the same quality for extended periods of time. However, for primary cell presence and quality testing, these results were promising.

The final testing was conducted on the ability to obtain contractile data using neonatal rat cardiomyocytes and our device. As stated in chapter five, this testing could not be concluded using the seeded threads that were provided. The main issue was that the seeded threads were immersed in media, in order to encourage some cell contraction before use. This immersion degraded the mechanical integrity of the seeded threads and when handled and loaded, these threads repeatedly broke. Before, when human mesenchymal stem cell seeded threads had been tested to solely observe if they could withstand the loading process, results had been encouraging. The seeded threads had been successfully loaded numerous times, so it seemed acceptable to assume that the neonatal rat cardiomyocytes seeded threads would have similar results. However, this was not the case and the team did not have the funds or time to test the neonatal rat cardiomyocytes again. Therefore, our team was not able to collect contractile data or video data. In the future, we would like to run additional tests using neonatal rat cardiomyocytes to obtain contractile data. Certain alterations would have to be conducted to produce these results, but based on our previous success and calculations, we expect our device can obtain this data.

Chapter 7: Final Design and Validation

7.1 Final Design Description

Our final design consists of three major components – a polycarbonate well plate insert, PDMS anchor points and cantilever beams, and cell-seeded fibrin microthreads. Together, these components can be used with a standard, disposable six well plate to quantify the force of contraction of the seeded cells on the fibrin microthreads. With the addition of pharmaceutical agents to the media, our device can be used to assess potential side effects of drugs on cardiac function.

7.1.1 Polycarbonate Well Plate Insert

Our polycarbonate well plate insert was designed to work with most standard, disposable six well plates. To construct the insert, 1/16” thick polycarbonate was purchased from Ultimate Plastics in Worcester, MA. The plate was then cut in the Higgins Laboratory Machine Shop at WPI with the assistance of a machine shop technician. To assist in the manufacturing, the following CAD model was developed, as seen in Figure 7.1.

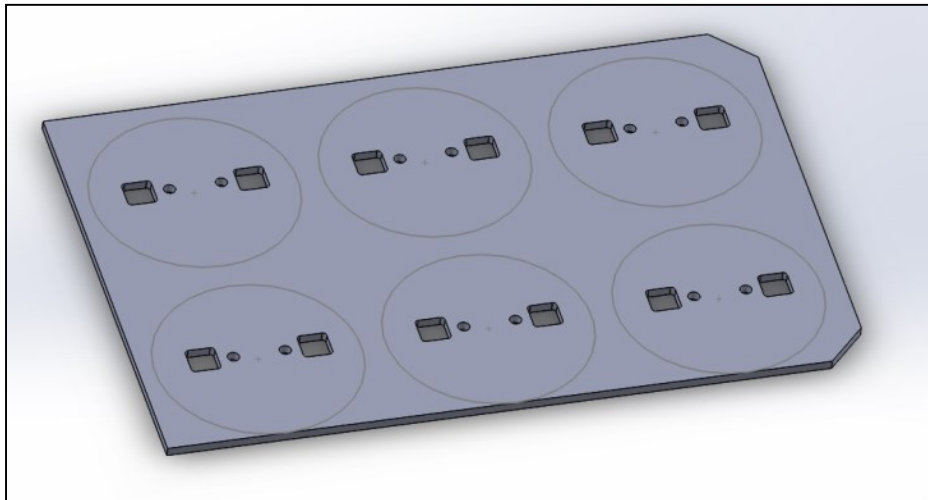


Figure 7.1: CAD model of polycarbonate well plate insert

As shown in the figure, the overall dimensions of the plate are 12.05 cm x 7.60 cm. The plate contains twelve 0.5 x 0.5 cm square holes, two in each well. These cuts are made for the insertion of PDMS anchor points and cantilever beams. There are also 0.2 cm diameter circular holes, made for the insertion of electrical wires for the stimulation of fibrin microthreads. The plate has two chamfered edges so that it can fit in multiple designs of standard six well plates. Computer Aided Manufacturing (CAM) software was used in conjunction with a computer numerical control (CNC) mill in order to make the cuts in the polycarbonate.

7.1.2 PDMS Anchor Points and Cantilever Beams

In order to suspend the fibrin microthreads in media within our device and be able to measure the contraction of the threads, we designed both PDMS anchor points and cantilever beams. PDMS is a soft, thermoset plastic, that is made by combining two liquid components into a mold in the desired shape. Using CAD software, we designed two aluminum molds for the purpose of creating the anchor points and cantilever beams, as seen in Figures 7.2 a, b, and c.

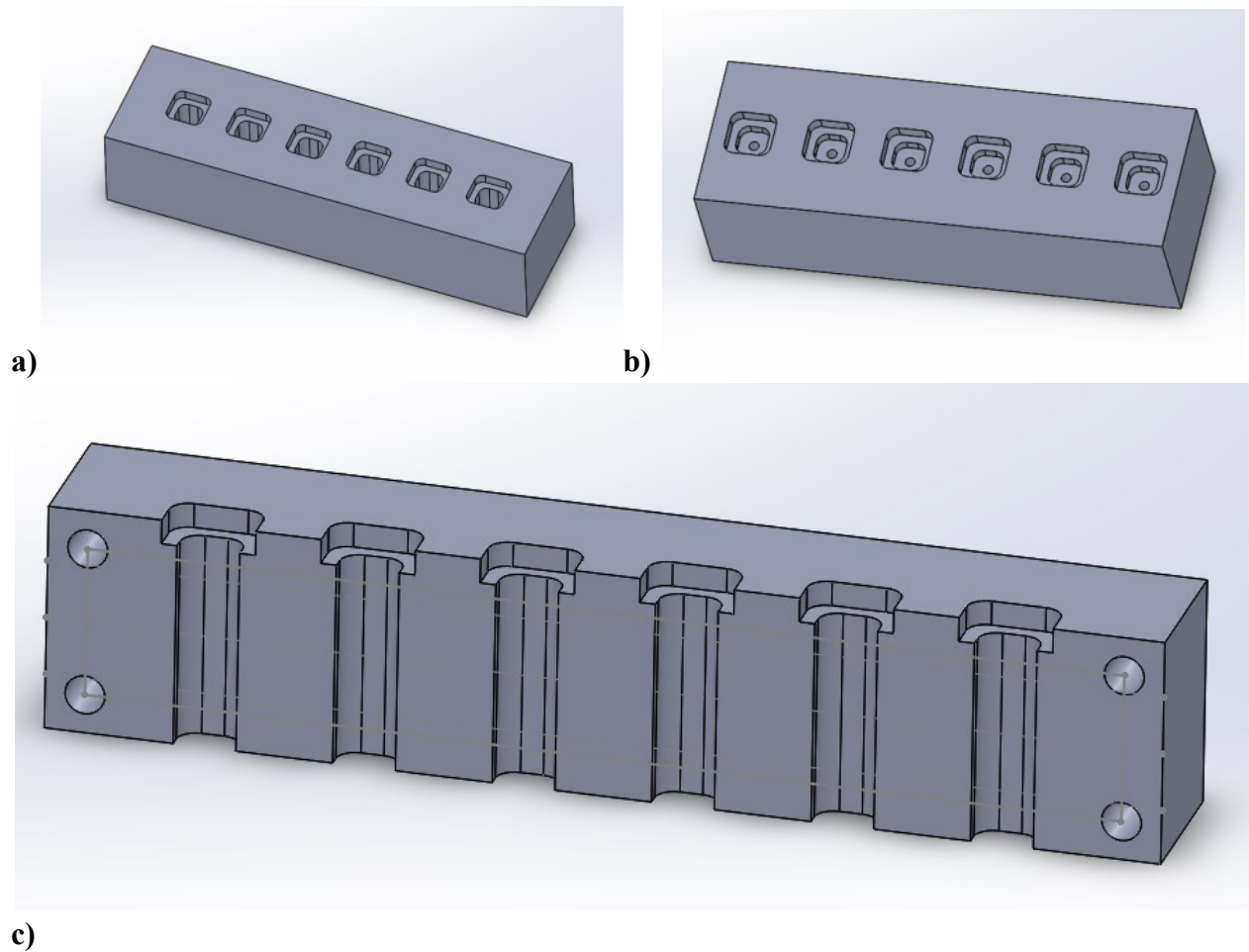


Figure 7.2: Complete molds for anchor points (a), cantilever beams (b), and half of the two-part anchor point mold (c)

As seen in Figure 7.2, each mold consists of six different wells for the insertion of PDMS so that one well plate insert can be completed with the preparation of one mold. Each mold is a two-part mold, as displayed in Figure 7.2c, so that the PDMS can be easily removed after it is poured. Each mold also has slits on the side in order to easily pull them apart to remove the PDMS. Figure 7.2a shows the anchor point mold. The total depth of the well is 1.85875 cm. The anchor point portion of the well measures 0.5 cm x 0.5 cm and is designed so that it protrudes 1.5 cm out from the polycarbonate well plate insert. In order to keep the anchor point from falling through the well plate, a thin layer was added to the top that measures 0.75 cm x 0.75 cm. Figure 7.2b shows the cantilever beam mold, which is designed with the same dimensions as the anchor

point mold, except that the cantilever beam portion of the mold is circular, with a diameter of .238 cm.

The molds were constructed out of aluminum. CAM software was used in conjunction with a CNC mill in WPI's Higgins Laboratory Machine Shop in order to make the proper cuts in the aluminum. Assistance was received from a trained machine shop technician. Directions for using the molds to create the PDMS anchor points and cantilever beams are found in Appendix E.

The entire assembly appears as below in Figure 7.3:

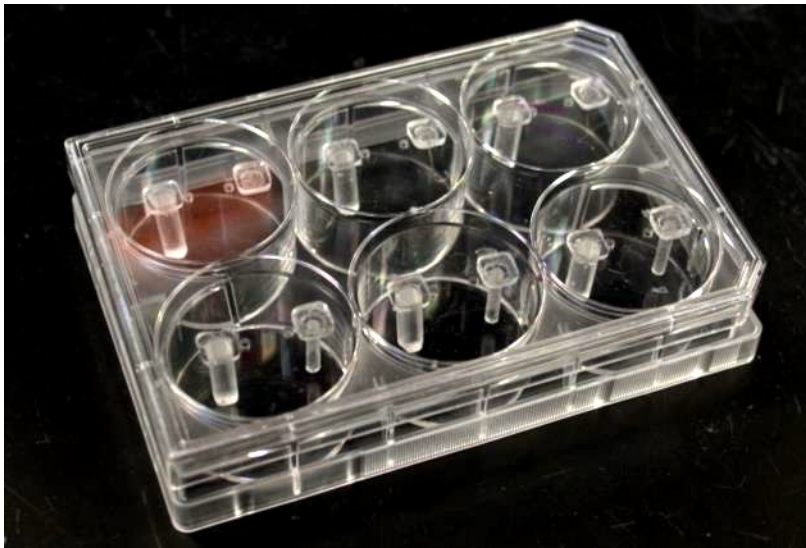


Figure 7.3: Completed assembly showing polycarbonate insert with PDMS anchor points and cantilever beams housed inside a standard, disposable six well plate

7.1.3 Cell-Seeded Fibrin Microthreads

Individual fibrin microthreads were provided by the Gaudette Lab and then bundled into groups of eight and cut to lengths of about 6-7 cm. To perform testing for our device, these microthreads were seeded with neonatal rat cardiomyocytes. The protocols for bundling and seeding the threads are found in Appendix E.

7.2 Loading of the Device

After the threads are seeded, they can be loaded into the device. It is important that the threads are loaded immediately after the seeding process is finished so that they maintain their mechanical properties during the process. The protocol for loading the loading process is also found in Appendix E.

7.3 Imaging of Contraction

Once the threads have been successfully loaded into the device, the assembly should be placed in a cell incubator. The threads will likely be ideal for imaging contracting about 48 hours after the seeding process is complete. Microthreads seeded with neonatal rat cardiomyocytes can contract spontaneously or with the aid of electrical stimulation. When the threads are ideal for contraction, they can be imaged using a standard laboratory microscope. The protocol for this process is found in Appendix E.

Chapter 8: Conclusions and Recommendations

Our device shows great promise in providing a method to quickly, reliably, and easily evaluate the contractile force and rate of cardiac cell-seeded fibers *in vitro*. Through validation testing, we have demonstrated our device's ability to reliably hold fibrin bundles without slipping or breaking, maintain cell viability for at least three hours, and allow for the capture of very high quality images and video. Although we were not able to conduct contractile tests using cardiac cell-seeded fibers in our device, our research and calculations suggest that the PDMS cantilever beams will deflect a distance that is easily detectable by a microscope. Composed primarily of polycarbonate and PDMS, our device is very cost effective, and all materials can be easily replaced or reproduced. Additionally, the design minimizes external costs by being compatible with standard disposable six-well plates and by reducing the amount of media required in each well. Finally, our device is highly user-friendly because the polycarbonate insert exhibits a unique inverted design that is removable from the six-well plate, allowing the user more room to easily secure the cell-seeded threads in the attachment system.

While we were successful in developing a proof-of-concept device that holds much promise in being used as a drug-screening platform, we were unable to definitively validate this proof of concept due to time and financial constraints. We recommend additional tests and design improvements before the device may be used in a pre-clinical environment. First, developments must be made toward improving the integrity and contractile strength of the cardiomyocyte-seeded fibrin bundle constructs. Further biocompatibility tests should be conducted to quantify and analyze the morphology, density, and uniformity of cells seeded on the fibrin bundles so that improvements in the fibrin bundle cell seeding process can be made. Additionally, electrical stimulation of cardiomyocyte-seeded fibers within our device should be performed to pace the

cells on the threads to promote a strong, uniform contraction of the bundle suitable for evaluation and testing. Once the attachment and qualitative evaluation of contracting cardiac cell-seeded fibers is achieved using our design, we suggest the implementation of software to be used in conjunction with our device in order to present a quantitative output of contractile rate and force exhibited by the fibers. Upon future testing of various pharmaceutical agents with our device, a numerical and graphical representation of contractile data in comparison with control data would ensure the quick and easy identification of potentially harmful compounds on the heart.

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Glossary

Bundle – A group of 4-12 fibrin microthreads that are twisted together.

Cantilever Beam – A structure that is anchored only at one end. A tissue can be attached to a cantilever beam in order to calculate exerted force by monitoring displacement.

Cardiotoxicity – The occurrence of cardiac physiological or muscular damage due to medication.

Fibrin Microthreads – WPI patented extruded protein fiber that is created using fibrinogen and thrombin. These threads are used in cell testing because they replicate a three-dimensional structure. This application is especially valuable in the field of cardiac testing.

hMSCs – A type of cells commonly used in cell testing because they exhibit properties of cells present in the human body.

Limit Switch – An off and on switch used in circuitry to implement a stop condition for evaluating an electrical signal.

PDMS – Polydimethylsiloxane (PDMS) is a silicone-based rubber that is created by mixing an elastomer base and curing agent. It is commonly found in laboratories, which is used for cellular testing because of its known biocompatibility.

Polycarbonate – A thermoplastic polymer that is durable with low-scratch resistance and is easily workable. Polycarbonate was the material selected for the insert in our device.

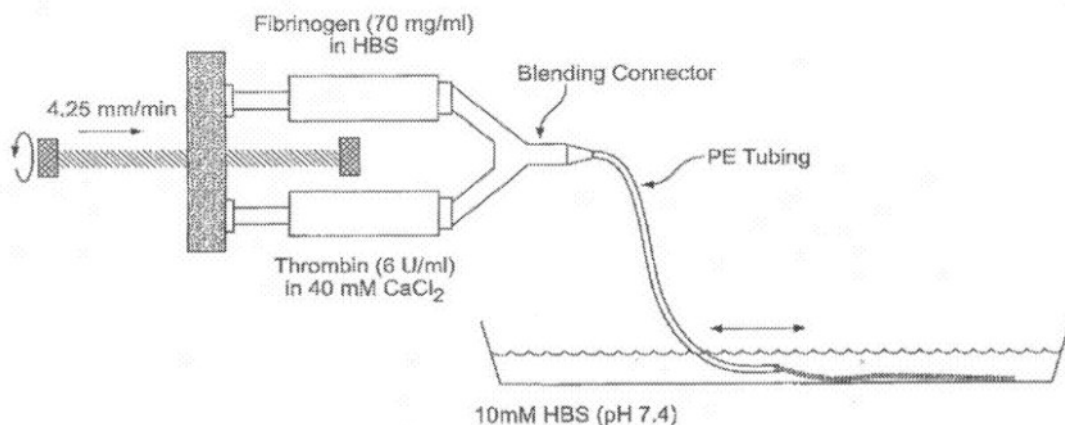
Suture – A fibrin microthread bundle that is folded through a surgical needle for use in cardiac cell therapy.

Transducer – A sensor used to compute energy and output a resulting measure. In this project, a force transducer was considered as a method for measuring contractile force of the seeded fibrin microthread.

Appendix A: Microthread Extrusion and Seeding Procedure

THREAD EXTRUSION AND BUNDLING

1. Combine 70mg/mL fibrinogen in HEPES-buffered saline (HBS) and 6U/mL thrombin in 40mM CaCl₂ using blending connector (figure 1).
2. Extrude protein blend at a constant rate of 4.25mm/min through small diameter polyethylene tubing into shallow 10mM HBS bath (pH 7.4).

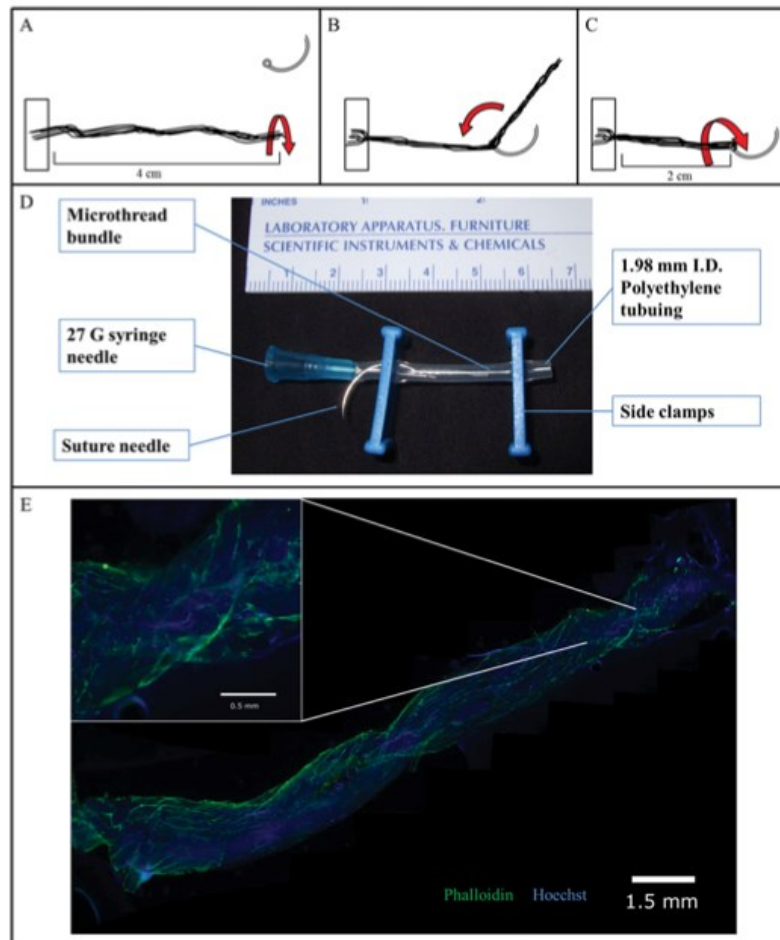


3. Remove extruded threads from HBS bath and stretch across hollow cardboard box or equivalent. Allow to air-dry overnight.
4. Rehydrate threads and twist together to form bundles. Bundles vary between four to twenty-four, with eight to ten being most common.
5. Stretch bundles and allow to air-dry overnight.
6. Store in desiccators until needed.

CELL SEEDING

1. Using surgical scissors, cut bundle into desired length (typically 4cm).
2. Place thread inside small diameter polyethylene tubing (i.d. ~2mm). Clamp off both ends of tube.
3. Using a syringe, inject media containing cells through one end of the tube, filling the tube and completely submerging the fibrin bundle.
4. Place setup in a 50mL conical tube.

5. Load conical tube in rotator and rotate on a 15 degree incline at 8rpm for 1-2 days.

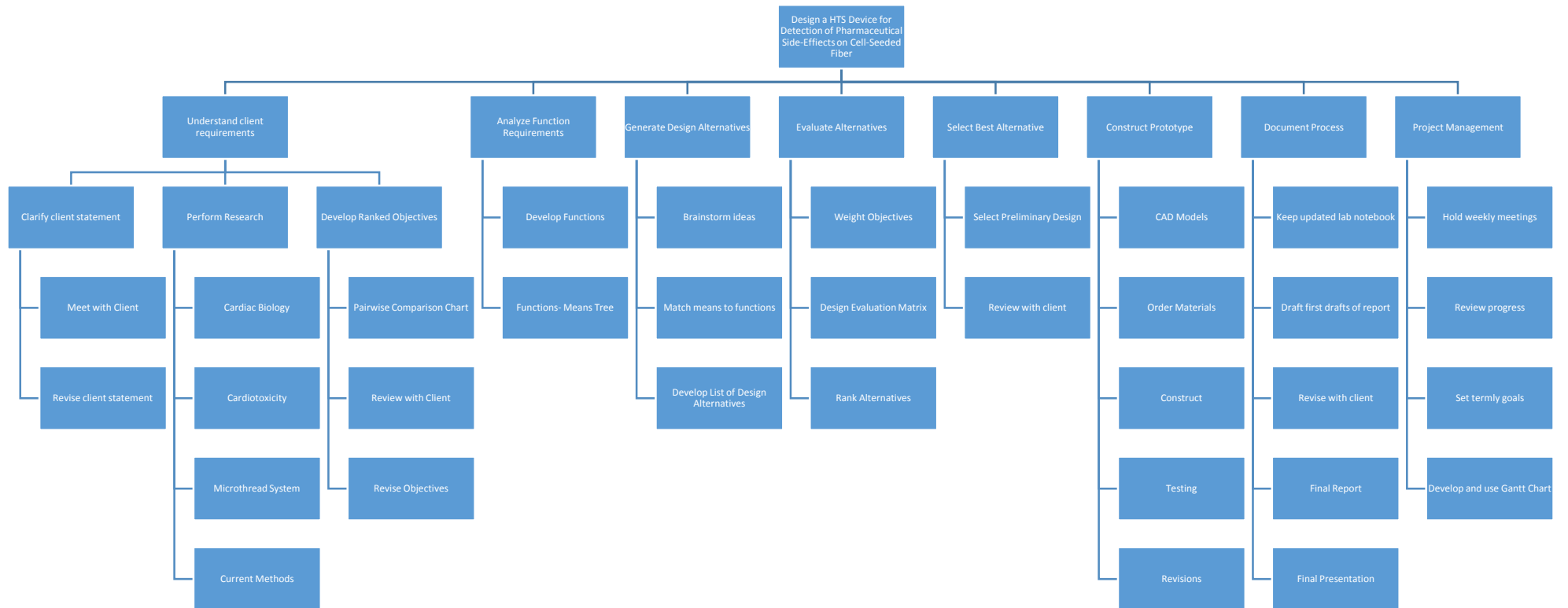


Cornwell, K., Pins, G., Billiar, K. "Collagen and Fibrin Microthreads In A Discrete Thread Model of In Vitro ACL Scaffold Regeneration". Duane Morris LLP - *Patent US2011/0034388 A1*. (Feb 10, 2011).

Appendix B: Pairwise Comparison Chart

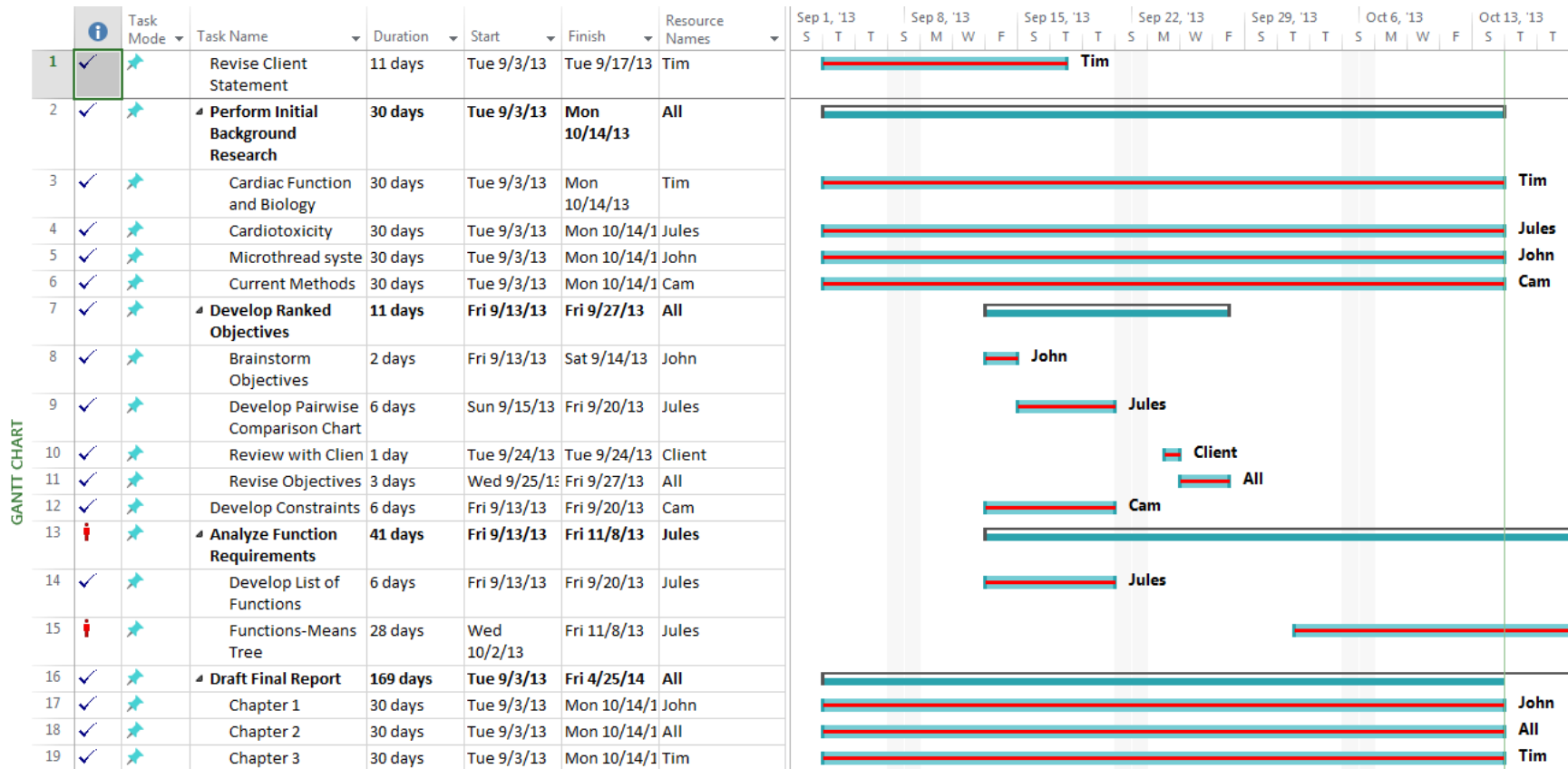
PRIMARY OBJECTIVE	Biocompatible	HTS	Safe for User	Easy to Use	Cost Effective	Total
Biocompatible		0	1	1	1	3
HTS	1		1	1	1	4
Safe for User	0	0		0	1	1
Easy to Use	0	0	1		1	2
Cost Effective	0	0	0	0		0

Appendix C: Work Breakdown Structure

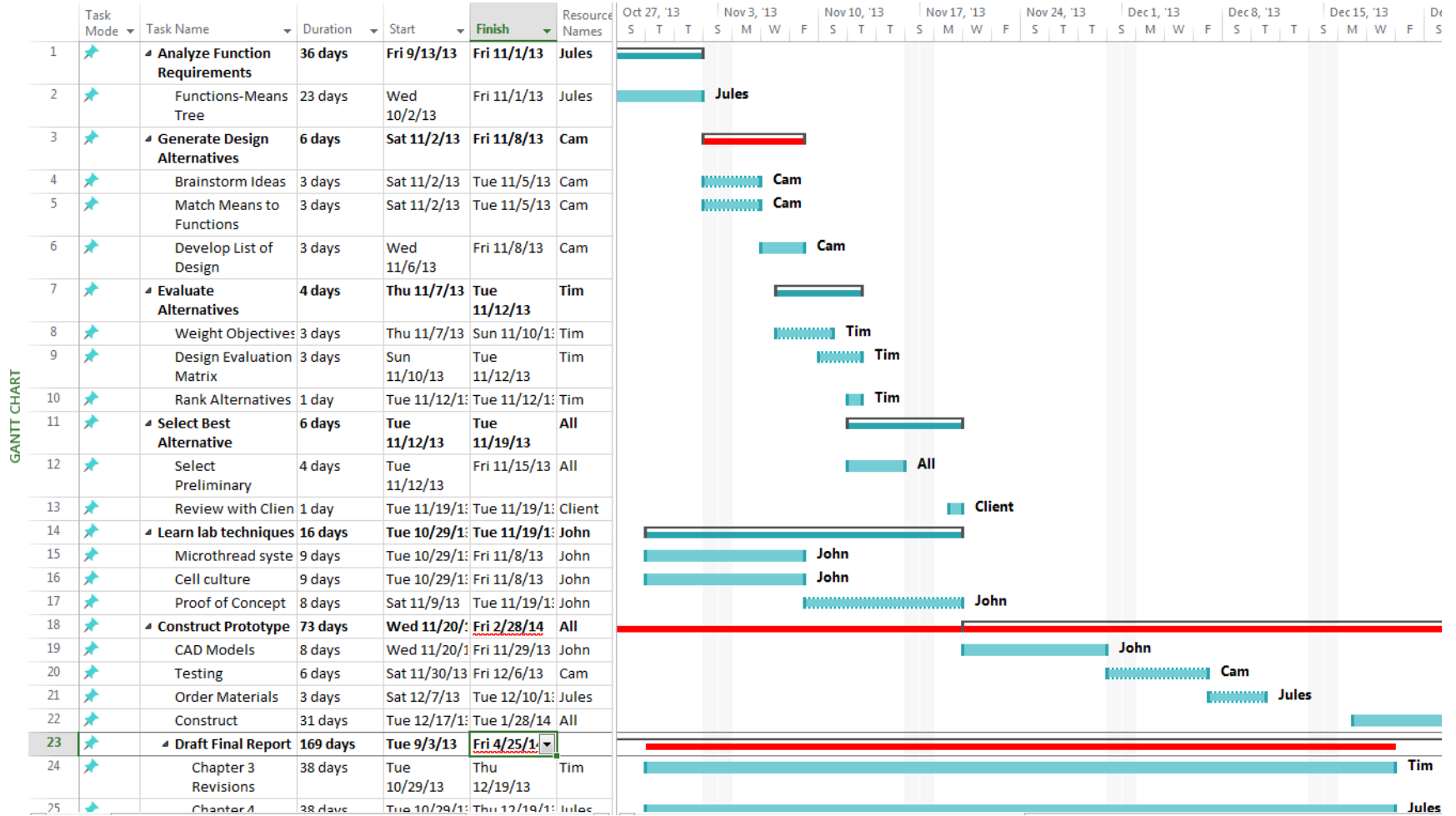


Appendix D: Quarterly Gantt Charts

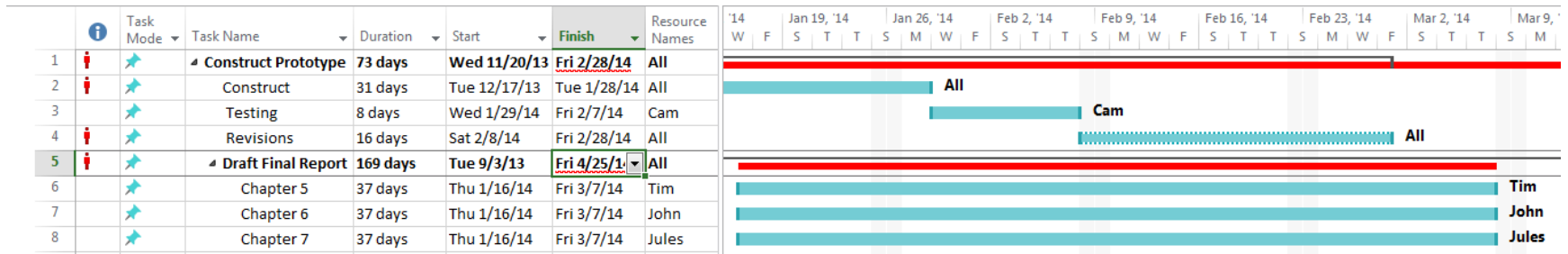
First Quarter



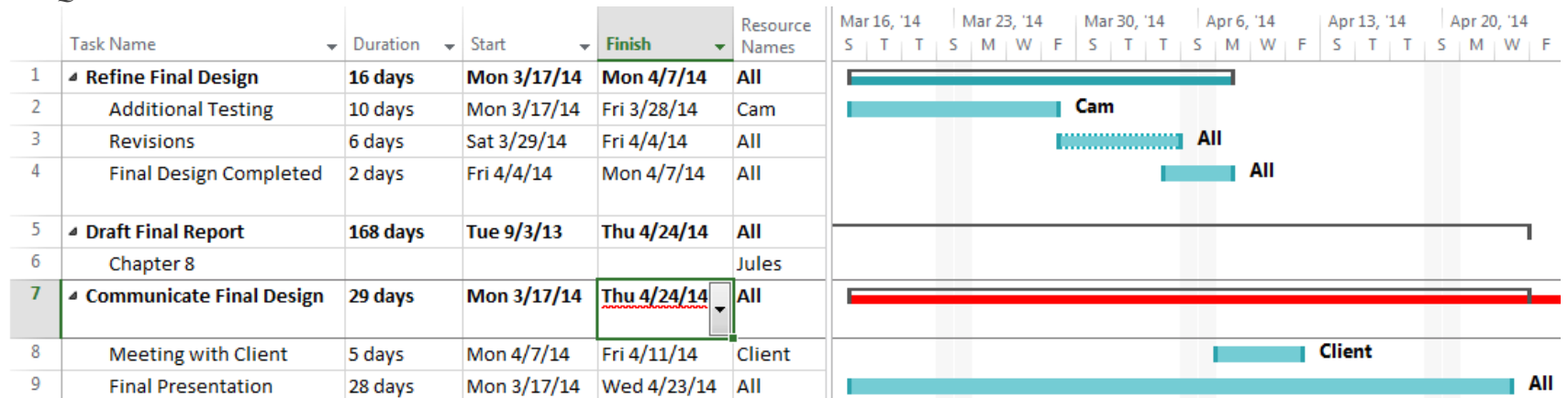
Second Quarter



Third Quarter



Fourth Quarter



Appendix E: Laboratory Protocols

Protocol for Seeding Fibrin Microthreads

Materials:

- Sterile thread-bundle in Bioreactor
- Sterile PBS
- Sterile 1 mL syringe (3)
- Cell Suspension (100,000 cells/100 μ L)
- 50 mL conical tube
- Tube rotator (fully charged!)

Procedure:

1. Use a sterile syringe to inject sterile PBS into bioreactor (by attaching syringe to the already inserted 27G needle).
2. Ensure all bubbles are eliminated from the bioreactor.
3. Attach slide clamp and remove/discard syringe. (Keep syringe needle in the bioreactor).
4. Allow 20 min for hydration
5. While bundle hydrates prepare cell suspension according to cell passaging protocol
6. Use new syringe to expel all sterile PBS from the bioreactor before seeding. For this, remove the slide clamp at the end opposite to the needle, draw air into a new sterile syringe and push the air into the bioreactor to expel all the PBS.
7. Use a new syringe (1 cc maximum) to inject cell suspension (100,000 cells / 100 μ L) into the bioreactor. For this, hold the bioreactor such that the open end of the bioreactor (one without slide clamp) remains elevated such that the cell suspension doesn't spill out while being injected. After 100 μ L of cell suspension is injected into the bioreactor, close the end opposite the needle by sliding the clamp onto the tubing.
8. After injecting the cell suspension and adding the slide clamp, remove the 27G needle from the bioreactor.
9. Place the bioreactor into a gas permeable 50 mL conical tube.
10. Place the bioreactor into the MACSmix tube rotator and rotate at 4 RPM (lowest setting) for 24 hours.

Protocol for Phalloidin/Hoechst Staining

Reagents:

- Phosphate Buffered Saline
- 4% Paraformaldehyde (Only needed for tissues/cells that have not been fixed);
- 0.25% Triton-X
 - 0.25% V/V Triton-X in PBS
 - 10 μ L Triton-X in 3990 μ L PBS
- 1% BSA
 - 1% V (W)/V BSA in PBS
 - 40 μ L in 3960 μ L PBS
- Phalloidin (AF 488 Phalloidin A12379, Invitrogen)
 - 2.5% V/V Phalloidin in PBS
 - 50 μ L in 1950 μ L
- Hoechst
 - 0.0167% Hoechst dye in PBS
 - 0.5 μ L in 3000 μ L PBS

For unfixed sections/cells:

1. Rinse in PBS x2
2. Fix in 4% Paraformaldehyde for 10 minutes
3. Follow directions for fixed sections

For fixed sections/cells:

1. Rinse with PBS x2
2. Triton-X solution for 10 minutes
3. Rinse with PBS x2
4. Block with BSA solution for 30 minutes
5. Phalloidin solution for 30 minutes
6. Rinse with PBS x2
7. Hoechst solution for 3-5 minutes (typically 3)
8. Rinse with PBS x2
9. Cytoseal and coverslip
10. Store frozen at -20 degrees C.

Results:

- F-actin is stained green
- Nucleus is stained Blue

Protocol for Live/Dead Assay

Solutions:

Solution 1:

1.0 mL Serum Free DMEM
1.0 μ L Ethidium Homodimer-1
1.0 μ L Calcein AM

Solution 2:

1.0 mL Serum Free DMEM
1.0 μ L Ethidium Homodimer-1
1.0 μ L Calcein AM
0.5 μ L Hoechst Dye

Concentrations:

300 μ L/thread

Process:

Incubate dead controls in 70% Ethanol for 30 minutes prior to experiment
Mix solution 1 within 1 hour of use
Incubate cells with solution 1 for 15 minutes
Mix solution 2 within 1 hour of use
Incubate cells in solution 2 for 15 minutes
Wash cells with 1x PBS 6 times
Fix cells in 4% Phosphate buffered formaldehyde for 10 minutes
Mount and coverslip cells on an uncharged microscope slide

Protocol for PDMS anchor points and cantilever beams to prepare well plate insert

Materials:

1. Sylgard Silicone Elastomer base (Ellsworth Adhesive #184 SYL ELAST)
2. Sylgard Silicone Elastomer curing agent (Ellsworth Adhesive #184 SYL ELAST)
3. Gloves
4. Spatula
5. Weigh dish
6. Scale
7. PDMS molds – for anchor points and cantilever beams
8. Clamps
9. Oven
10. Teasing needle w/ wooden handle
11. Well plate insert

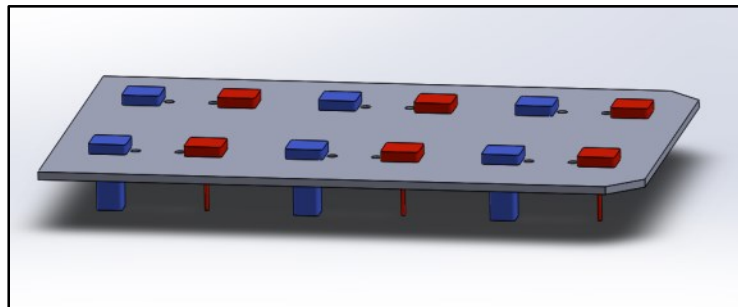
Methods:

1. Before mixing the PDMS, use two clamps on each mold to ensure that the pieces of the mold are tightly held together and the PDMS will not leak out, as shown in the picture below:



2. Mixing and Pouring PDMS
 - a. Wearing gloves, weigh 8 ounces Sylgard silicone elastomer base and 0.8 ounces Sylgard silicone elastomer curing agent. Note: do not mix the stock solutions!!! Use separate weighing materials for each reagent.
 - b. Pour reagents together and thoroughly mix the elastomer base and curing agent with a spatula.

- c. Using the spatula, pour the well-mixed solution into each of the two molds. Fill each mold to just below the top, as the PDMS expands upon heating.
 - d. Degas the PDMS by putting it into a vacuum chamber for 1 hour.
 - e. After degassing, visually inspect the PDMS to ensure that there are no more bubbles. If there are, repeat steps 2d and 2e.
 - f. Cure the PDMS by placing the molds into an oven set to 60°C for 1 hour on a sheet of aluminum foil.
 - g. Remove the molds from the oven being careful to only touch the aluminum foil.
 - h. Let the molds sit for 24 hours.
3. Removing the PDMS from the molds
- a. Examine the top of the mold. If PDMS has dried on the top layer of the mold, use a teasing needle to gently remove the excess PDMS.
 - b. Use a flat head screwdriver to pull apart the two molds by applying pressure to the notch cut out on each side of the top of the mold.
 - c. Remove each anchor point or cantilever beam individually.
 - d. Clear off any extra thin film of PDMS by gently removing it with your hands or with the assistance of a teasing needle.
4. Insert each PDMS piece into the corresponding opening in the well plate insert, as seen below. Each anchor point and cantilever beam should fit securely within its corresponding hole in the polycarbonate insert.



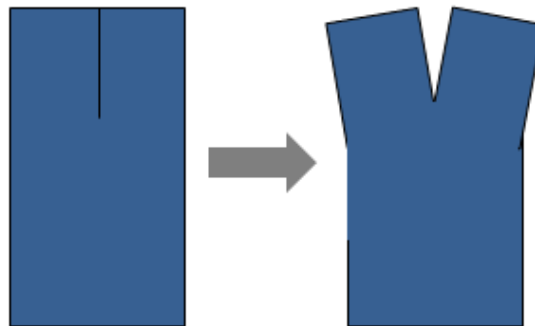
Protocol for Loading Microthreads in the Device

Materials:

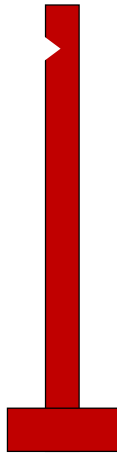
1. Polycarbonate insert with pre-loaded PDMS anchor points and cantilever beams
2. Packaged, disposable six well plate
3. Razor blade
4. Cell-seeded fibrin microthreads in media
5. Fine-tipped forceps (2)
6. Gloves
7. Ruler
8. Marker
9. Eye dropper
10. Media
11. Automatic pipette

Methods:

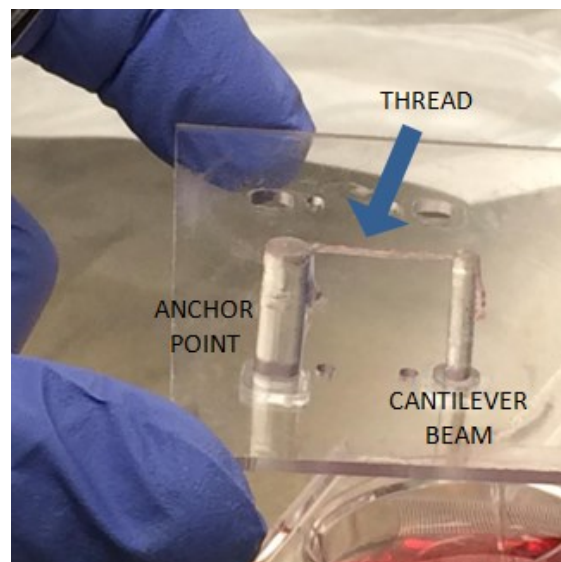
1. Prior to the loading process, ensure that all materials are sterile and that all steps of the following procedure are completed in a sterile fume hood. The insert and PDMS should be sterilized with ethylene oxide gas. All other materials can be sterilized with 70% ethyl alcohol before being placed in the fume hood.
2. Remove the disposable six well plate from the package and fill each well with 5 mL of media with the use of the automatic pipette. Replace the lid upon completion.
3. Using a ruler and marker, draw a line 0.1 cm from the top of both the PDMS anchor point and cantilever beam.
4. Using a razor blade, cut a slit down the middle of the top face of the anchor point, as shown below, to the 0.1 cm depth identified by the marker line.



5. Again using the razor blade, cut a horizontal notch at the marker line in the cantilever beam, as shown here.



6. Repeat steps 2-4 for each necessary pair of anchor points and cantilever beams.
7. Place the polycarbonate insert in its inverted orientation so that the anchor points and cantilever beams can be easily accessed.
8. Remove a fibrin microthread from a petri dish by grasping it at one end with the forceps.
9. Using both pairs of forceps, insert this end of the microthread into the vertical slit in the anchor point with a careful, flossing action.
10. Grasp the other end of the thread and wrap it around the cantilver beam so that the middle of the thread rests in the horizontal slit.
11. Repeat step 7 with the other end of the thread.
12. If the microthread is not taut, use the forceps to pull each end of the thread through the anchor point until it is tight, while ensuring the cantilver beam is still perfectly upright. A correctly loaded microthread should appear as below:



13. Repeat steps 7-11 for the necessary amount of threads. (Media may need to be periodically added to the loaded threads to prevent them from drying out. This can be done with the eyedropper and the excess media from the petri dish.)
14. Once all loading is complete, remove the lid of the six well plate and place the insert on the plate so that the microthreads are immersed in media. Replace the lid of the six well plate.

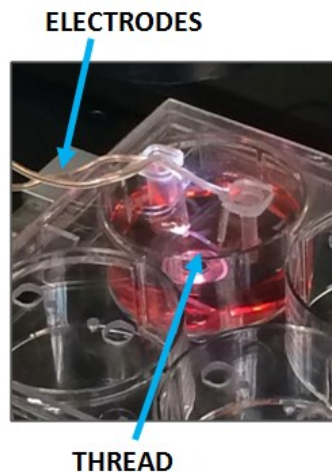
Protocol for Imaging the Microthreads in the Device

Materials:

1. Well plate and insert assembly with loaded microthreads
2. Laboratory microscope with video imaging capabilities
3. Grass stimulator
4. Computer

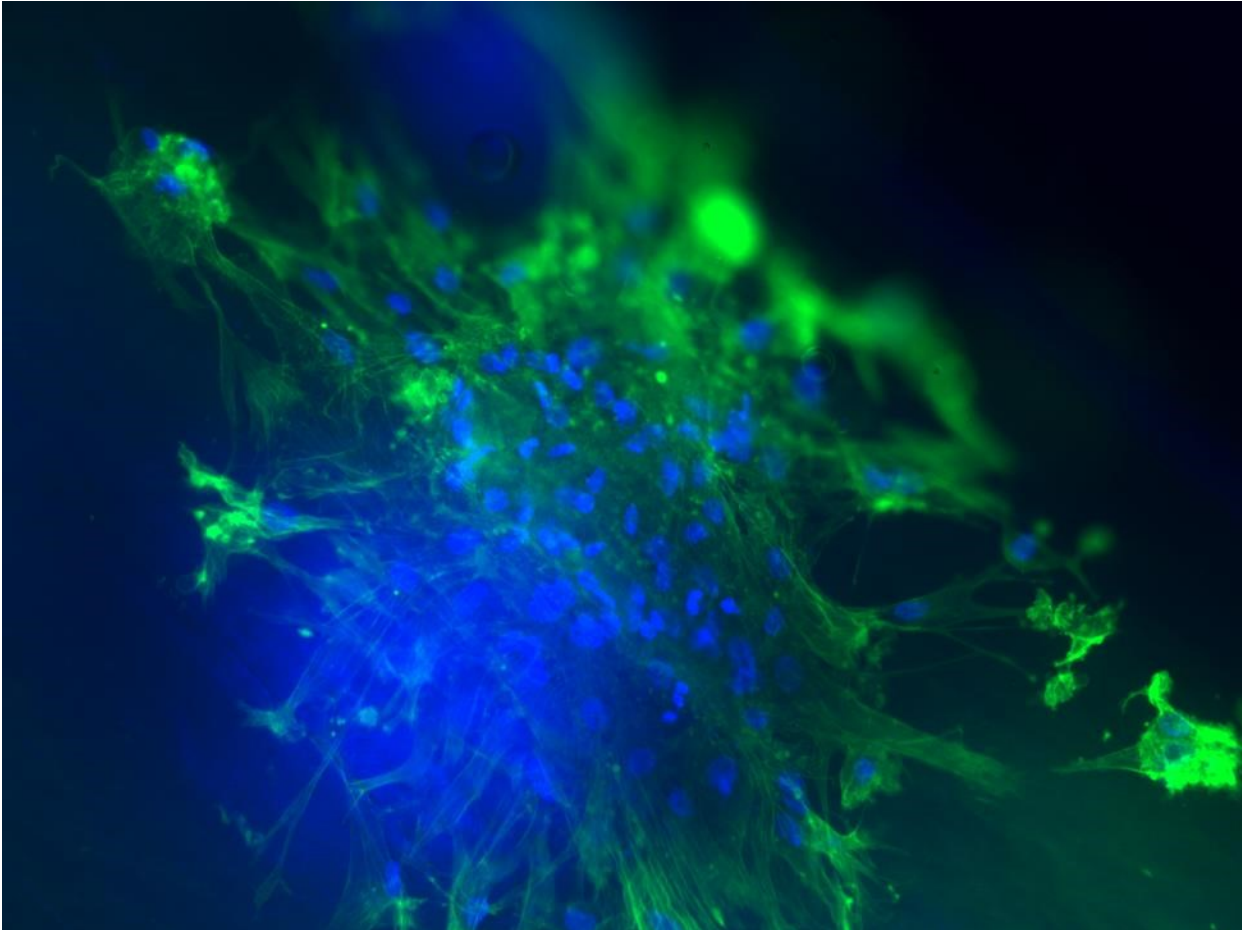
Methods:

1. Place the six well plate with the contained loaded microthreads on the microscope platform so that the appropriate well is seen in the scope.
2. If imaging the thread itself, travel along the length of the thread with the scope until a section of contracting fiber is visible. This contraction can be recorded with the use of a Fastec HiSpec4 4G camera and Matlab script to obtain AVI files.
3. To obtain quantifiable contraction data, image the cantilever beam by moving the well plate until the cantilever beam is in view. Record a video of the deflecting cantilever beam using a Fastec HiSpec4 4G camera and Matlab script to obtain AVI files. The deflection can be used to calculate the force of the microthread as described in Chapter 5.
4. For electrical stimulation of the microthreads:
 - a. Remove the lid of the six well plate.
 - b. Insert an electrode into each of the two circular holes in the polycarbonate insert so that an electrode touches each end of the loaded microthread.
 - c. Replace the lid on top of the six well plate.
 - d. Adjust the settings on the Grass stimulator to obtain the desired voltage, frequency, etc.
 - e. Image the thread and cantilever beam as described in steps 2 and 3. The correct set up can be seen in the image below:

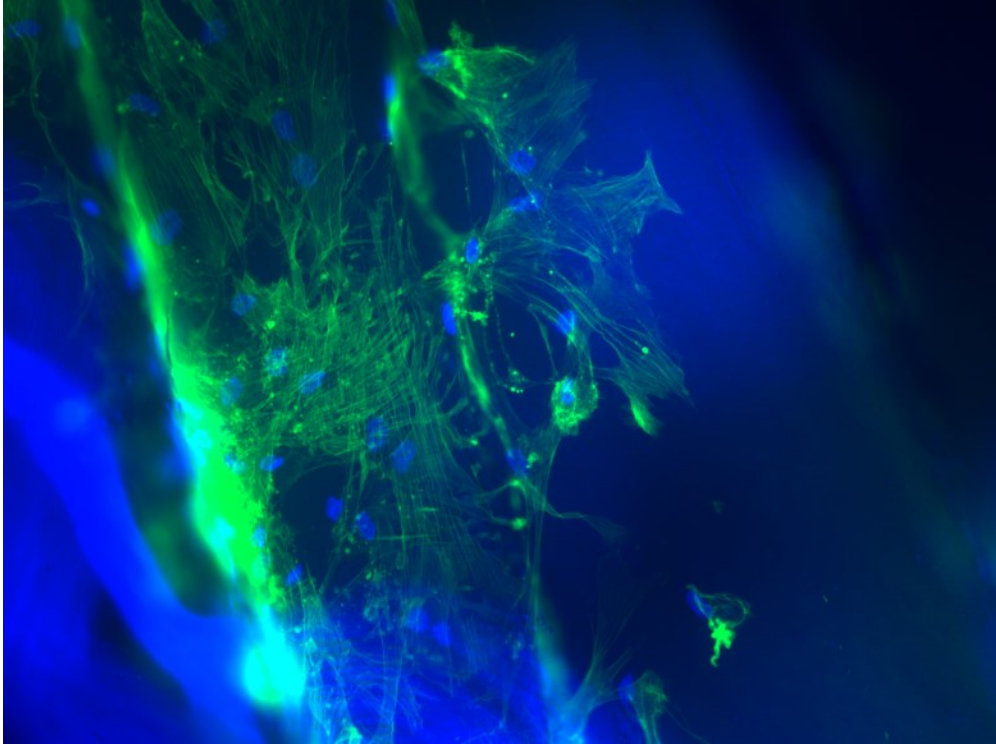


Appendix F: Staining Images

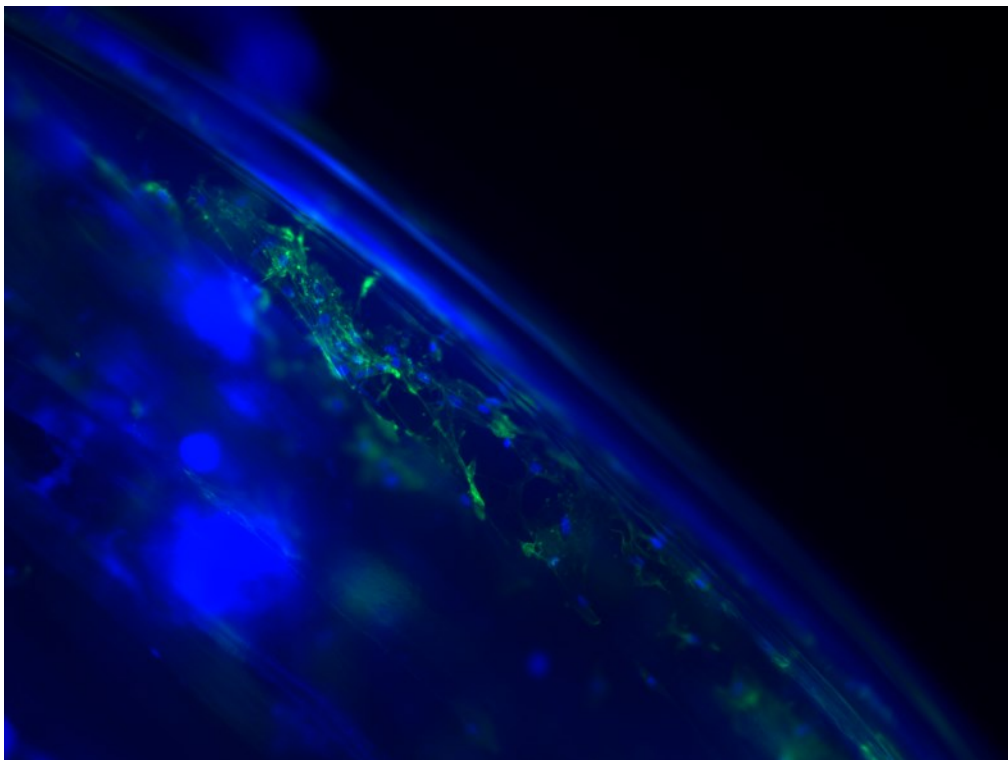
Phalloidin/Hoechst Stains



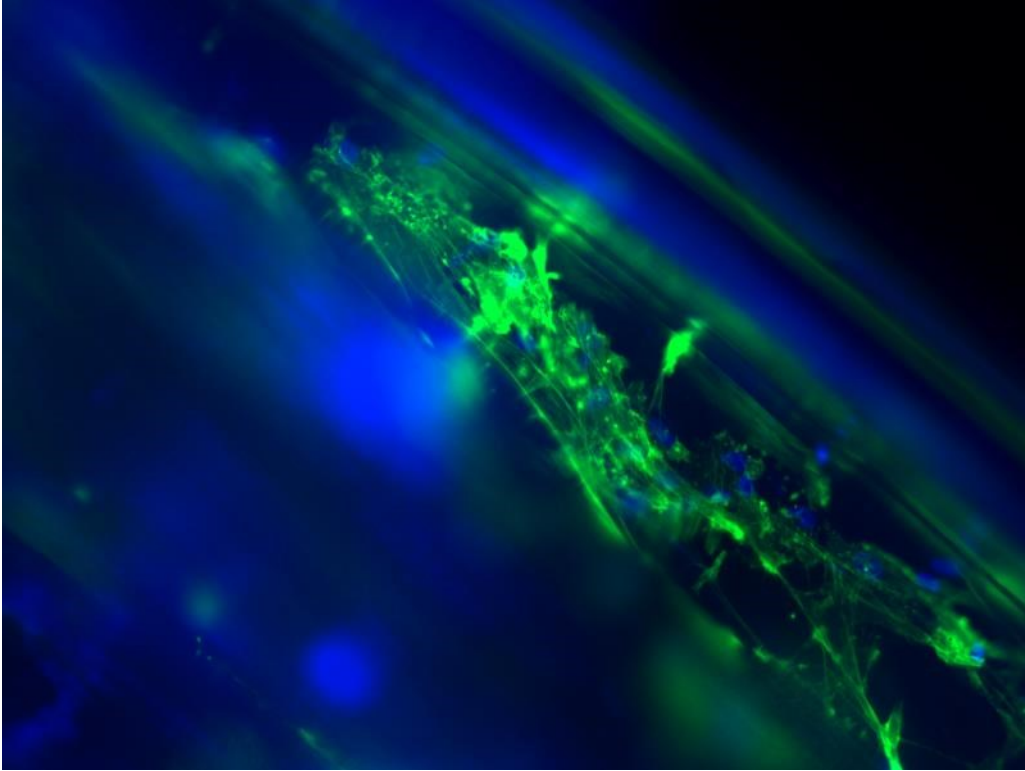
Time = 0 (10X)



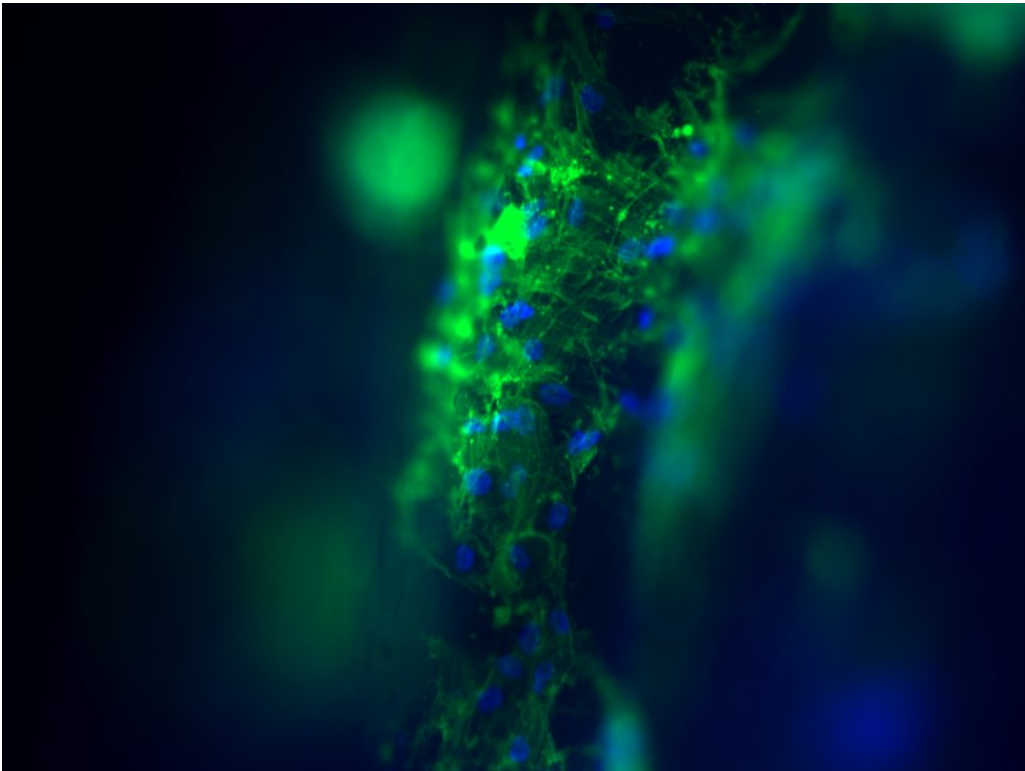
Time = 0 (20X)



Time = 3 hours (10X)



Time = 3 hours (20X)

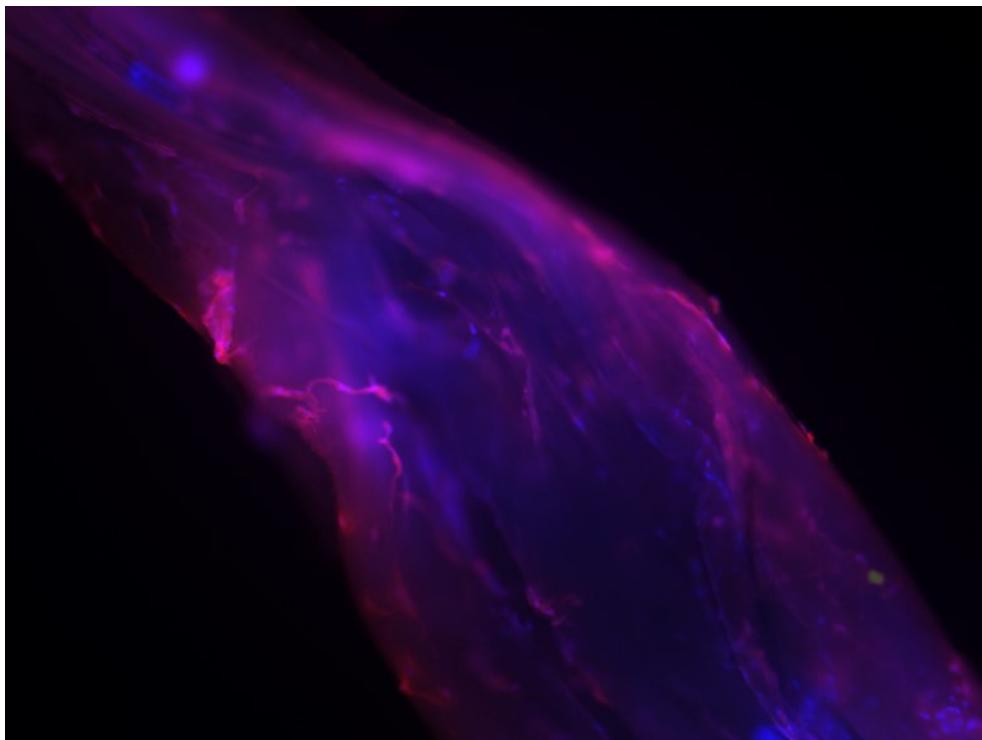


Time = 3 hours (20X)

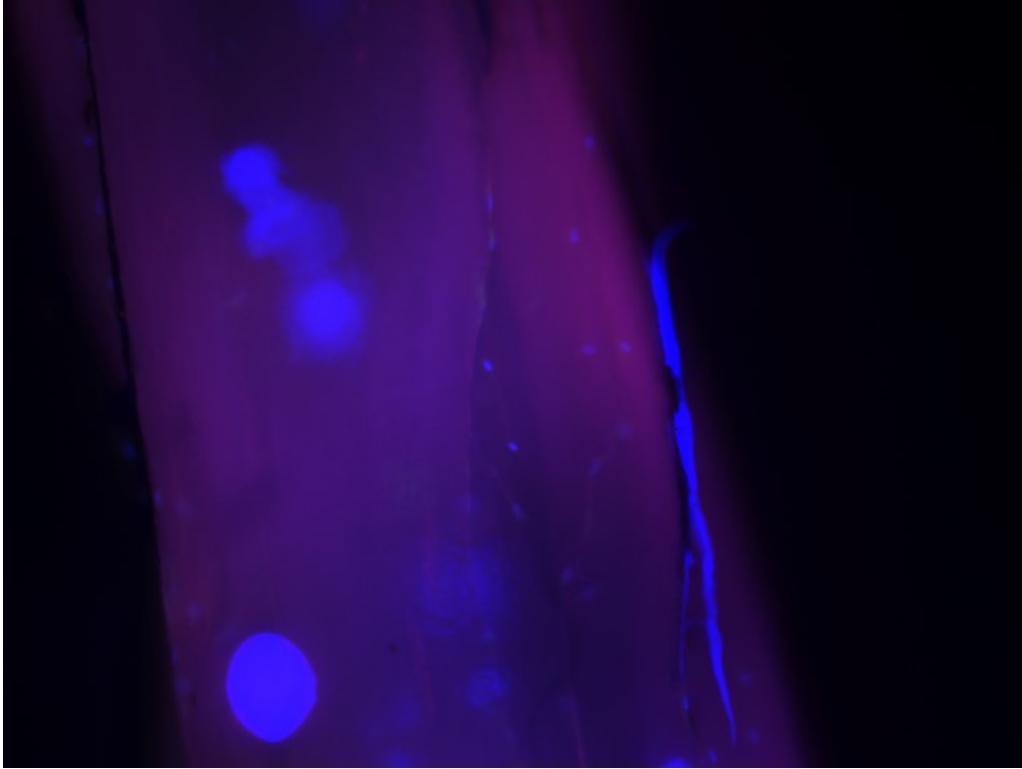
Live/Dead Assay



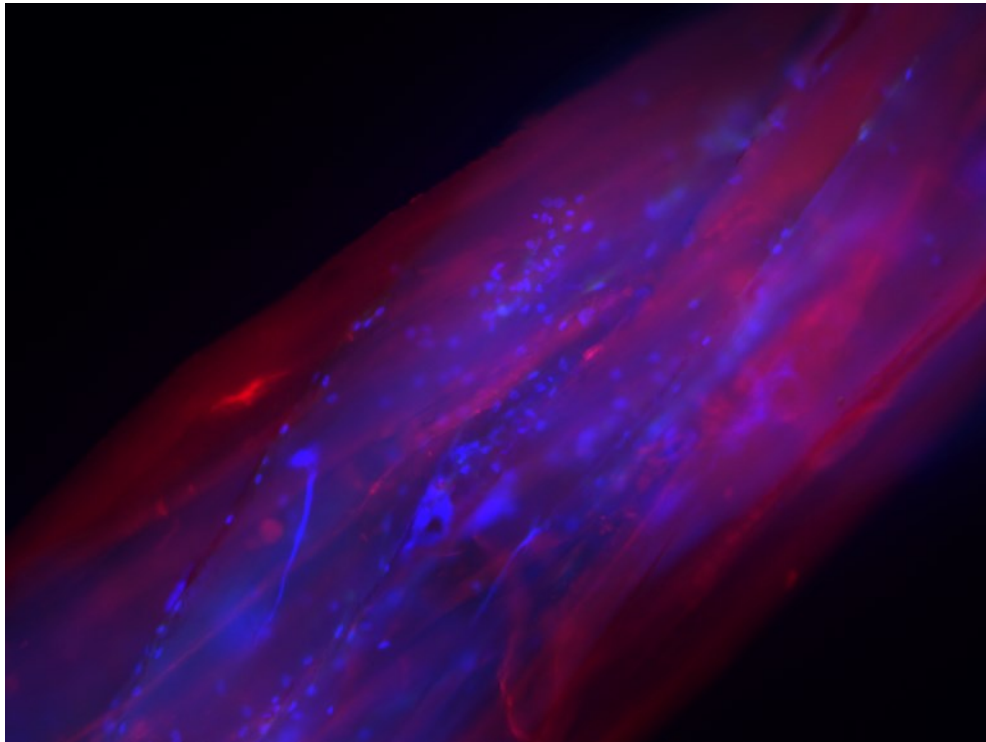
Time = 0 (Dead Control, 10X)



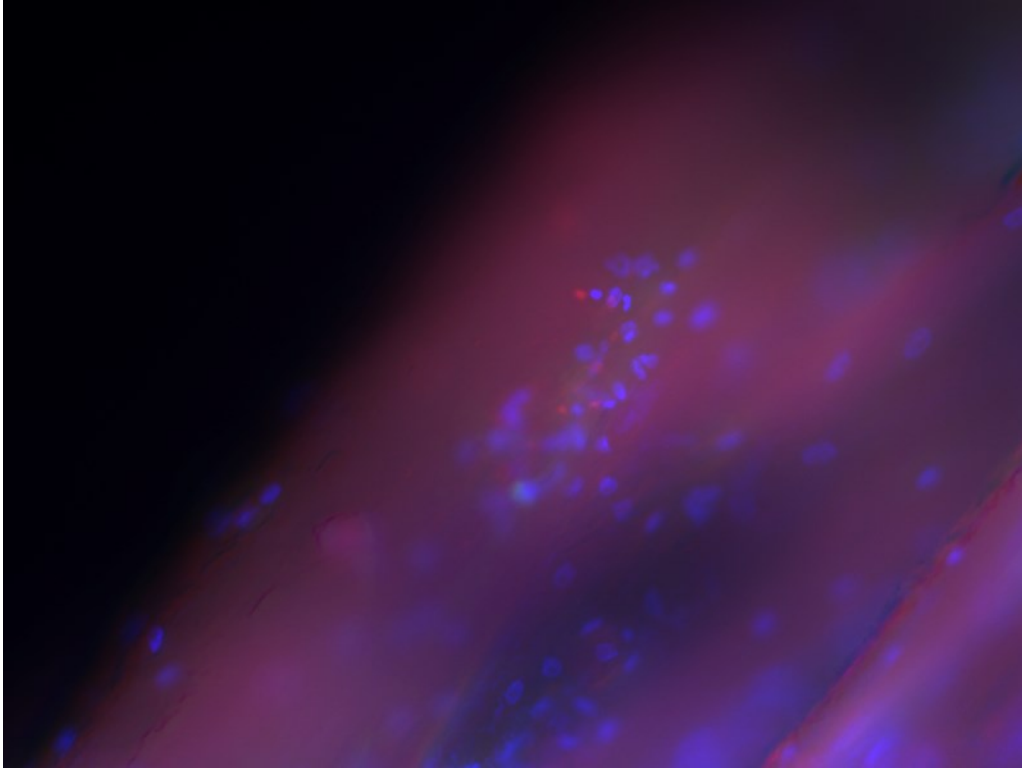
Time = 3 hours (Dead Control, 10X)



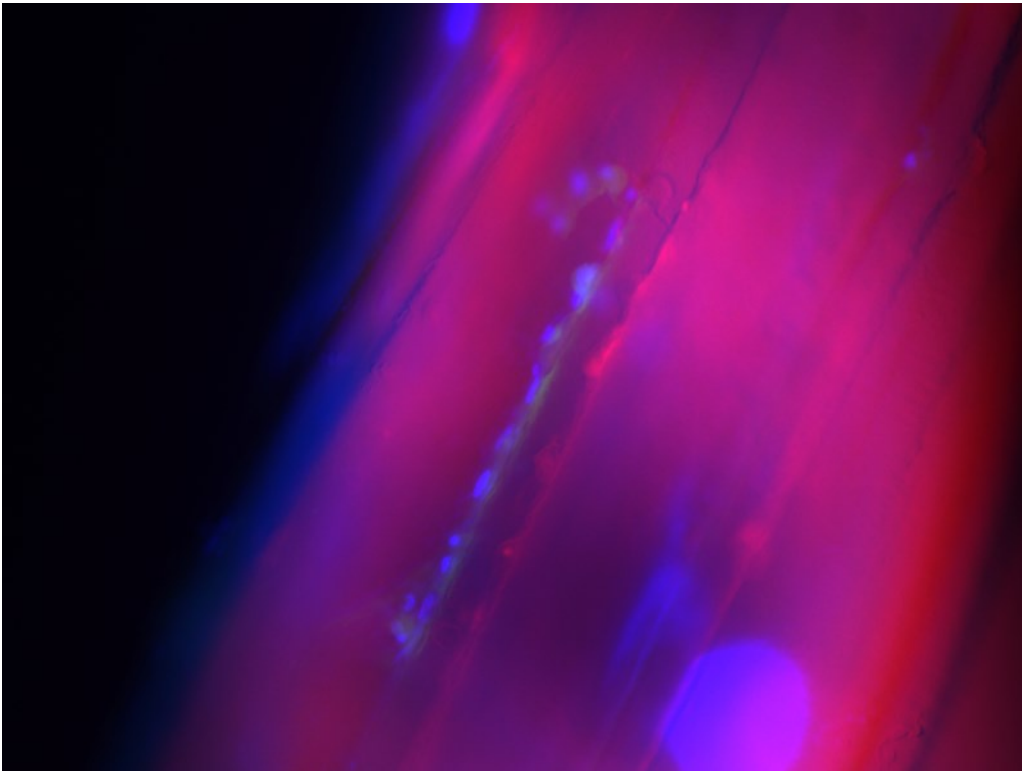
Time = 0 (Experimental, 10X)



Time = 3 hours (Experimental, 10X)



Time = 3 hours (Experimental, 20X)



Time = 3 hours (Experimental, 20X)