Cell Seeding on Bioscaffolds:

Bioreactor to Load Human Mesenchymal Stem Cells onto Fibrin Microthreads

A Major Qualifying Project Report:

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Abstract

Heart disease is the leading cause of death in the United States. Currently there is no effective method to repair heart tissue that becomes damaged. Cell therapy, particularly the use of stem cells, shows promise in restoring mechanical function to damaged areas of the heart. The existing methods of cell delivery to the heart provide too low of engraftment rates for clinical use, however, the use of bioresorbable fibrin sutures to deliver human mesenchymal stem cells (hMSCs) to the heart provides a new opportunity. The problem is loading the cells onto the suture for engraftment into the heart is currently inefficient. This project created a system that uses a mixture of static and dynamic seeding methods to load hMSCs onto four fibrin sutures simultaneously. The system consists of a syringe pump capable of infusion and withdrawal attached to a 3D printed cartridge with four parallel channel. A cell suspension is passed through the cartridge with the sutures loaded into it. This device has shown the ability to deliver cells to fibrin sutures and the possibility that cells not loaded may be recycled for future use.

Chapter 1: Introduction

Heart disease is the leading cause of death in the United States and the world. In 2011 there were 7 million deaths associated with heart disease; that amount accounts for 11.2% of reported deaths worldwide. Heart attacks (myocardial infarctions) are a common form of heart disease with someone in the US suffering from a heart attack every 34 seconds[1]. A heart attack occurs when blood flow to the heart is restricted. This can be as a result of narrowing of the arteries, blockages or blood clots. When the blood flow to a portion of the heart in inadequate a shortage of oxygen occurs causing the death or damage to the affected tissue. Currently this damage to cardiovascular tissue is permanent. The heart is incapable of regenerating tissue by itself and this leaves the heart structurally damaged. Such damage will leave the heart more susceptible to further disease and damage and puts the patient at risk for cardiac arrest.[1]
Research is ongoing as to methods to help regenerate cardiovascular tissue that was lost to myocardial infarctions. Cell therapy is a promising field to achieve this aim. In general cell therapy is any treatment involving the insertion of cellular material directly into a patient. A significant aspect of cell therapy relates to the use of human stem cells to regenerate damaged tissue within the body. Stem cells are human cells with the capability to differentiate into a multitude of different cell types given their environment, as such stem cells possess the ability to regenerate cardiovascular tissue. This project focuses on the use of mesenchymal stem cells which can be harvested for use in a patient from their own bone marrow. These cells have been shown to improve function of heart tissue after a heart attack but are currently difficult to implant into the heart. Current methods of implantation show engraftment rates of 1-13%.[2]

The use of fibrin microthreads show potential as a scaffold to deliver human mesenchymal stem cells (hMSCs) to the heart. Current fibrin microthread scaffolds can only be loaded at an efficiency of about 33% and with a very labor intensive process. In order for this therapy to be practical, there needs to be increased stem cell loading efficiency onto the fibrin microthread scaffolds. Thus it is the goal of this project to design a device or process capable of consistently loading cells onto fibrin microthread scaffolds with a good loading efficiency in a timely manner.[3]

Chapter 2: Literature Review

2.1 The Heart

The heart is a four-chambered double pump located in the center of the circulatory system. The heart works in a network of blood vessels to supply blood to the entire body. Blood serves as the transport medium while the blood vessels provide the pathways to the entire body and organ systems. The heart is responsible for both the systemic and pulmonary circuits. The systemic circuit provides oxygenated blood to the entire body allowing for the exchange of oxygen to power cellular processes. The pulmonary circuit is responsible for the pumping of oxygen-poor blood to the lungs where oxygen binds to red blood cells and is then pumped back to the heart.[4]

The heart is composed of cardiomyocytes, which contract systematically giving the heart its mechanical function. The sinoatrial (SA) node in the right atrium of the heart is the "pacemaker" which coordinates the contraction of all cardiomyocytes to provide proper contraction of the heart's chambers.

The heart is the central organ which must function correctly in order to keep the body alive. If any complications were to occur preventing the heart from performing its normal function, serious health complications or even death may occur.

2.2 Heart Disease and Failure

According to the American Heart Association, in 2012, approximately 1 in 4 deaths in the United States were directly linked to heart disease. Specifically, Myocardial Infarctions, or heart attacks, affect an estimated 1.2 million Americans each year. Heart failure due to a MI occurs when 25% (~1 billion) of the ventricle's cardiomyocytes die compromising the mechanical function of the heart and in turn decreasing the cardiac output.[5] In serious cases of heart failure, donors are needed, but there are only about 2,000 transplants available each year. With 4,000 people waiting for a donor heart on any given day, this develops a need for alternative treatment options.[6]

The heart would benefit greatly from regenerative therapy given the frequency of deaths due to heart disease and the lack of its regenerative ability. Since it is one of the least regenerative organs in the body, it poses one of the greatest challenges primarily due to the inability of adult cardiomyocytes to sufficiently proliferate and generate new heart tissue.[7]

2.3 Cell-Based Therapies

Current treatment methods for heart failure are limited and traditional approaches, including the use of medication and surgery to increase blood flow are only slightly effective. With the increasing occurrences of cardiovascular disease in the United States, alternative methods to address MI and heart disease have been in development. The area of cell based cardiac repair has been given growing attention with recent developments in research. Initially, cell based therapy was not openly accepted but evidence has shown that implanted cells could create new tissue and improve mechanical function of an afflicted heart.[5]

In recent years, various cell based therapies have been developed. The use of skeletal myoblasts was explored with the expectation that the cells would transdifferentiate into cardiomyocytes when implanted into the heart. Unfortunately the cells remained differentiated as skeletal myoblasts and began to form mature skeletal muscle in the heart.[8] Aside from skeletal myoblasts, other cell lineages were explored including embryonic stem cells, endothelial progenitor cells and adult stem cells. Both endothelial progenitor cells and embryonic stem cells showed potential for the use in cardiac biomedical applications, they each have their own drawbacks. Ethics behind using embryonic stem cells is in question an endothelial progenitor cells are difficult to harvest.[9]

Using adult stem cells for cardiac regeneration has shown promise and is advantageous due to their inherent cellular plasticity and their availability in all mature humans. These cells remain undifferentiated meaning they have not generated structures or manufactured proteins of a specific cell type.[30] These cells are widely available in human tissue or organ and can readily differentiate to yield major cell types such as neural, bone, cartilage, fat, muscle, marrow stroma, digestive tract, and cardiomyocytes. These cells are ideal since their primary endogenous function is to maintain and repair the tissue in which they reside so theoretically transplanting them to a wound site should yield positive results.[10, 11]

2.4 Human Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) are found in the bone marrow of femoral bones[12] and are capable of differentiating into a wide range of cell types and tissues. Some of these types and tissues are fat cells, muscle cells, skin cells, nerve cells and in rare occasions cardiomyocytes.[11] Researchers have enough information on this stem cell type in order to produce cell lines in culture. Recently, it has been proven that cardiomyocytes can enter the cell cycle[13, 14] and also stem cells enhance myocyte mass in the heart by inducing native myocytes to proliferate through paracrine signalling.[15,16] This signaling occurs due to the secretion of a number of angiogenic cytokines and growth factors, which induce changes in neighboring cells which have positive effects on damaged tissue. These factors include: VEGF, bFGF, IGF-1, SDF-1, TGF-β, HGF, PDGF, etc., which all play different roles in the body, from the promotion of angiogenesis to regulation of cell proliferation.[31]

Dissimilar to other adult stem cells, mesenchymal stem cells can easily be attained in appropriate quantities for clinical applications. Also, techniques for isolation and amplification of this type of stem cell in culture have been determined. These techniques allow the stem cells to be easily maintained and for propagation control over a long duration without losing the ability to differentiate. Moreover, these cells have been shown to be advantageous in clinical research as they have high proliferative and renewal capabilities allowing for lasting effects in clinical studies. Since understanding the culture conditions has been so easily attained, scientists are now progressing in understanding the molecular pathways that control the growth and differentiation. Some clear benefits of this stem cell type include its ability to act appropriately when introduced to specific genes and the capability of MSCs to be frozen in preservation without losing their original functional purpose.[11] Trials using animal specimens are underway involving the reconstruction of heart muscle after infarct tissue forms. Improvements in angiogenesis[17] and myocardial wall movement[18] have been attained using stem cell delivery methods to the site of infarcted tissue. These studies are providing great hope for human implications. Finally, and possibly the most important benefit of mesenchymal cells, is their ability to be derived from a patient's bone marrow,

multiplied in culture, and then re-implanted in the same patient, completely avoiding all immune rejection consequences.

2.5 Cell-Delivery Methods

It is critical to ensure that an efficient and consistent stem cell delivery technique is chosen for this heart regeneration process. Even if the appropriate number of cells is cultured in vitro that is needed to completely regenerate the infarct heart, this doesn't necessarily correspond to a successful treatment in vivo. The stem cells must be delivered to the heart with a high certainty of them actually proliferating and migrating on the infarct tissue. This novel approach provides hope to improve the amount of cells that reach the site of infarct tissue by allowing for localized application. The suture gets stitched onto the dead tissue directly.

The delivery methods that are currently being used for heart regeneration have proven to be ineffective to return the heart to its full physiological function. One of these methods is injection. There are three types of injection; intramyocardial, intracoronary and intravascular Intramyocardial injection has proven to unsuccessful because the cells are lost at the injection site as they do not engraft to the heart and this process can also loose cells due to cells dying because of the applied shear stress of injection and/or hypoxia.[20] Intracoronary injection leads to a high amount of cell death too. If the correct amount of cells is not delivered and the infusion characteristics are not properly determined a high risk of additional heart tissue death arises. In addition to this negative aspect of IC injection, this type of cell delivery correlates to a decrease in coronary blood flow.[21] The final injection method, intravascular, leads to a problem in localized delivery. The stem cells have a very low engraftment efficiency using this technique because they do not even make it to the infarct tissue. This has been assumed by a study that focused on cell homing to non-cardiac organs.[21]

The efficiency and consistency of cells that are delivered to the heart is critical to ensure the proper number of cells engraft and proliferate leading to regeneration. This is why a localized delivery method holds so much promise for this application. A very low engraftment percentages result from the three injection methods mentioned above; 3-12%.[2]Leaving this process to chance is not acceptable; direct application of stem cells can lead to a higher engraftment rate with more control of the amount of cells delivered upon optimization.[16]

2.6 Biosutures as a Delivery Device

Recently, biomaterials in the form of sutures have been explored as a delivery method for cell therapy. Some potential materials for this application include silk, collagen and fibrin. All of these materials have their advantages and disadvantages. Silk is biocompatible yet it is non-absorbable since is it not a natural material in the body and doesn't degrade at the wound site. Collagen is biocompatible and bio-resorbable yet it takes a long time to break down the collagen fibers and degrade out of the heart. Fibrin, on the other hand, has desired degradation characteristics and breaks down in the body in a few days effectively delivering the cells to the wound site.

The suture material that is being used in this localized stitching delivery technique is fibrin. This material is bundled to form a microthread and biological microthreads have been proven to be successful scaffolds.[22]The affinity of hMSCs to fibrin microthreads is higher than any other scaffold materials because fibrinogen and thrombin are the two ECM proteins involved in the early stages of

wound healing. These two ECM proteins combine to make fibrin.[23-26]The number of threads bundled and how they are bundled allows for control over the strength and size of the suture. Biological microthreads mimic the structure of natural tissue, are biocompatible and biodegradable. However, fibrin biologically degrades much faster than other scaffold materials; which is desired for this application.[22] Cells also orient themselves along the long axis of microthreads and migrate well on them; which both enhance tissue regeneration.[22]

2.7 Current Method and Improvements

2.7.1 Seeding Techniques

Cell seeding for tissue engineering is really a two part process. The first part involves the loading itself where the cells are attached to the scaffold in use and the second portion is the culture where the cells are allowed to proliferate and spread. These steps can be done together or separated. Each can be done a variety of ways but the two major categories are static and dynamic. In a static method the scaffold and cell suspension remain unmoved. In dynamic either one or both of the components is in motion. As might be expected, the type of technique used has effects on the ways cells attach and proliferate.

Static varieties do not vary much and largely involve the scaffold be placed in cell suspension with a cell culture well and allowed to sit. This kind of technique can be used for the loading and/or culture portions of the cell seeding process. When used for the loading portion of the process static techniques may generate aggregations of cells rather than an even distribution.[27] These techniques still do often lead to stable cell growth and low cell damage making them much more viable for culture purposes after a different method has been used to distribute the cells across the scaffold.

Dynamic seeding methods vary much more widely. Again it can be used for the loading or culture process but with different results. Dynamic methods were shown to provide even cell loading but less significant cell proliferation during culture than static methods.[27] Dynamic methods also have two major subdivisions: turbulent and laminar flow. In theory laminar flow is more predictable and likely less damaging where turbulent may fill gaps present in laminar flow and give cells more opportunity for attachment. It was found, however, that turbulent flows generally produced lower cell densities on scaffolds and produced more cell damage than laminar flow.[28] Dynamic methods can also vary based on which part of the apparatus is in motion: the scaffold, the cell suspension, or the whole device. These methods are more dependent on the exact type of scaffold and the device and therefore their effectiveness cannot be easily generalized. However when designs are created they can theoretically be modeled to predict flow behavior and loading effectiveness.[29]

2.7.2 Current Process

The current method used in the lab of Professor Gaudette involves a dynamic seeding method. In the current method, the suture is loaded into a gas permeable syringe tube which is first loaded with PBS or saline to hydrate the microthreads. The cell suspension is then loaded into the tube with the suture. The tube a suture apparatus is then loaded into a 15 mL conical tube with a filter cap which allows for gas exchange. Several of these conical tubes can then be loaded onto a rotator at a slight angle. The entire rotator is placed into the incubator for 12 hours and is ready to use upon removal.

In this method the sutures are loaded with 100,000 cells in a suspension of 100 μL. The average yield on the 2 cm long suture is 16,313 cell/cm. This means the current method is resulting in a loading efficiency of only 33%.

Chapter 3: Project Parameters

3.1 Initial Client Statement

During preliminary communication with Professor Gaudette, the team was presented with the following initial client statement:

"Design a device or system to increase the seeding efficiency and decrease the seeding time for seeding cells on fibrin microthread sutures. The device or system should be easy to use (including loading and removal), provide a sterile environment and have a small footprint. The design should also allow for the reproducible loading of cells onto the sutures. The device or system should hold 4 sutures."

The goal of this project is to design a device that increases the efficiency of cell seeding onto fibrin microthread as compared to current methods used in Professor Gaudette's lab.

3.2 Project Approach

3.2.1 Technical Approach

By conducting an extensive review of the literature and analyzing the problem, the team revised the initial client statement and compiled a list of objectives and constraints. Objectives were ranked in order of importance by the client and designers in a Pairwise Comparison Chart as shown in Figure 1. Functions and specifications for the device were made. In order to determine how key functions of the design would be carried out, a Function-Means chart was developed and can be seen in Appendix D: Functions-Means Tree. This chart allowed for the easy creation of design alternatives through the grouping of various means.

Conceptual design ideas were generated in various brainstorming sessions using the Collaborative Sketch and the Gallery methods. Initial designs were presented and discussed with the client over the course of several meetings. The team also met with a PhD student working on bioreactors to discuss different cell seeding methods as well as particular design details such as fluid flow, viscous forces, and cell adhesion. Method testing of fluid flow using a syringe was conducted on a rough model. A design evaluation matrix was created to assess whether or not the design alternatives met the objectives, shown in Appendix E: Design Concepts/ Evaluation Matrix. Designs that did not comply with the objectives were eliminated or adjusted. A set of metrics using a numerical grading system were also created to measure how well a design met each objective detailed in Appendix F: Metrics on Objectives. Various aspects from different design alternatives were improved, modified, and combined to create a preliminary design. The team engaged in ongoing research concerning device materials.

A CAD drawing of the initial design selection was prepared and discussed with the client. The device design was modified in CAD per the adjustments suggested and a prototype was made using rapid prototyping. The team assembled a system composed of the prototype, a thin sheet made of silastic plastic, tubing, a syringe and a syringe pump. The syringe pump perfused the device with liquid through the tubing that was connected to the head of the syringe. The silastic plastic sheet was utilized to cover the prototype and prevent leakage when flowing liquid through the system. Device performance was optimized during some bench top testing.

Glass microspheres were obtained to mimic the size and movement of hMSCs in the device. With the help of a PhD student in the Gaudette lab, the team imaged the flow of the microspheres in water through the system at a flow rate of 1 $\frac{mL}{min}$ using an upright microscope to visually determine the flow pattern and distribution throughout the inlet and microchannels. More detail about this flow testing can be found in Chapter 4: Design Alternatives. Based on observations made during this testing, the team identified areas for improvement in the device. Subsequently, the prototype was redesigned to further enhance flow with a smaller inlet and microchannel diameter as well as a cover and was produced via rapid prototyping. Specifics on different design modifications in addition to the progression of device designs are described in Chapter 4: Design Alternatives.

Concurrently, hMSCs were obtained from the Gaudette lab. The team prepared the cell media and fed and passaged the cells according to protocols listed in Appendix G: Protocols for Cell Culture. Additionally, the team acquired fibrin microthreads from the Gaudette lab and followed the protocols in Appendix H: Fibrin Microthread Suture Protocols for suture fabrication.

Using Computational Fluid Dynamics and the computer program ANSYS Fluent, fluid flow was simulated through the final prototype at flow rates of 0.5 mL/min, 1 mL/min, 1.5 mL/min, and 2 mL/min. This allowed the team to characterize the flow behavior through the device at the different flow rates by analyzing velocity contours and path lines as well as to examine particle distribution which could be correlated to cellular motion. Additional information about this ANSYS Fluent testing can be found in Chapter 5: Design Verification.

Three biocompatible models of the final prototype were produced via rapid prototyping and used to test cell seeding in the final device with subsequent Hoechst staining and imaging. Different ratios of dynamic to static seeding were tested in order to determine the most favorable seeding ratio as well as duration of overall seeding time was tested at time points of 1, 3, 6 hours as detailed in Chapter 5: Design Verification. The results were evaluated and the team identified areas for improvement and future design modifications. The team finished documenting the design process, reported the findings, and delivered a final presentation to the Department of Biomedical Engineering.

3.2.2 Managerial Approach

The design team determined the necessary tasks to be completed in the design process and organized them into a hierarchical structure categorized by task group. This work breakdown structure consists of the following task groups: understanding the client statement, determining design parameters, generating and evaluating designs, creating the preliminary design, testing, creating the detailed design, creating the final design, documenting the design process, and project management, that are further broken down into subtasks as shown in Appendix B: Work Breakdown Structure.

Managing thinking as well as the organization aspect of the design process is necessary for its successful implementation. We designate a chairperson and secretary for our meetings. The purposes of the meeting as well as discussion points are outlined in an agenda. The chairperson makes sure conversation stays on topic. The design process is tracked in the laboratory notebook.

As scheduling is an important part of planning the design process, the designers projected how much time would be required to complete project goals and plotted the design tasks on a timeline. This assists the designers in budgeting time, keeping track of what has been and what needs to be

completed at any point in the design process, and finishing all necessary tasks by the deadline. In this Gantt chart represented in Appendix C: Gannt Chart.

3.2.3 Financial Approach

This section shows the team's expenditures and financial breakdown for the design process. This includes the cost of materials, prototyping, testing, and design fabrication. Currently, from the budget of $624, $545.67 was spent on the rapid prototyping, and $100 was spent as a fee to use the lab resources in GH0006. The remainder of the expenditures that exceeded the budget were paid for by the Gaudette lab.

Over the course of the design process the team was careful when deciding on materials and methods used to construct the device in order to minimize cost and maximize the effectiveness of the given budget. The device will not be manufactured for commercial use but rather for the sole purpose of aiding research eliminating the need to calculate manufacturing costs.

3.3 Assumptions

Several assumptions were made during the formulation of the project approach:

- Fibrin microthread with needle attached will be provided prior to seeding
- User will be familiar with common biological laboratory practices
- Threads will be used within 36 hours of hydration

3.4 Design Parameters

3.4.1 Objectives

A brainstorming session was conducted by the team to determine what attributes and performance characteristics that the stakeholders want in the design. A list of objectives was created and organized into an objective tree depicted in Appendix A: Objective Tree. In no particular order, the top level objectives and corresponding secondary objectives were narrowed down to that the device should be:

- Safe
    - Safe for the User
    - Safe for the Cells
- Easy
    - Portable
    - Accessible
    - Compatible
    - Easy to Use
- Consistent
    - Cell Attachment
    - Time
    - Hydration
- Resource efficient
    - Minimum Waste
- Time efficient

    - Minimum Seeding Time
    - Minimum Preparation
    - Maximum Sutures
    - Scalable
- Effective
    - Cell Loading
    - Seeding Efficiency

In terms of safety, the device should be safe for the user who handles it and safe for the cells that are loaded into it. This means no sharp edges such as an exposed needle or possible risk of electrocution. Also, there should be no forces or flow rates within the system great enough to harm or lyse the cells. Another important objective is that the device should be easy. This stipulates the creation of a device that is portable from the lab to surgery, compatible with the current lab equipment, and makes the sutures accessible for loading and removal. Ease of use is also an essential aspect of this design objective. Regarding consistency, the device should allow for consistent cell seeding which specifies that a reliable number of cells attach to the fibrin microthread sutures within a predictable amount of time when comparing suture to suture as well as trial to trial with the device. There should also be even hydration of the cells throughout the thread and from thread to thread. Subsequently, the device should be resource efficient requiring that it has efficient cell loading and produce the minimum waste possible. Furthermore, an additional design objective is time efficient which can be broken down into minimizing seeding and preparation time, seeding the maximum number of sutures, and being scalable in terms of number of sutures seeded in an amount of time. In order to prove the device's efficacy, the objective of the device being effective can be broken down into successful cell loading and improving seeding efficiency on fibrin microthreads.

A Pairwise Comparison Chart or PCC was created to score the objectives against one another and rank them accordingly. The design team completed the Pairwise Comparison chart as did the client and a user. The PCC is shown in Figure 1.

	Safe	Easy	Resource Efficient	Time Efficient	Consistent	Effective	Total
Safe		0	0	0	0	0	0
Easy			0	0	0	0	1
Resource Efficient				1	0	0	3
Time Efficient					0	0	2
Consistent						0	4
Effective							5

Figure 1: Pairwise Comparison Chart for Design Objectives

The scores from the PCC were used to rank the objectives. In order of importance, the objectives can be ranked in the following order:

1. Effective
2. Consistent
3. Resource Efficient
4. Time Efficient
5. Easy
6. Safe

3.4.2 Constraints

A list of constraints was devised by the team to later apply to design alternatives and restrict the final design choice to an acceptable alternative with suitable performance. The constraints are that the device must:

- Withstand being placed in an incubator at 37 C for up to 36 hours
- Fit in an incubator with the dimensions 17" x 20" x 20"
- Minimize the stresses on the suture to maintain mechanical integrity
- Allow for sufficient hydration of the microthreads for ≥ 10 minutes
- Must be composed of biocompatible materials

The constraints listed herein refer to strictly the design itself .Some constraints on the design process include:

- Project be completed by the end of the 2013-2014 academic year
- Project cost be ≤ $624, $156 per team member

Failure to comply with these constraints in their entirety will result in an unsuccessful device and project.

3.5 Revised Client Statement

Based on the above-mentioned design parameters, the client statement was revised as follows:

"This project aims to design a device that allows for consistent cell seeding onto fibrin microthread sutures with a focus on mesenchymal stem cells. This device should be able to support at least four sutures which are easily accessible and removable in a clinical environment. The design should decrease seeding time and increase cell seeding efficiency on the suture compared to the current method."

Chapter 4: Design Alternatives

In order to start their design alternative generation process, the team began by creating a list of desired functions and specifications.

4.1 Functions, Needs Analysis and Specifications

4.1.1 Functions

A group of functions were developed to define what the device is supposed to do. This list outlines the actions of the device that will comprise the collective action or purpose for its creation and use. The functions include:

- Load Cells
- Add and Remove Fluid
- Seed Cells
- Support Sutures
- Maintain Cell Viability
- Allow for Gas Exchange
- Reduce Production Time

The functions were used to generate means as depicted in the Function-Means Tree (Appendix D: Functions-Means Tree) that will later be the basis for design alternatives.

4.1.2 Needs Analysis

Upon meeting with the client, the group was able to put together a "Needs Analysis". This analysis consists of a differentiation between what the seeding technique needs to do versus what the client wants it to do. Ideally the device would be capable of the following specifications:

- This device would hold 8+ threads as the current process prepares 4
- Cell count totals must have a standard deviation of less than 20%
    - This applies to each 0.5 cm on each suture and from suture to suture
- The seeding efficiency should ideally be greater than 16,000 cells/cm; with a very low standard deviation
- The entire seeding process should take under 8 hours
- The cell viability should be greater than 95%

4.1.3 Specifications

These specifications were set established after negotiating with the client. These will help the team determine when the device has reached expectations. The main specifications for this device are:

- Efficiency must be greater than 20%
    - This means that over 20% of the cells used in loading must adhere to the suture
- Volume used must be less than 2 mL per thread
    - This means for hydration no more than 2 mL of PBS per thread and for seeding no more than 2mL of media per thread
- Must hold 4+ sutures
- Hydration must be even
    - After hydration the swell ratio must be even along the length of the suture

- Cell count totals must have a standard deviation of less than 20%
    - This applies to each 0.5 cm on each suture and from suture to suture
- Cell count must be at least 10,000 cells/cm
- Cell viability must be greater than 90%
- Must accommodate hMSCs and fibroblast cell types
- Whole seeding process must take less than 12 hours

These specifications must be met to have a successful device capable of performing the functions previously outlined at the level expected of the client.

4.2 Design Alternatives

4.2.1 Conceptual Designs

The team, using the Function-Means Tree found in Appendix D: Functions-Means Tree, generated four conceptual designs to start the design generation process. A means for each function was chosen and put together as a design alternative. The four conceptual designs are shown and explained below.

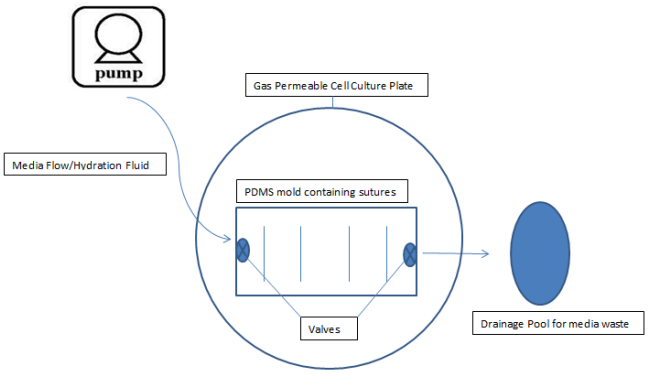

Figure 2: Conceptual Design #1

Conceptual design #1 uses a PDMS mold to contain sutures (Figure 2). A pump induces flow of the cell containing media over the static sutures. A drainage pool is present after the fluid passes over the fibrin microthreads. This process allows for dynamic cell seeding as the pump pushes the media over the sutures. A proper flow rate and tube diameter would need to be determined in order to provide the optimal amount of seeded cells. A gas permeable cell culture plate that is structurally altered to accommodate the tubing from the pump would be used to contain the PDMS. Valves would be placed on both sides of this plate to allow for static seeding as well.

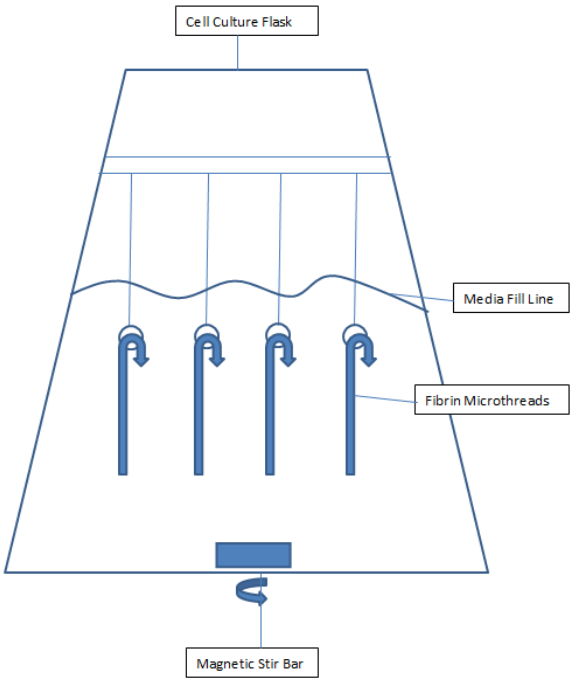

Figure 3: Conceptual Design #2

Conceptual design #2 (Figure 3) involves sutures being hung from a rod inside of a large cell culture flask. The needle would be hooked around an O-ring to allow for suspension into the media solution. The media fill line would be well above the sutures to ensure they are never exposed to air pocket at the top of the flask. The lid would be made of an oxygen permeable filter with twist on and twist off capabilities to allow for reuse. Static and dynamic seeding options are available as allowing the sutures to remain immobile in the stagnant media is possible, and a magnetic stir bar would be used to keep the cells in suspension providing a dynamic vortex of flow.

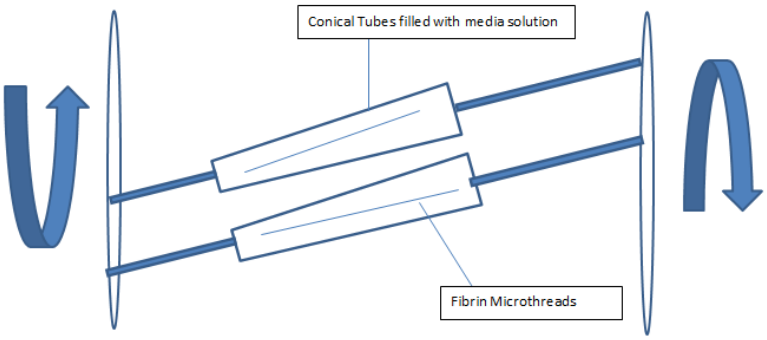

Figure 4: Conceptual Design #3

Conceptual design #3(Figure 4) consists of loading the fibrin microthreads in conical tubes with oxygen permeable caps. These tubes would then be supported in an apparatus to allow for rotation on a varying axis. The fibrin microthread would be encapsulated individually in a cylindrical tube, inside which would be the media solution. This tube would be static inside the larger conical tube.

The angle of which the axis is varied on would be able to be adjusted for optimization testing. This would be a dynamic seeding technique.

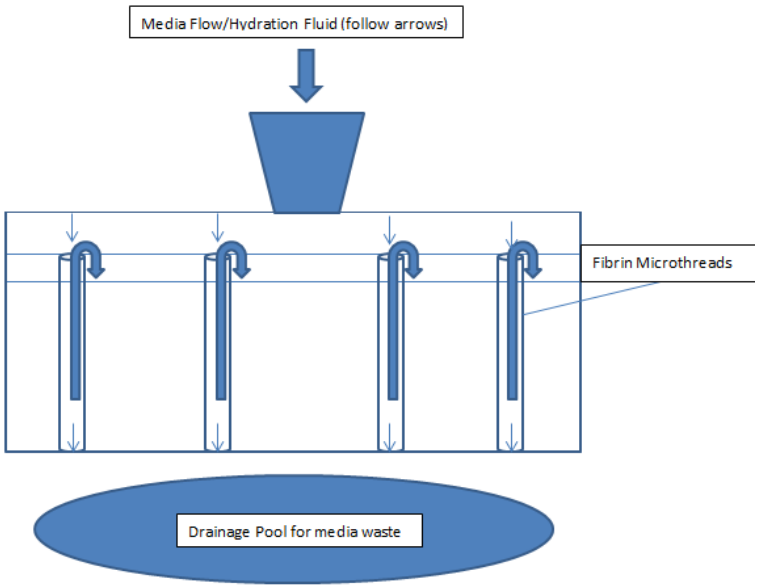

Figure 5: Conceptual Design #4

Conceptual design #4 (Figure 5) is a box with an entry funnel and a reservoir layer for the media entrance. Each fibrin microthread is hung in a cylindrical column that is core drilled in the solid portion of the box at a slightly larger diameter than that of the thread. Media would pass through each individual column with gravity being its only driving force, in what the group envisioned as a dripping method. A drainage pool for media recycling or disposal would be provided at the bottom of the apparatus.

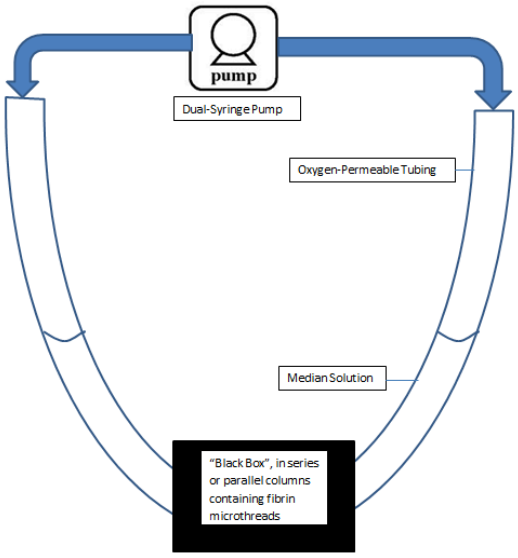

Figure 6: Conceptual Design #5

Conceptual design #5 (Figure 6) uses a dual-syringe pump to push and/or pull cell containing media through a removable piece that contains columns either in series or parallel. The tubing is permeable to oxygen to maintain cell viability. The sutures are laid in these columns and kept in place by using some sort of rubber stopper to penetrate with the needle or utilizing an attached O-ring to hook the need on in each column. This method allows for both dynamic and cell seeding and precise control over the flow rate of the media.

4.3 Decisions

Going forward the conceptual designs were analyzed to make an initial design selection. The conceptual designs were compared using a design evaluation matrix (Appendix E: Design Concepts/ Evaluation Matrix). The designs were also discussed with the client who helped to specify which designs, or aspects of designs, appealed to him. The result was determining piece by piece which design components would be most beneficial for the design.

The first design aspect that was decided was that the chosen design should be capable of both static and dynamic seeding. This decision was made based on research mentioned in Chapter 2: Literature Review. This decision meant that the most practical designs would be those which utilized a pump, since that would allow for programming alternating dynamic and static seeding without supervision.

Discussions with the client suggested that the use of a cartridge to hold the threads that would be removable from the full device would be extremely desirable. The client indicated that the decision to create a reusable or disposable cartridge was up to the design team.

At this point, the team needed to pick a pump type to work with. The two main options, peristaltic and syringe pumps, were decided by availability. The use of a peristaltic pump would mean a complete circuit with fluid moving in one direction. The syringe pump would be a closed end tube with fluid alternating in movement direction. The team, after discussion with the client, decided on the syringe pump because having only one direction of flow as with the peristaltic pump could cause an undesirable gradient to form along the length of each suture with the end closest to the fluid source attaining a higher cell density as compared to the far end. The team expects that alternating flow from each direction will limit this gradient.

Then, the design was to use a syringe pump attached to a “U” shape piece of tubing. The cartridge that holds the threads would attach at the bottom of the “U” (Figure 6). Further design decisions mostly regard the cartridge portion of the design. The team had to choose whether to place the sutures in series in one channel in the cartridge, or to place each suture in its own channel in a parallel configuration. Discussions with graduate student Sam Acott, on loan from the University of Bath to Professor Gaudette’s lab, revealed that the best choice for consistency would be to use a parallel channel format. This design is shown by the CAD drawing, made using the SolidWorks software, in Figure 7 and Figure 8.

The cartridge shape also came into question during the design process. Initial modeling showed the cartridge being composed of two half cylinders that, when placed together, would create a full cylinder. The benefit of such a design being that the cartridge may be capable of spinning if that was later preferred by the team or the client. Also, this would allow the two pieces to be easily sealed using a pair of basic hose clamps. For ease of use, cartridge shape was changed to a single

rectangle with troughs cut into it with these channels being covered with a sheet of gas permeable silastic film. The film could then be affixed with simple c-clamps or a more elaborate method could be created if needed.

Figure 7: Preliminary Design of Removable Cartridge from Conceptual Design #5 (top view)

Figure 8: Preliminary Design of Removable Cartridge from Conceptual Design #5 (side view)

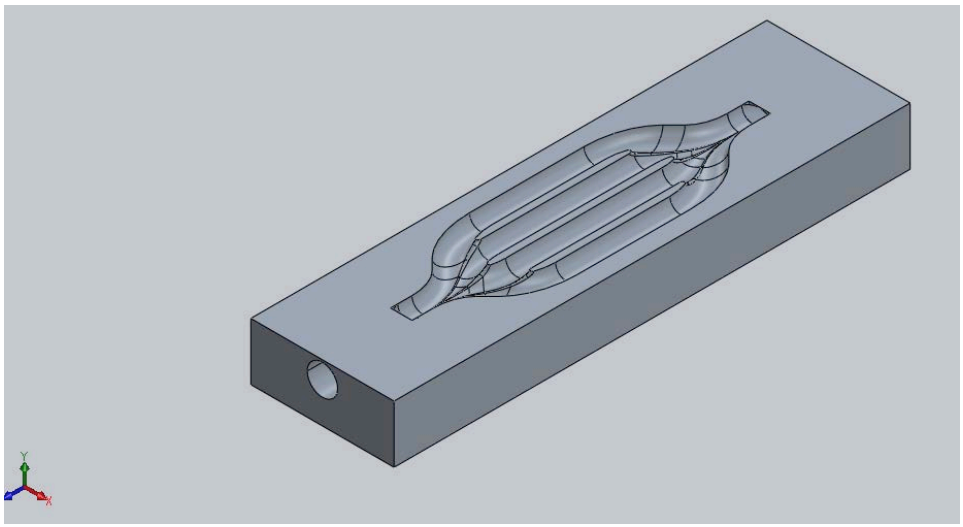

Figure 9: Preliminary Design of Cartridge

Figure 9 shows the preliminary cartridge design created by the team. This design will be modeled, prototyped, and undergo preliminary tests. Further adjustments to the design will be made based on the results of these tests along with feedback from the client.

4.4 Design Calculations

Figure 10 and the subsequent calculations show how the diameter of the inlet, outlet and microchannels were determined:

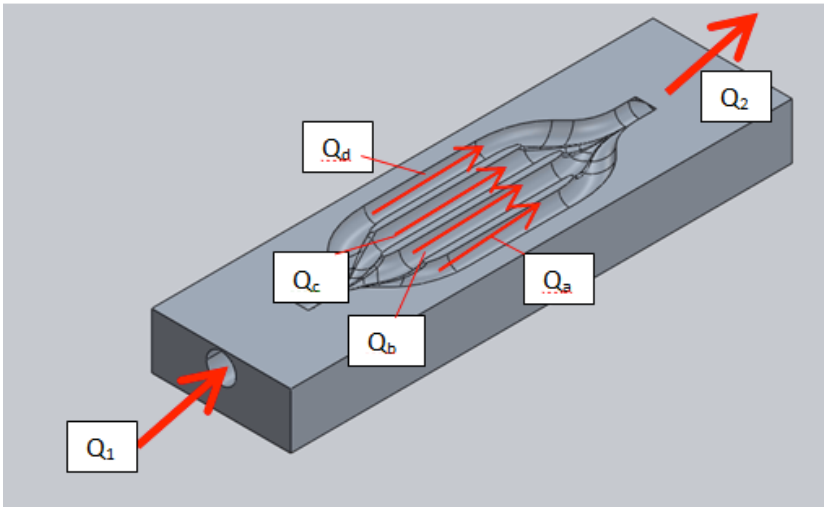

Figure 10: Determination of Channels Diameters

$$Q = vA$$

$$Q_1 = Q_a + Q_b + Q_c + Q_d = Q_2$$

$$A_1 = A_2$$

$Q = volumetric\ flow\ rate$
$v = velocity$
$A = cross\ sectional\ area$

Desired:
Equal velocity in all channels

Assumptions:
-Cylindrical channels
-frictionless

$$A_a = A_b = A_c = A_d$$

$$v_1 = v_2 = v_a = v_b = v_c = v_d$$

$$v_1 A_1 = v_a A_a + v_b A_b + v_c A_c + v_d A_d = v_2 A_2$$

$$\sqrt{\pi r_1^2} = \sqrt{4 r_{a \to d}^2}$$

$$r_1 = 2 r_{a \to d}$$

4.5 Rapid Prototype #1

The design, shown in Figure 9, was 3D printed using the Objet 260 Acrylonitrile Butadiene Styrene (ABS) Connex™ rapid prototyping machine. As shown above by the flow calculations the inlet and outlet diameters we chosen to be 1.06cm in order to allow for a press fit of tubing with an OD of 7/16". The microchannels diameters were machined at 0.5 cm, approximately half of the inlet and outlet diameter. The overall dimensions of the cartridge were designed to be 10cm x 5cm x 2cm. The material chosen for printing was VeroClear™, a rigid plastic with similar properties as Acrylonitrile Butadiene Styrene (ABS). This model was printed to be used for conceptual flow testing therefore was not printed with a biocompatible material.

4.6 Conceptual Testing: Flow

4.6.1 Experimental Set-Up

Rapid prototype #1 (RP1) was covered with a piece of piece of glass cut to 10cm x 5cm for imaging purposes. Using clear vinyl tubing with an OD= 0.8 mm and ID=0.5 cm and polytetrafluoroethylene sealing tape to ensure a water tight seal, the device's inlet and outlet tubes were secured. A KDS 200 syringe pump was used and programmed accordingly to the desired design parameters. An upright microscope and high speed camera were utilized to image the flow of the particles. The experimental set-up is shown in Figure 11.

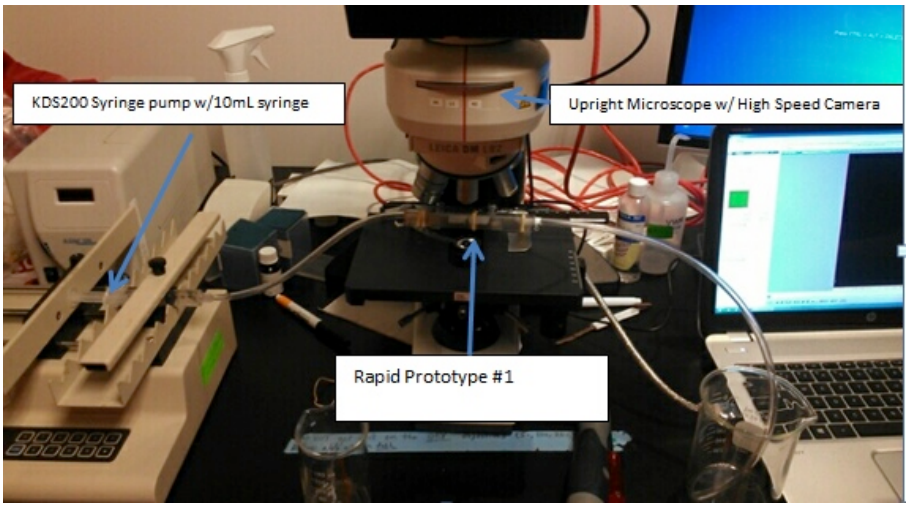

Figure 11: Experimental Set-Up for Flow Testing

The standard curve below was used to determine the microsphere concentration used in solution with the appropriate volume of deionized water (see Table 1). This curve takes the density of the Soda Lime Microspheres from Cospheric and particles per gram data provided by Cospheric as a function of the particles diameter.

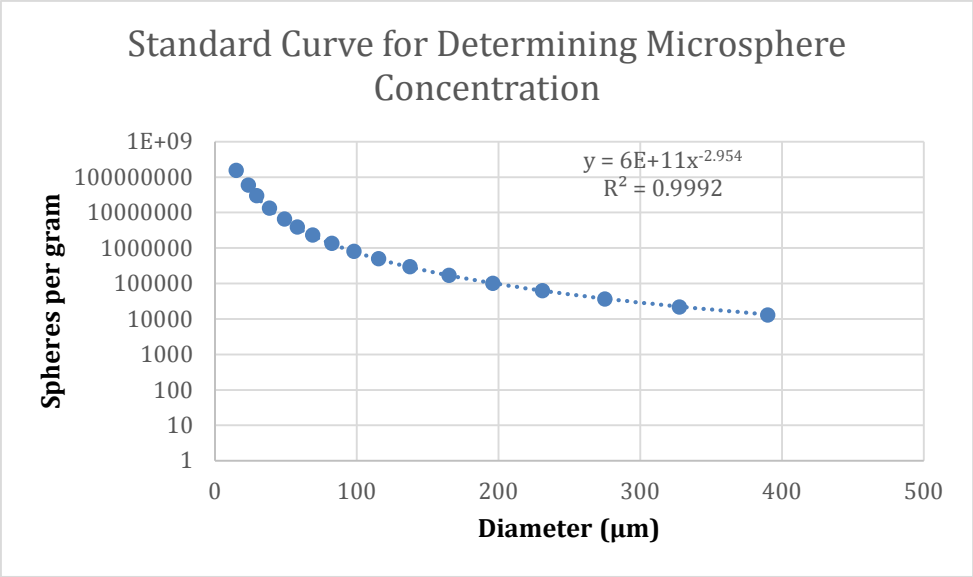

Figure 12: Standard Curve for Determining Microsphere Concentration

4.6.2 Flow Testing Observations

For imaging purposes, the concentrations of microspheres were increased in comparison to the parameters that will be used when testing with stem cells is done (400,000 cells/4mL). Table 1 shows the testing parameters and observations made after each test:

Table 1: Flow Testing Parameters and Observations

Trial	Microsphere Concentration	Particle Size	Flow Rate	Volume of Solution	Observations
1)	900k	11 µm	0.25 mL/min	8.3 mL	White particle on white background, focus issues
2)	1 million	20 µm	1 mL/min	9.2 mL	Laminar flow, velocity profile estimate with a lower velocity along the base of channels
3)	1 million	20 µm	1 mL/min	9.2 mL	Flow imaged at mouth of channels , particles observed to distribute evenly to all four channels

Laminar flow was observed with even flow through each of the four channels. Upon imaging the inlet mouth of the device (the area before flow breaks into four channels) it was observed that microparticles we distributing to each of the four channels based off of the direction of flow clearing splitting. The flow was definitely inhibited by the bottom surface of our device with some microparticles becoming embedded in the striations. This is shown in Figure 13.

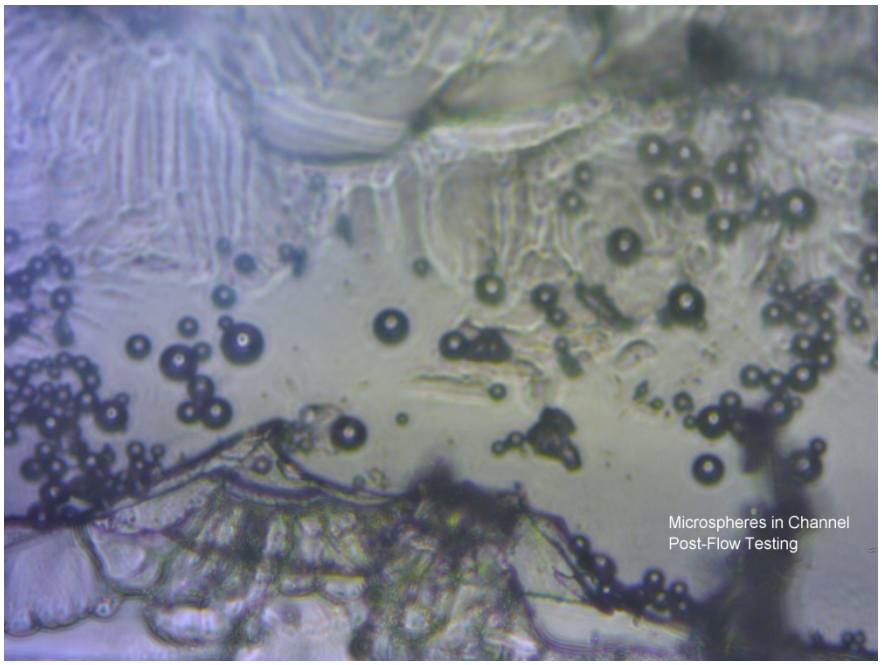

Figure 13: Microspheres Embedded in Striations at Bottom of Microchannels

4.6.3 Flow Testing Conclusions

The striations need to be removed to try to rid or reduce the number of microspheres that embed themselves into the base of the device. The microchannel diameter needs to be reduced to decrease the total volume of the channels and to reduce the inlet/outlet diameter to ensure a press-fit water tight seal. The plan is to pierce the silastic film with the sutures on the end of the microthreads, therefore the idea of a cover has been brought up to increase user safety by removing exposure to open needles and to maintain sterility during usage and transport.

4.7 Rapid Prototype #2

The revised design depicted in Figure 14 and Figure 15 encompasses the new features:

- Cover: 1) To allow for "glossy" finish during 3D printing on the entire top surface of device, removed most striations
  2) Ensures sterility in incubator and safety for user by removing the exposed needle
  3) Two posts that leave room for silastic film and actually press down on it to increase water tightness and to inhibit device rotation.
  4) Inlet/outlet pegs to complete the entry and exit holes.
- Microchannel, Inlet and Outlet Diameter:
  1) Reduces overall volume needed to fill device allowing for more cells to be exposed to microthreads.
  2) To better fit tubing ensuring water tight press fit.
- Increased surface area of top surface not microchannels:
  1) To allow for footprint for posts
  2) Allow enough surface area for biocompatible glue in case device remains non-water tight.

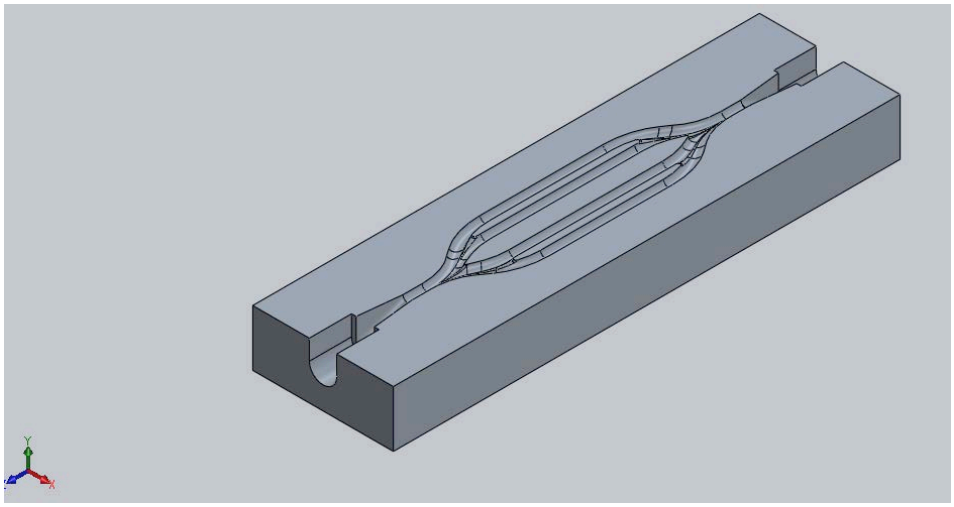

Figure 14: Revised Design, Base-microchannels

The new inlet/outlet diameter is 0.65 cm to ensure a press-fit water tight seal as mentioned above. The channels now have a 0.22 cm diameter to reduce to total volume. The overall dimensions of the device are 12x3.33x1.33 cm.

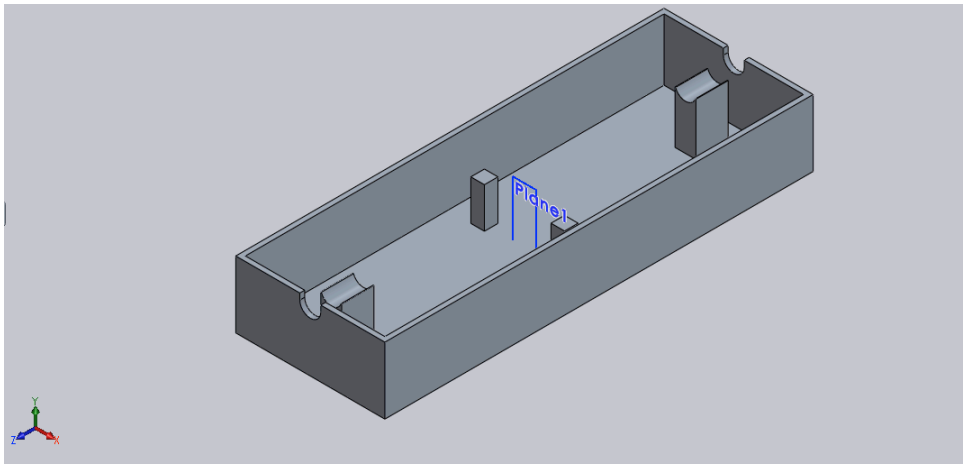

Figure 15: Revised Design, Cover

The overall dimensions of the cover are 13.3x4.45x2.066 cm. The plug diameter that finishes the circular entrance and exit has the same diameter as the hole it plugs in to of 0.65cm. The posts are 0.421x0.421in terms of area and 1.25cm in length. Figure 16 shows the entire assembly put together as a whole:

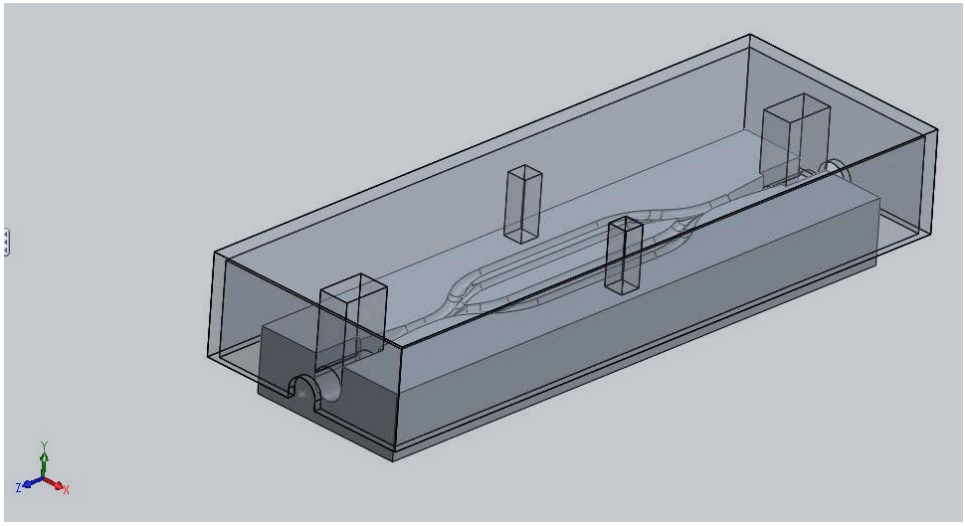

Figure 16: Revised Design, Complete Assembly

4.8 Rapid Prototype #3

A few changes were made to rapid prototype #3 (RP3). These changes were made because the device was still not water tight after the design of RP2. The semi-circular pegs that were press-fit around the tubing at the inlet/outlet were not sufficient in completely sealing the entry/exit points of the device. The silastic was also lifted off of the top surface of the device due to the middle posts applying a great deal of pressure at their footprint on the film itself. Semi-circular caps were used in place of the pegs as this still allowed for the press-fitting around the tubing and for the use of a biocompatible silicon adhesive. This adhesive ensured a water tight seal upon curing. More posts were also added under the cover in order to have a more distributed pressing force upon "clamping" or taping the cover to the base of the device. The top surface was also covered with the silicon adhesive upon which the silastic was place. This provided a strong bond between the two and completed the sealing of the device. RP3 is displayed in Figure 17.

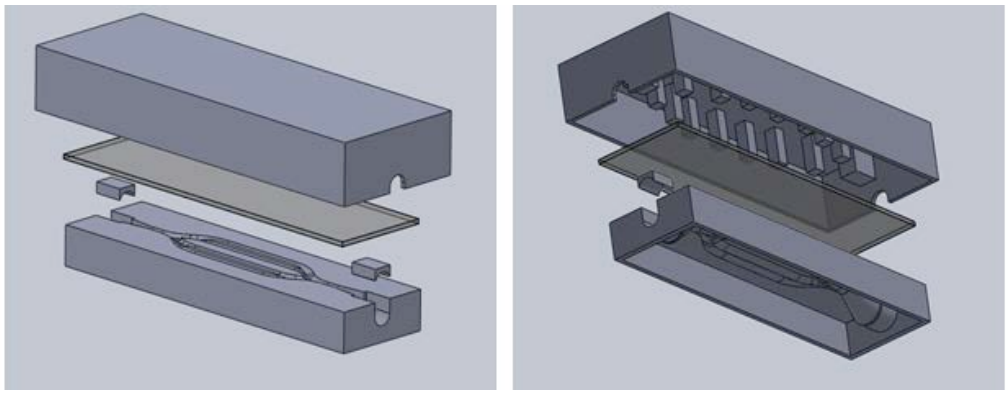

Figure 17: Final Bioreactor Design, RP3

Chapter 5: Design Verification

5.1 ANSYS Fluent Computational Fluid Dynamics Model

A Computational Fluid Dynamics (CFD) study was performed as part of the design verification process. This technique is widely applicable in the field of engineering and involves computer modeling software to solve flow fields. It does this by solving the underlying differential equations of the flow phenomena by using the finite volume method. The goal of this CFD analysis was to verify the device design by analyzing flow discrepancies, velocity hot and cold spots, and any undesirable turbulent areas.

5.1.1 Methodology

In order to conduct the CFD study, the Computer Aided Design (CAD) model of the device previously created needed to be exported. Before the export, however, it was necessary to invert the geometry so that the fluid volume is available to be interpreted by the CFD software. Since the model exhibits axial symmetry, the model was halved in order to save on computation time. The inverted fluid volume is represented in Figure 18.

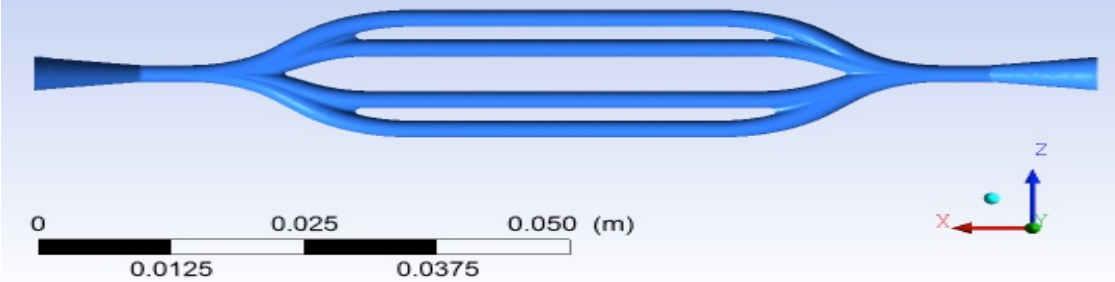

Figure 18: The inverted CAD geometry showing the fluid volume

The inverted CAD design was then exported. ANSYS Workbench 14.5 was the software package of choice for this CFD study, with the Fluent solver. The exported fluid volume was imported and the geometry was meshed using tetrahedral cells. The finished mesh was then interpreted by the Fluent solver and the study was set up. The default units for ANSYS are meters, so the mesh was scaled using a factor of 100 to convert from centimeters to meters. The general options for the solver were set-up, which included a pressure-based, absolute velocity formulation and transient flow study. The viscous model was activated with all other models left off, since no heat transfer or other phenomena was modeled in this study besides fluid flow. The viscous model was set to laminar flow because of the low Reynolds number due to the low velocity flow and the experimental results from the laboratory. The transient flow parameter ensures that fluent is still able to model any turbulent areas and vortices should they exist. The fluid material was defined as water, liquid and the predefined properties from the fluent database were utilized. The imported volume was set as the fluid zone, and the boundary conditions were defined. One end of the model was defined as a velocity inlet and the other end as a pressure outlet. The symmetry plane was defined as a symmetry boundary and the remainder of the model was defined as a wall boundary.

In order to monitor the convergence of the solution and verify the CFD result, solution monitors were set up. This included plotting of the solution residuals and plotting area-weighted averages of velocity at the outlet. A solution is converging if the residuals are decreasing and the area-weighted outlet velocities approach those of the inlet velocities after each iteration.

The solution was initialized with a gauge pressure of 0 pascals and a zero velocity vector. The solver was set to auto-save data after a reasonable number of iterations to create smooth solution animations at medium frame rates. The goal was to create equal number of data sets for the four different flow rates shown in Table 2, therefore the time needed to flow from the inlet to the outlet was divided by the time step size to calculate the auto-save intervals. The time step size was set to 0.1 seconds and the inlet velocity was set to one of the four values shown in Table 2.

Table 2: CFD Study Flow Rates

[ml/min]	**[m^3/s]**	**[m/s]**	**[m^3]**	**[s]**
Flow Rate	Flow Rate	Inlet Velocity	Volume Fluid Region	Time to flow from inlet to outlet
0.5	8.33333E-09	0.00030151	7.34E-07	88.093338
1	1.66667E-08	0.00060302	7.34E-07	44.046669
1.5	2.50000E-08	0.00090452	7.34E-07	29.364446
2	3.33333E-08	0.00120603	7.34E-07	22.0233345

The solution iteration was then started with the number of iterations set so that during the final iteration the flow-time is equal to or greater than the time it takes for one fluid molecule to travel from inlet to outlet.

After the iterations were completed, post-processing was performed to generate solution animations and graphics in order to analyze the results. Among others, animations and graphics of velocity contours, pathlines colored by velocity, velocity vectors, and vorticity vectors were obtained.

5.1.2 Results and Discussion

Several results were obtained. The first topic of discussion are contours of velocity overlaid onto the symmetry plane as shown in Figure 19. The velocity contours for the four flow rates are shown, starting with 0.5 ml/min at the top left and ending with 2 ml/min at the bottom right. It can be seen that velocities are equal throughout the 4 channels and no hot spots or stagnant velocity areas exist. Not that the velocity at the walls is zero since the no-slip wall boundary condition was used. The velocities were slightly higher at the necks of the inlet and the outlet due to the smaller diameter. This trend is consistent throughout all 4 solutions, where the maximum velocities are higher for higher flow rates, as expected. All solutions shown are at the final time step of iterations.

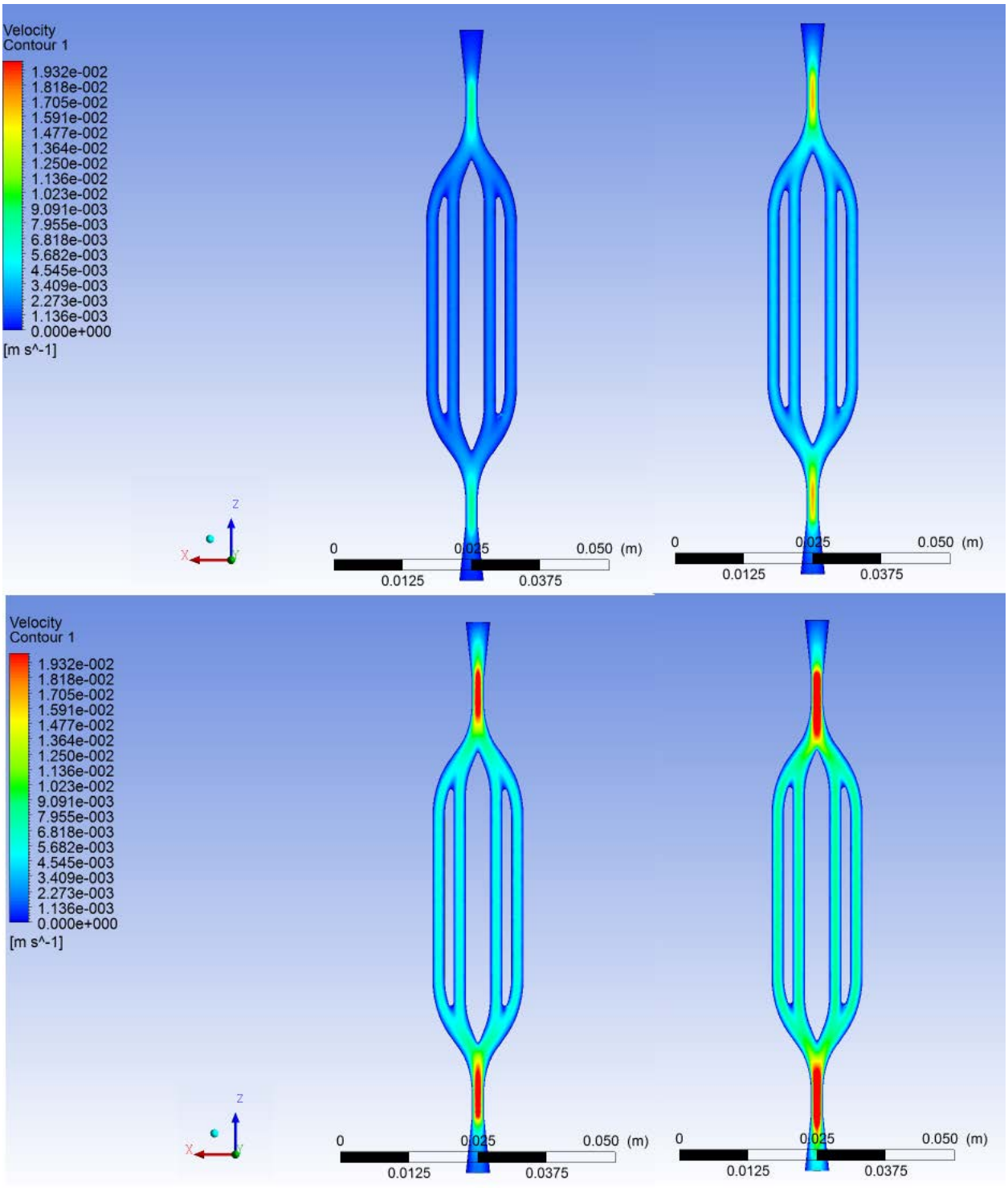

Figure 19: Velocity contours overlaid onto the plane of symmetry

Pathlines colored by velocity were also obtained from the results, displayed in Figure 20. Again, the graphics are arranged from the lowest flow rate in the top left to the highest flow rate in the bottom right. Pathlines are most valuable in identifying any possible area of turbulences and vortices. From the graphic, it can be seen that no turbulent areas exist and the flow is 100% laminar. From the

velocity contours, the inlet and outlet were identified as areas of elevated velocities which could have been a sign of turbulence, but the pathlines clearly show that this is not the case. While the velocities at the inlet and outlet are higher than in other parts of the device, relatively speaking they are extremely low and produce Reynolds numbers of well below 3,000. Again, as the flow rate increases so does the velocity, but no turbulent flow appears as a result.

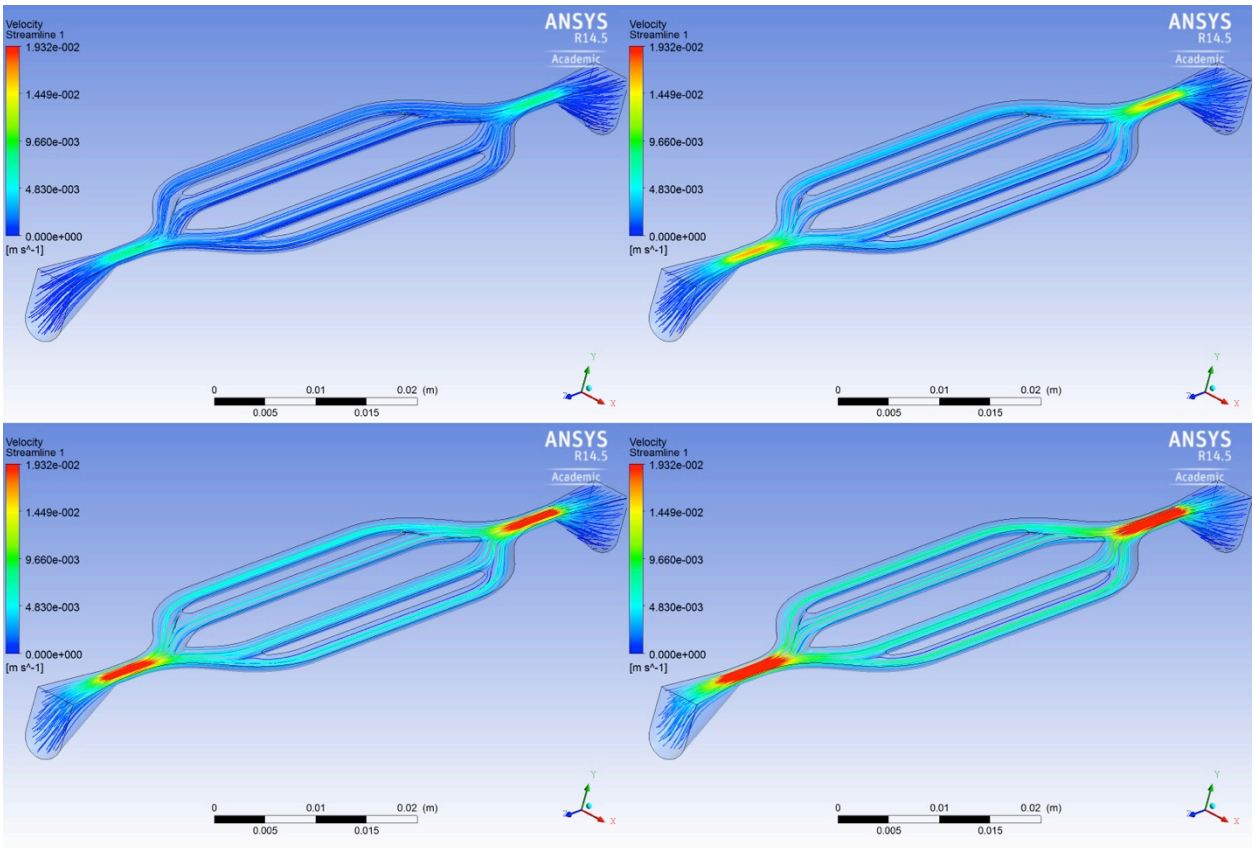

Figure 20: Pathlines colored by velocity

Next, vectors of velocity are considered. Velocity vectors are simply scaled vectors of velocity shown throughout the fluid volume to visualize the flow field and the fluid forces. These are displayed in Figure 21. Again, the different runs are organized from lowest flow rate on the top left to the highest flow rate on the bottom right. For the most part, velocity vectors are along the x-axis indicating laminar, undisturbed flow. When fluid particles change direction because of the 1 channel to 2 channels to 4 channels split, the velocity vectors point into different directions since a direction change has to occur for a fluid particle to travel into one of the 4 channels. Still, only minor velocity deflections occur and as shown by the pathlines no turbulence is created as a result.

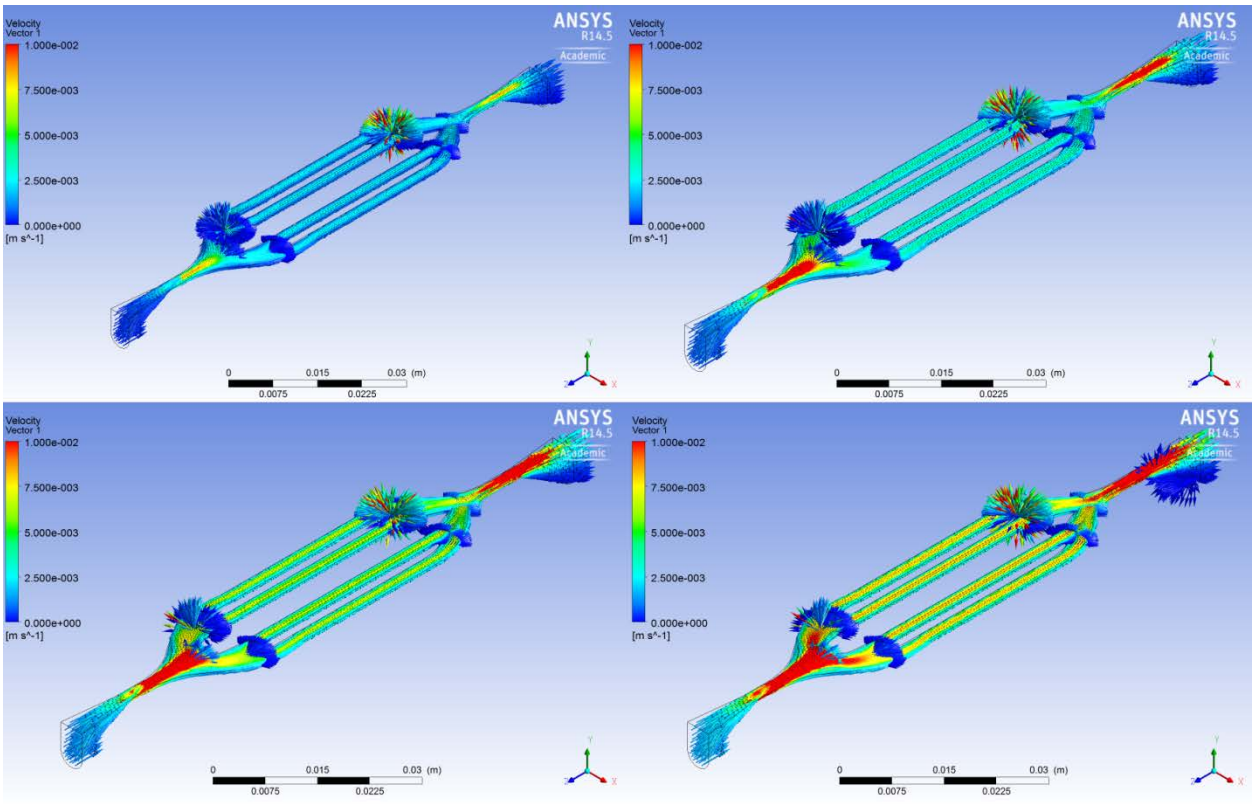

Figure 21: Vectors of velocity

Lastly, particle tracks are analyzed as displayed in Figure 22. These display the number and distribution of particles if released at regular intervals from the device inlet at the time of the final iteration. Again, the graphic is arranged so that the top left shows the lowest flow rate and the bottom right depicts the highest flow rate. It can be seen that particle distribution throughout the four channels is even, proofing that the manifold mechanism of the 1-into-4 distribution functions properly. Because these fictional particles were released from the inlet in the same interval regardless of flow rate, there appear to be less particles in the 2.0 ml/min flow rate than in that of the 0.5 ml/min flow rate. This makes sense because the higher the velocity is the faster the particles travel through the modeled device and exit through the outlet at a faster rate. It is important to remember that in actuality there are the same number of fluid particles in the device at all time since the fluid modeled is an incompressible fluid.

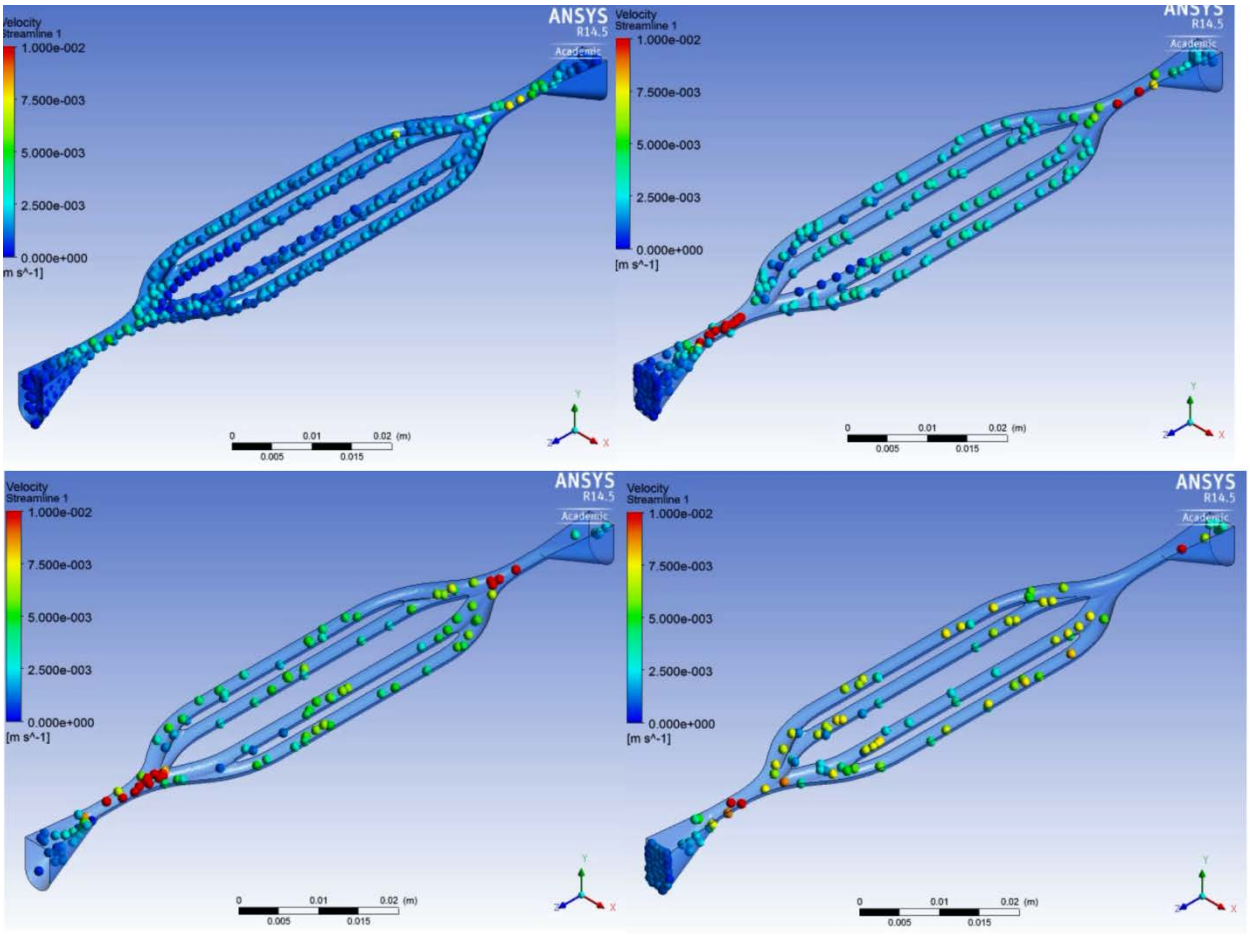

Figure 22: Particle Tracks

5.1.3 Conclusion

The effectiveness of the design was verified by this CFD study. The design was confirmed in that there were no velocity hot or cold spots in unexpected areas that could negatively affect cell seeding. There were also no sharp edges or cut-off faces discovered that could lead to design problems, both the 1-into-4 and the 4-into-1 manifolds had smooth curves which lead the fluid into the proper paths. No areas of turbulence were discovered, the entire fluid volume was laminar at the flow rates studied. The particle distribution was even throughout the 4 channels, leading to the most effective cell seeding possible. The CFD study confirmed this device and encourages further studies.

5.2 Final Design Testing: Experimental Methods and Testing

After analyzing the Fluent models and coming to a consensus that the current design would achieve our intended results (even distribution to all four channels and non-turbulent flow to avoid damaging the cells), the final design was 3D printed with a biocompatible material. The final assembly can be seen in Figure 23 and includes all of the components except for the tubing at the inlet and outlets.

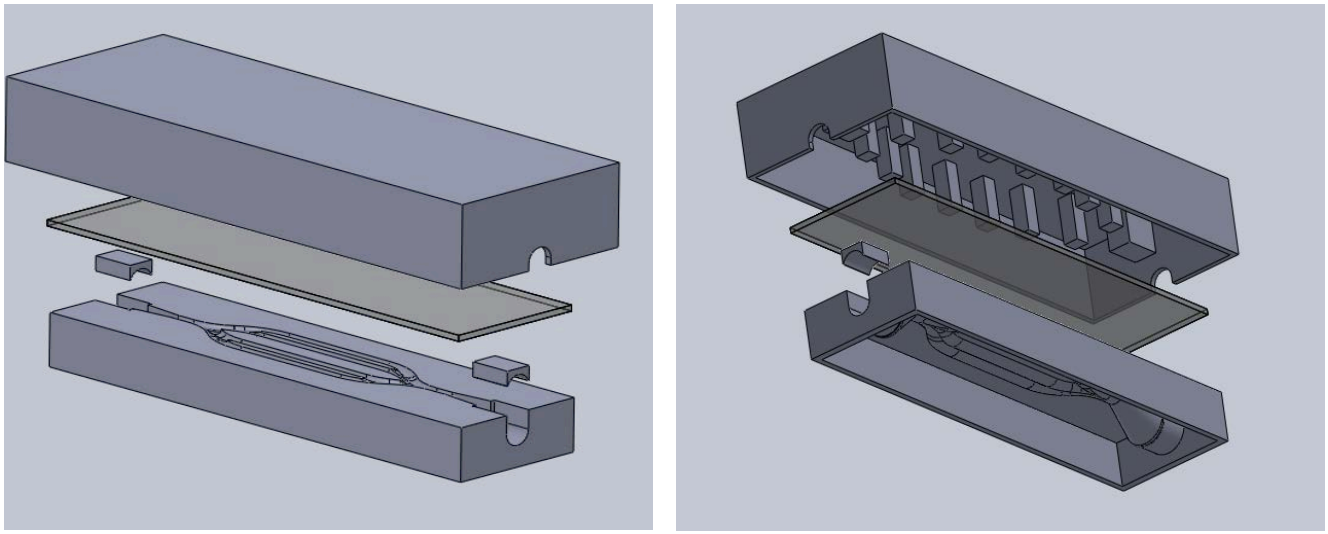

Figure 23: Final Prototype Assembly

5.2.1 Test #1 Static vs. Dynamic seeding time ratio

With different seeding strategies producing different results, the first experiment performed was a short test with variables of the "time seeded statically" and "time seeded dynamically." With this in mind, we decided on 3 different groups, the first group being a 1:1 static to dynamic ratio, the second being a 1:2 static to dynamic ratio, and the third being a 2:1 static to dynamic ratio. This would allow us to determine which proportion of seeding strategies to use for a longer seeding time in subsequent tests.

5.2.1.1 Programming of the syringe pump

The seeding technique would be controlled with the program SyringePumpPro and would be characterized in the following way:

Dynamic Seeding- is defined as any time the syringe pump was infusing or withdrawing media.

Static Seeding- is any time the syringe pump was not infusing or withdrawing media.

It was also decided that the infusion and withdrawal rate would be set to 0.5 mL/min and each group would undergo seeding for 1 hour. In order to create the program the amount of media would have to be defined beforehand and a volume of 6mL was chosen to create a cycle time of 12 minutes. A cycle was defined as the entirety of the media being infused or withdrawn into the device.

With the parameters defined, three programs were created to model these three scenarios. These programs are created through Microsoft excel and can then be converted into a .PPL file that the SyringePumpPro software would read and execute the proper commands to the pump (Figure 24).

An example of one of the excel files is shown below. In this, a command called "pumping rate" is partnered with a volume per minute, in this case 0.5mL/min and an infuse/withdraw option. These commands then comprise the time of dynamic seeding. A command called "pause" would then stop the pump for a designated amount of time. These commands would encompass the static seeding times. The amount of infuse/withdraw and pause commands could then be changed to satisfy the three seeding ratios.

New Era Pump Systems Inc. Any other usage, including reverse engineering, not authorized by New E
(631) 249-1392 **PUMPING PROGRAM CREATOR** V2.3

Any values in GREEN BOXES can be changed. ~~Strikeouts~~ indicate non-applicable parameters. RED boxes indicate invalid data.

Save this file with your project name, then select worksheet PPL and follow instructions to generate pumping program.

*** Note: This spreadsheet has only limited error checking. Verify that values entered are within pumping limits.

Phase Label	Notes	Phase #	Program Function	Pumping Rate, Value, Phase # or Label	Pumping Rate Units	Volume to be Dispensed (mL). 0 = Continuous Pumping	Time (minutes : seconds)	Pumping Direction
		1	Pumping Rate	0.5	mL/min	5.500		Infuse
		2	Loop Start		----			----------
		3	Pumping Rate	0.5	mL/min	3.000		Withdraw
		4	Pause (seconds)	60	----			----------
		5	Pause (seconds)	60	----			----------
		6	Pause (seconds)	60	----			----------
		7	Pause (seconds)	60	----			----------
		8	Pause (seconds)	60	----			----------
		9	Pause (seconds)	60	----			----------
		10	Pumping Rate	0.5	mL/min	3.000		Infuse
		11	Pause (seconds)	60	----			----------
		12	Pause (seconds)	60	----			----------
		13	Pause (seconds)	60	----			----------
		14	Pause (seconds)	60				----------
		15	Pause (seconds)	60	----			----------
		16	Pause (seconds)	60	----			----------
		17	Loop Count	2	----			
		18	Pumping Rate	0.5	mL/min	3.000		Withdraw
		19	Pause (seconds)	60	----			----------
		20	Pause (seconds)	60	----			----------
		21	Pause (seconds)	60	----			----------
		22	Pause (seconds)	60	----			----------
		23	Pause (seconds)	60	----			----------
		24	Pause (seconds)	60	----			----------
		25	Beep		----			----------
		26	Stop Pump		----			----------
		27	----------		----			----------
		28	----------		----			----------

Figure 24: Excel file to tailored control pump in 1:2 dynamic to static test

5.2.1.2 Device Preparation

Once the programs were created and converted to .PPL files the cartridge was then prepared (Figure 25). The devices, tubing, sutures, and silastic sheeting were sterilized the night before by Ethylene Oxide and brought into the biosafety cabinet where they would remain sterile when they were removed from the packaging. With the use of a silicone adhesive, tubing was inserted into the inlet and outlet holes and the two caps were then placed on top with more silicone adhesive to ensure a watertight seal. The tubing was cut at a length of 8 inches for the inlet hole; this would allow the device to attach to the syringe pump, which is placed outside of the incubator due to size restrictions. For the outlet hole, a length of tubing was cut to 20cm; this would function as a reservoir for the media that was infused through the device.

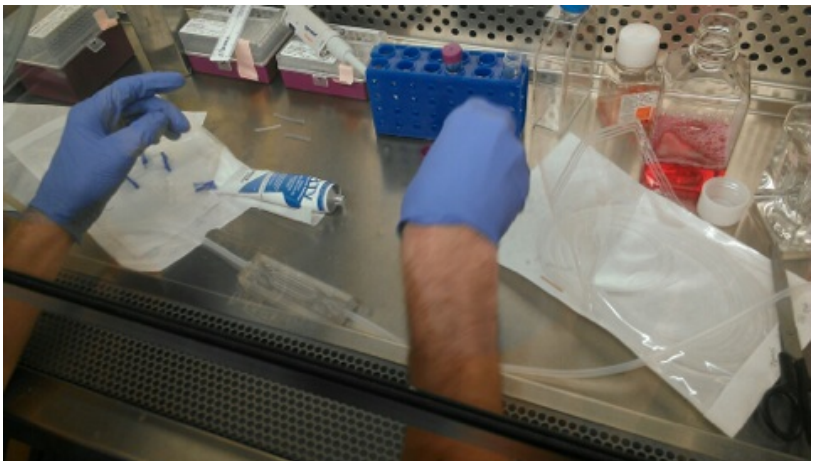

Figure 25: Workstation in biosafety cabinet

Next, the fibrin microthreads were then inserted into the silastic sheeting and spaced in a manner so they will lie in the channels when the silastic was placed over the device (Figure 26). The silastic was then carefully placed over the device in a manner so that the threads would lie down in the parallel channels. This was held down with the silicone adhesive to ensure that the entire device remained watertight.

With the device sealed and watertight, the final component is the cover. This was designed to apply pressure around the channels to help hold down the silastic while also protecting the user from the needles which are now protruding upward from the device. The cover also functions as a means to preserve sterility on the transfer from the biosafety cabinet to the incubator. The cover is then held tightly to the device with the use of tape to apply a constant pressure and ensure that it doesn't slip off when handling the apparatus.

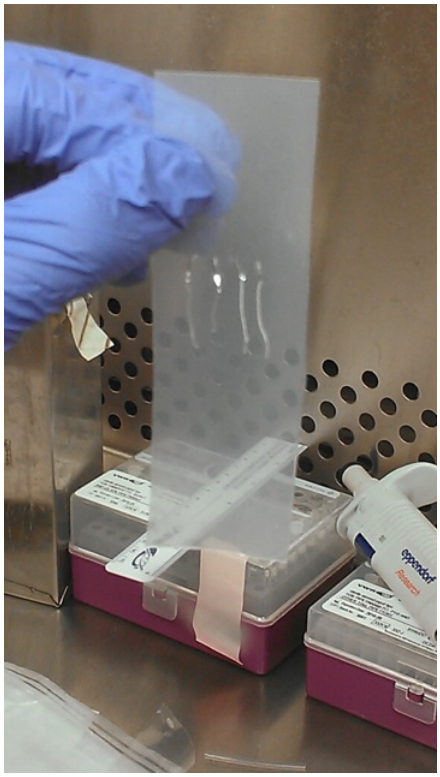

Figure 26: Sutures inserted into silastic sheet

With tubing attached, sutures in place, and cover over the device, the preparation of the cartridge is complete. Figure 27 shows the overview of a device ready for seeding.

Figure 27: Device after preparation

5.2.1.3 Cell Suspension Preparation

When the device assembly is complete, the silicone adhesive should be given ample time to dry to ensure complete water tightness and prevent slipping of any of the parts. While this is drying the cell suspension can then be created. For each device, a suspension with 400,000 cells in 6mL of media will be put into a 10mL syringe. This number of cells was chosen, as is the same number of cells per thread used in the current method.

This suspension is created by following the standard hMSC passage protocol and stopping when the suspension is centrifuged and a pellet is produced. From this, a cell count is taken and an appropriate amount of media is added to create a concentration convenient to isolate 400,000 cells. When the cells are isolated and put into a separate conical tube enough media is added to supplement the 6mL needed for the syringe pump program to run properly.

The final step before beginning the test is to hydrate the threads. This can be done by injecting PBS into the cartridge and allowing 5-10 minutes for the threads to hydrate. When this is complete the PBS can be withdrawn and all of the components for the experiment are ready to proceed.

5.2.1.4 Experimental Preparation

Once the device and cell suspension are prepared and threads are sufficiently hydrated they can be removed from the biosafety cabinet and into the incubator/syringe pump apparatus. In order to maintain sterility, Parafilm should be put over the syringe outlet and ends of the tubes when being transferred. The device is then placed into the incubator and the short length of tubing is fed through the hole in the back where it can reach the syringe pump. The cell suspension is then loaded into the syringe pump and the tubing leading to the device can be attached. Figure 28 shows the experimental setup.

Figure 28: Syringe Pump/Incubator Apparatus

Once the cell suspension is loaded into the pump and the device is attached to the syringe the program can be initiated.

5.2.1.5 Post-experiment procedure

When the experiment is complete, and the pump stops running after the designated time, the media can be withdrawn out of the device and the device can be removed from the incubator. It was then transported to the workstation where the threads could be stained. When the cover is removed it is evident that the threads were exposed to media, as they are now pink in color supporting the fact that our device worked as intended (Figure 29).

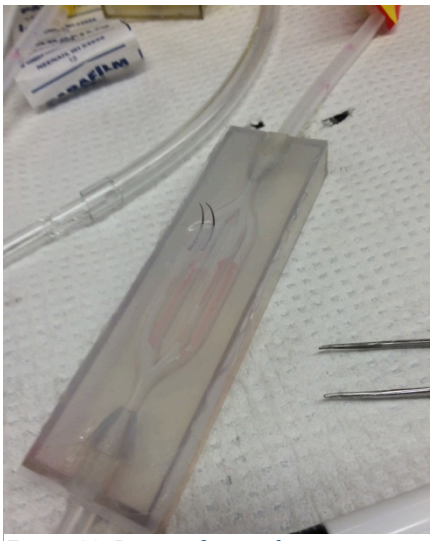

Figure 29: Device after seeding

To proceed with staining the threads must be removed from the device. This is done by simply cutting the silastic sheeting and removing it where it should have 4 sutures still attached to it. From here the sutures can then be removed with needle drivers and are ready to be stained.

Hoechst staining was utilized for this experiment to determine the amount of cells on the threads. Hoechst is a fluorescent stain, which highlights nuclei of cells when imaged. The staining setup and image of one of the microthreads is shown in Figure 30 and Figure 31.

Figure 31: Staining of microthreads

Figure 30: Hoechst stained thread with cell nuclei highlighted

5.2.1.6 Experiment #1 Results

Since the threads were only seeded for 1-hour, an extremely short time, high cell counts on the threads were not expected. Instead this test was designed to determine the best ratio of dynamic to static seeding time to be used in longer experiments. After staining the threads and analyzing them under the fluorescent microscope we were able to determine that the best ratio to use was a 1:2 dynamic to static ratio. These threads had the most cells on them when observed under the microscope so it was decided that we would proceed with this ratio for prolonged seeding time testing.

5.2.2 Test #2 Time Dependent Seeding

After deciding to proceed with a seeding ratio of 1:2 dynamic to static three times were chosen to seed the cells for an extended period of time. One control and two experimental groups were chosen. The control would be 1 hour like the first test; the two experimental groups would be 3 and 6 hours. Each group would consist of one device and four threads would be seeded on each.

5.2.2.1 Experimental Methods

Much like the first experiment, a program was created for the syringe pump. This program then had to be extended to run for 6 hours instead of the one-hour time interval in the previous test. All three devices would be seeded simultaneously and an audible beep would occur at 1 and 3 hours to signal that the corresponding device should be removed from the incubator.

The goal of this experiment was to quantify the amount of cells and see if the amount of time needed to seed the cells with this new method could be decreased from the current seeding time of 24 hours.

5.2.2.2 Experimental Preparation

The preparation for this experiment is identical to the first test with the only exception being that three devices will be prepared and seeded simultaneously. Once all three devices are prepared, the threads are hydrated, and 3 suspensions of 400,000 cells in 6mL of media are made they can be transferred to the syringe pump/incubator apparatus. Once everything is attached the program can be initiated and the experiment will run for the next 6 hours.

At the 1-hour time interval a beep will occur signaling that the first device can be removed. This will happen at 3 and 6 hours when the other two experimental groups are finished.

5.2.2.3 Post-experiment procedure

Like the first experiment, the devices were stained with Hoechst to be fluorescently imaged. This is done 3 separate times, immediately after each experimental group is removed from the incubator. The same thread removal and staining procedure from the first experiment is performed.

5.2.2.4 Experiment #2 Results

It was conjectured that the threads that were seeded for a longer time would results in more cells on the threads due to more opportunities for the cells to come into contact with the threads. However due to a very small sample size it is not practical to draw conclusions as to which time was better for seeding efficiency. When the threads were imaged a much smaller number of cells were found on the threads than anticipated making it difficult to quantify the exact amount of cells that were seeded onto the microthread.

Instead what we were able to obtain was images from multiple threads that showed cells were successfully seeded onto the suture and that this method of seeding is a practical and can achieve the intended function of the device and seeding method. Figure 32, Figure 33 and Figure 34 are images at various magnifications and seeding times depicting cells that had successfully attached to the fibrin microthread.

Figure 32: Thread at 10x magnification

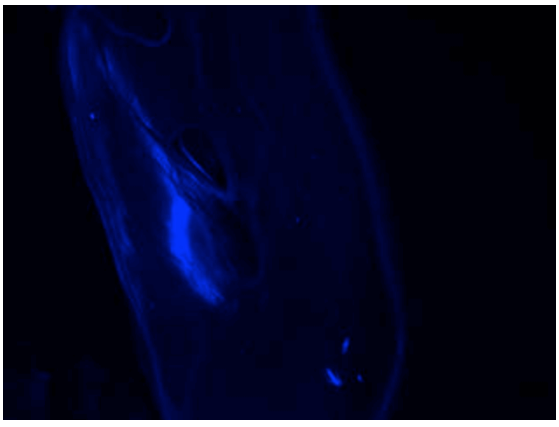

Figure 33: Thread at 5x magnification

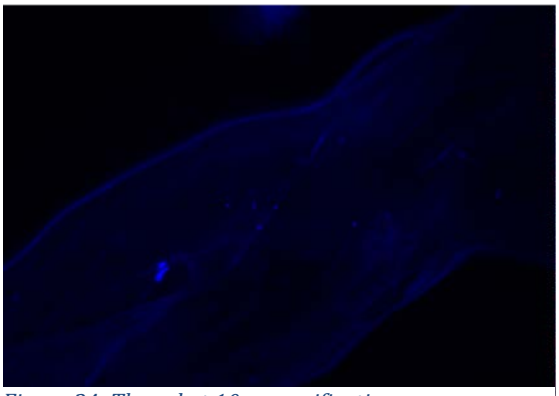

Figure 34: Thread at 10x magnification

Chapter 6: Discussion

After testing there was a basis to examine the effectiveness of the device. Looking back it was possible to reconcile the results of testing with the objectives that had been established early in the design process. The first objective laid out was safety. Having performed the seeding process using the method already used in the lab, the major safety concern that was noticed was the use of needles. During the original lab seeding process the user must regularly insert a 20 gauge needle into a piece of silastic tubing only 1-2 mm wide. This leads to regular pricking of the user's fingers, this sort of injury can also occur because in the former process the suture's needle is uncovered for much of the handling. The process designed for this project alleviated both of these issues. The new device does not require the use of a 20 gauge needle so there is no risk of injury from one. As for the suture needles they are placed through the silastic film, meaning the film can be handled instead of directly manipulating the needle. The needles are also covered during the seeding process by the lid for greater safety. The device also needed to be safe for cells. The device was constructed out of biocompatible material and thus was assumed to be safe, however, the process added factors that could potentially harm cells. Tests showed that when the media from the 6 hour test was placed in a T25 flask and replaced in the incubator that on inspection days later there were living cells adhered in the flask. The suggests that the cells are alive and viable for further use after the seeding process and thus are not seriously harmed during the seeding process.

The next objective was that the device be easy to use. The point of comparison is of course the current seeding method. The current process is very labor intensive and requires a great deal of time on each tread which must be prepared individually. The new device allowed for the simultaneous preparation of 4 sutures at once. The new process removes the high dexterity procedures such as the insertion of a needle into thin silastic tubing. The most difficult part of the new process is using the silicone adhesive to ensure a water tight seal, if this is not performed correctly it can lead to leakage during the seeding process. The device is extremely portable which will be particularly useful for transfer of the sutures to surgery. There is currently a major drawback in the ease of use of the device and that is the cleaning process. The silicone adhesive used to maintain the seal of the device is very strong and is difficult to remove from the device after use. The sutures can easily be removed by cutting the silastic film and removing the portion containing the threads. The remaining silastic is difficult to remove and requires a fair bit of labor, the remaining silicone residue can also be arduous to remove. These ease of use for the device will come with more iterations and practice, currently it provides the opportunity to prep 4 sutures with little difficulty. The device also interfaces with equipment already in use including compatibility with the lab's syringe pump and incubator. The advances this device provides for prep and transport are forth the added effort needed for cleanup.

The testing done could not confirm that cell counts would be consistent from suture to suture but the analytical data collected suggests that conditions within the device are relatively similar from channel to channel. Due to a lack of cellular testing the Fluent modeling of the device is the best source of information on the consistency of the device. What the model shows is that the flow profiles through each cannel look very similar. This suggests that in testing applications the 4 threads will see extremely similar cell concentrations and flow velocities. These assumptions are support by the particle distribution tests in the model which show no obvious bias of the particles toward any channel in particular. The conclusion to be drawn from this is that very similar cell seeding numbers can be expected in all sutures seeded in the same device. As for consistency from

trial to trial, this is best achieved through standardization of the lab protocol and the minimizing of variables that could change the outcome of the trial. One such factor is the construction of the model. These are done through 3D printing and with the high level of dimensional accuracy and minimal user input there is little room for variation from one model to the next. Another way in which the process suggests consistency is the way in which the syringe pump is used. First the actions of the pump are controlled by a pre-made computer program and if the program is unchanged from trial to trial this will limit any variation in the fluid dynamics between tests. The fact that the syringe pump is capable of holding multiple syringes at once means that more than one model can be used at a time without any variance in the pump. The largest area of variance is the formation of the threads but this factor was not within the scope of this design. The process designed by this project was created with a high level of consistency.

The device was also created with the efficient use of resources in mind. The most valuable resource to conserve through this process are the cells. The current method of seeding does not allow for the reuse of cells that do not adhere during the seeding process. In the current technique that means about two thirds of the cells are wasted. The new design allows for the cell suspension to be withdrawn from the device at the end of the seeding period and can be placed into a T25 flask. The cells were seen to have adhered and this means they were still viable after the seeding process. Further tests would need to be done to establish the health of the cells and to determine their sterility. If the cells passed these tests they could be reused in later seedings. This has the potential to seriously increase the efficiency of cell use and preserve a vital resource.

Time is always an important factor when performing an experiment. The first time element to consider is prep time. The ability of the device to take 4 sutures simultaneously will lead to faster prep times. Especially because in the current surgery procedure 4 sutures are needed, thus the use of one model can efficiently provide the necessary sutures for one surgery. The seeding time is where the majority of the time is used. There is insufficient cell data to speak to the ability of the device to provide similar seeding efficiencies at lower times. The advantage the device has is the ability to adjust seeding time and ratios easily through the use of the syringe pump pro software. The program can easily be modified to change the static and dynamic seeding times. A one hour sequence can then be created and looped any number of times to create a full seeding period. This is a more precise method than simply timing a seeding while the threads are in a rotator because it keeps the exact mechanics the same from test to test. This also makes it easy to test different times and through this process and ideal ratio and time could be discovered that would ideally cut down time from the current method.

The final objective set forth was for the device to be effective. In order to consider the device effective it was necessary to prove that the design could actually load cells onto threads. The proof of concept testing that was performed with cells validated the ability of the device to do this. The results of the Hoechst staining show that with incubation times as low as one hour there were still cells present on the device. Throughout the testing the device performed mechanically as expected and remained fully operational after testing. The fact that the Hoechst staining then indicated the presence of any cells constitutes a successful test. These results show us that the device is a functional platform from which the Gaudette lab will be able to optimize their seeding procedure. The cell counts were low on the threads likely due to the low seeding times used but no actual cell count was determined. Due to time constraints and the scope of the project the loading of any cells was sufficient to classify the device as effective.

Chapter 7: Final Design and Validation

As mentioned the client desired a novel bioreactor to seed human mesenchymal stem cells onto to fibrin microthreads in order to regenerate heart function after a myocardial infarction. This bioreactor and process in which seeding occurred should decrease cell seeding time and increase the efficiency and consistency of seeded cells over the current method. The group went through a design process including conceptual and preliminary testing in an attempt ensure this desired functionality was met.

7.1 Design Process

The team followed the Gantt chart shown in Appendix C for the design process. Conceptual designs were established from which two main ideas were drawn: the device needed to act as a removal cartridge and a pumping system was going to expose the threads to the cell suspended media in a dynamic and static fashion. The pump chosen was a programmable dual syringe pump that allowed for control over infusion and withdrawal rates and allowed for controlled dwell times for static seeding. The team decided that the removable cartridge was going to incorporate some sort of channel system in this stage of the design process. Parallel channels were chosen over in series channels because the former allowed for each thread to have an equal opportunity to be exposed to media at the same time for the same duration of time. What follows are the progressions the team made for this removable cartridge leading to the final design and how it has been verified.

7.2 Rapid Prototyping- 3D Printing

Using the computer aided design software SolidWorks and the Objet260 Connex (3D printer) a series of prototypes were created for conceptual and preliminary testing. Figure 35 shows the teams flow through prototype designs.

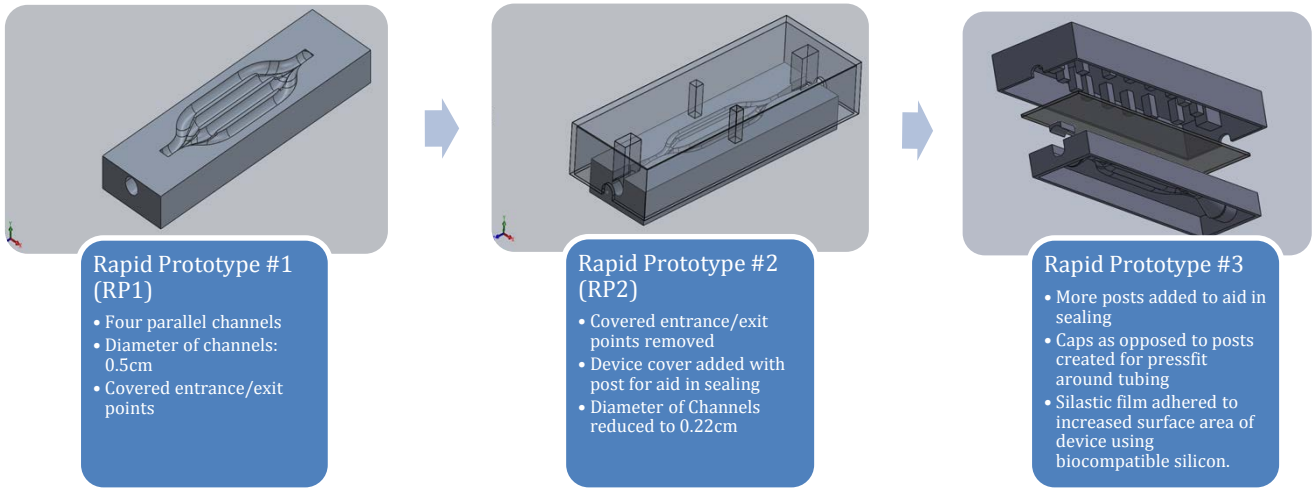

Figure 35: Flow through prototypes

Our first prototype (RP1) utilizes four parallel channels with a diameter of 0.5cm. This device was not finished with a gloss since the points of entry/exit were covered by an upper surface layer inhibiting the Objet260 connex from applying this finish.

Rapid prototype #2 (RP2) was constructed as a result of the flow testing experiment on RP1 which will be discussed below in Table 3: Experimentation Breakdown. The cover at the entrance and exit points was removed so the glossy finish could be applied so the roughness of the channels could be reduced. These covers were replaced by posts under the newly designed device cover that had a semi-circular bottom to finish the circular entrance and exit. This allows for a press-fit application around the tubing to increase the water tight seal at those locations. The cover was added to the device to increase user safety as it would enclose the exposed needles and ensure the maintenance of sterility during transport. The diameter of the microchannels were adjusted to decrease the total volume of the device and to increase the surface area of the non-channelized surface. This increase in surface area gave more adhesion points for the silastic film and allowed for enough footprint space for the added posts in the middle of the device under the cover. The posts aided in the sealing of the device and also prevented rotation of the cover itself.

Rapid Prototype #3 (RP3) was constructed after manual flow testing was conducted to observe the degree of water-tightness. Leakage of water was still observed on RP2. This final design added more posts under the cover aligning the channels entirely to decrease the "lift off" effect that one post caused. The force of one post depressed the silastic film at the post base and lifted the surrounding area of the film where posts were not located under the cover in RP2. In addition the semi-circular finished posts were replaced by semi-circular caps on to which silicon adhesive could be used to seal the entrance and exit points. They were separated from the cover so that the accessibility of the fibrin microthreads after seeding was easy and did not involve exerting a great deal of force to remove the cover. The silastic also was adhered to the surface and was then cut off used a blade around the channels to allow for ease of access to the microthreads. This final design could be rigorously cleaned and used again, however, the team designed this device to be disposable. Preliminary testing on this final design is discussed in Table 3.

7.3 Breakdown of Experimental Methodology

Conceptual testing and preliminary testing was conducted on each device to different degrees. Flow testing using a controlled system, manual system and a modeled system were all conducted. In lab testing with hMSCs was also conducted to attempt to collect and analyze seeding data. This was separated into testing of dynamic vs. static seeding and seeding time comparison. Table 3 presents a summary of each of these testing methods and what was observed and adjusted on the tested prototype and parameters.

Table 3: Experimentation Breakdown

Experiment	Prototype	Testing Observations	Adjustments Made on Prototype/Experimentation
Flow Testing: Using High-speed camera and Soda lime microspheres	RP1	• Laminar flow observed • Even distribution of particles to all four channels • Rough surface of channel lead to embedment of microspheres in striations • Device was not watertight • Channels too large in diameter	• Inlet/outlet upper surface removed to allow for glossy "finish" to reduce striations • Incorporation of cap to decrease risk of contamination during transport and to aid in watertight seal • Decrease in channel size decrease volume of device
Water-Tight Testing: Manual Flow	RP2	• Device was not water tight, leakage was observed at inlet/outlet and at point of contact of the middle posts on the silastic • No bubbles formed as water was infused and withdrawn through the device	• Caps replaced posts to allow for silicon adhesion of tubing-cap interface at entry/exit points • Additional posts added under cover to allow for more even pressure application along the channels • Use of biocompatible silicon adhesive on non-channelized surface of device to ensure water tight seal • Next prototype printed using biocompatible material
AnsysFLUENT Modeling	RP3	• Laminar flow with no stagnation point or areas of turbulence • Even distribution of particles to each channel with equal velocities at a flow of 0.5mL/min	• No adjustments were made on the device based on this testing because this model validated our concept in terms of even, laminar flow and even particle distribution
Ratio Testing for Dynamic vs. Static seeding	RP3	• Longer static seeding time more beneficial for cellular adhesion on threads • Experimental set up more user friendly than current protocol • Mechanical functionality of the device proved • Cells were seeded after very short incubation period • Viable process as cells that did not adhere where plated post	• Dynamic to static ratio 1:2 will be used for longer seeding time testing

		seeding and exhibited further proliferation	
Seeding Duration Testing (1,3,6hrs)	RP3	• More cells observed on 6hr seeding cycle • Cells can be recycled and used again proving process to be resource efficient in terms of cells (contingent upon contamination testing)	<u>Final Conclusions</u> • Platform of seeding process to be passed on to Gaudette Lab at WPI for optimization • Incubator compatible syringe pump could lead to increased cells seeding on bioscaffold • Test longer incubation times (8, 12, 16, 20hrs) • Incorporate different dynamic: static seeding ratios to find optimal relationship

Chapter 8: Conclusions and Recommendations

The procedure developed in this project is an effective platform which can be optimized to improve the performance of cell seeding in the Gaudette lab. The tests done showed that the design was usable but did not provide the necessary seeding quantities for implementation in the laboratory setting. What was accomplished was the ground work for a system that can be utilized effectively. The experimental method and the device design have been validated as useable. However, there are a great deal of variables that need to be refined in order to maximize the efficiency of the prototype and the seeding process. Further testing could be used to determine the most effective ratio of static to dynamic seeding. Ideally these tests could be done with a long total seeding time to ensure that cell attachment numbers would be large enough to quantify and compare. Once the ideal ratio was empirically determined, testing could be used to find the seeding time that both exceeded the needed number of cells per suture and provided the best ratio of cells seeded per unit time. These tests would help to determine the way to maximize the return on resources and time from the seeding process. Also testing should be performed on the cells that can be plated after the completion of the cell seeding process. Testing is needed to ensure that these cells are not damaged or contaminated in any way before they could be recycled for future seeding.

The design of the device was functional in proof of concept testing and was seen to perform as expected from a flow perspective as well as in its ability to maintain a water tight seal. The main issue is that the seal relied too heavily on the generous use of the silicone adhesive. The adhesive was very strong and this made it very good for sealing any poor interfaces between pieces of the device. Removing the adhesive proved to be quite difficult. This is an issue as it limits the ease of use of the device and may lead to undue wear and tear on the device through multiple uses. Future research may focus on eliminating the need for this adhesive through a change in the design of the model itself that helps to create a tight seal. The design could also be modified to just more easily facilitate the use of the adhesive to ensure the adhesive does not interfere with the functionality of the device. In lieu of either of these options, the process could at least be improved through the development of a better means to remove the silicone adhesive from the device.

The results of this project were a cartridge and paired system by which cells can be loaded onto fibrin microthreads. There is still a great deal of testing and modification that can be done to improve the system. This project represents the foundation of this platform and can be expanded on by future projects to produce even better results.

8.1 Impact of device

8.1.1 Economics

The short range economic effects would be felt by the Gaudette lab itself. This project has the potential to save a great deal of resources that are normally used during the cell seeding process, including time. By cutting down waste, particularly of the hMSCs used within the lab, costs for the seeding process and thus the associate research can be cut down. There are also more long range effects if this device helps to bring the loaded fibrin suture therapy to the clinical setting. In this case the economic impact is brought straight to patients. These patients would be put in position to save considerable amounts by cutting out years of therapy that controls their condition with a one-time surgery capable of curing their condition.

8.1.2 Environmental Impact

The main way that this device impacts the environment is trough reusability. In its current form the device can be reused over and over again and this will help to limit the plastics waste of the lab. Once the model is created the main resource that will be used up is silastic filming which is damaged during the process and cannot be reused future iterations of the device could prevent damage to the silastic and allow for it to be cleaned and reused with the rest of the device. Again here the ability to recycle resources like hMSCs from the process is valuable.

8.1.3 Societal Influence

The device may not directly affect society but if it helps build the research of hMSC loaded fibrin sutures it would be significant once the treatment hits the market. The introduction of a therapy that is capable of regenerating damaged heart tissue would be ground breaking in the US. This would help to extend the lives of many Americans who suffer from heart disease and are put at significant risk for heart failure. At some point almost every American will come in contact with someone who suffers or even dies from heart disease. The ability to fight back against these illnesses would have far reaching effects through society.

8.1.4 Political Ramifications

The major politics involved here relate to device regulation. The system designed here could potentially add a needed layer of consistency to the research of this therapy. Consistent results will help to show the safety of this treatment and help to bring it to the clinical setting. Ideally the research gained from this system will not only be used to show compliance with FDA regulations but also regulatory industries around the world. This, however, brings in another political matter and that is the way in which medical therapies appear in international markets and those decisions are effected by the governments and regulatory agencies of the foreign countries involved.

8.1.5 Ethical Concerns

This device helps to avoid the ethical concerns that are often associated with stem cell research. In this research the only stem cells used are human mesenchymal stem cells which are retrieved from full grown adult donors with no negative side effects to them beyond pain at the injection size. The usual concerns come with the use of embryonic stem cells because of the way they are derived, no such issue exists with hMSCs as they are present in usable form within the adult human body.

8.1.6 Health and Safety Issue

There are a great deal of issues that must be considered with any proposed medical treatment. There are a few that can be directly associated with the system developed in this project. The first issue is sterility. In all medical implants, and especially where cell culture is present, contamination is a major concern. In the process performed in this project all the pieces that came in contact with cells was sterilized and the utmost care was taken to maintain that sterility throughout the seeding process. If the batch if contaminated in any way, it could lead to the incubation of bacteria during the seeding process instead of hMSCs which would make it extremely dangerous to a patient. This brings up another factor related to the seeding process, quality control. It must become possible to check the final product of the seeding process before use to ensure that the treatment is going to be both safe and effective. This is benefitted by the 4 suture batches which allow the testing of 1 suture to shed light on the status of 3 other sutures.

8.1.7 Manufacturability

The current method by which the seeding cartridge is created is 3D-printing this allows for the creations of models in a relatively short amount of time with a high level of dimensional accuracy. This is reasonable as long as the cartridges are being made in small quantities and being reused for extended periods of time. If there is to be a large amount of cartridges made or if the lab would prefer to switch to a one use only system for the cartridges then it would become necessary to find a new means of production. If the amount to be made is sufficiently large it may be possible to create a mold of one and have a bulk order of the cartridges made by a third party manufacturer.

8.1.8 Sustainability

The design of the device uses little material or resources that would make it difficult to sustain. Much of the apparatus in use is completely reusable and many other components used in the process are recycled. The only loss of material comes from the silastic film which poses no serious risk of limiting the long term use of the device. The cartridge base and tubing are reused and the other components are common lab equipment which is often times reused or recycled. The demands of this product are very little beyond the normal day to day operations of the lab.

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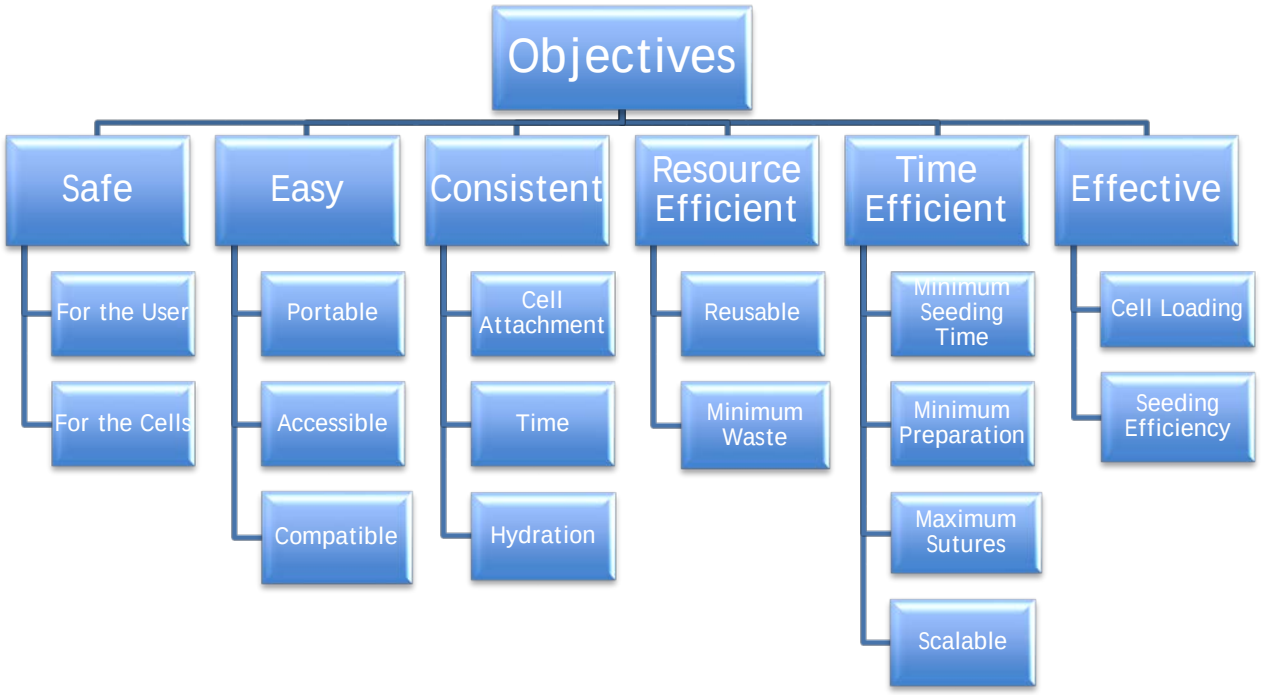
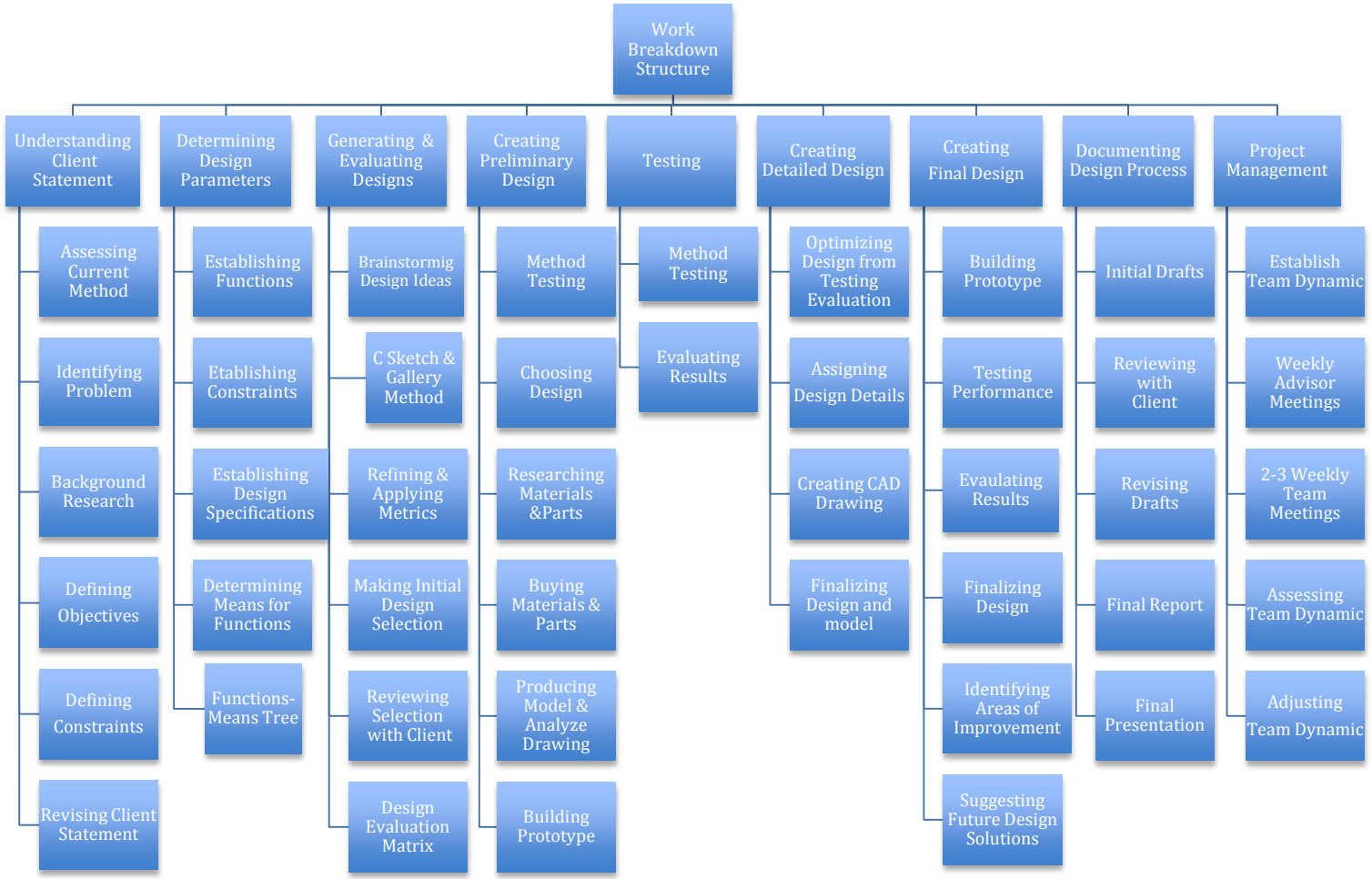
Appendices

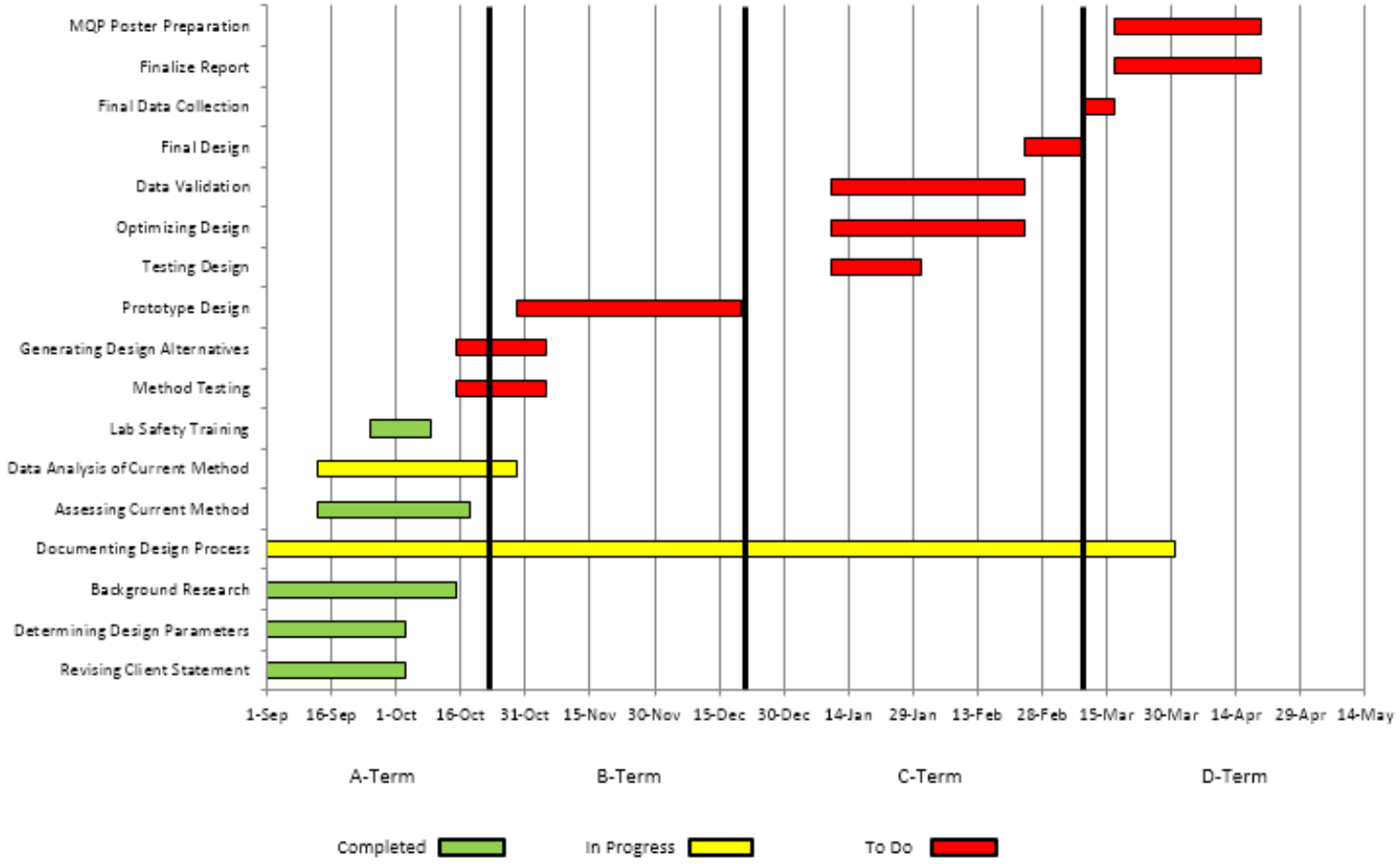
Appendix A: Objective Tree



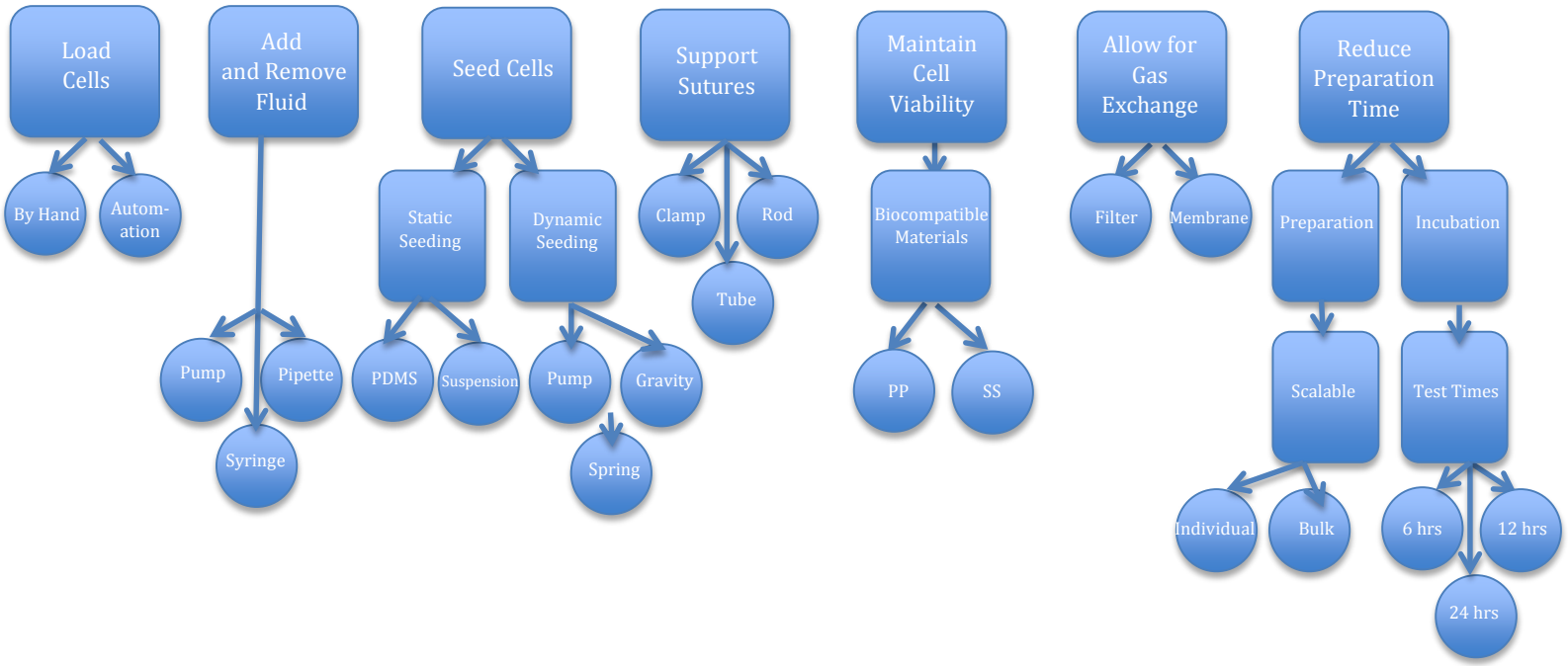
Appendix B: Work Breakdown Structure



Appendix C: Gantt Chart



Appendix D: Functions-Means Tree



Appendix E: Design Concepts/ Evaluation Matrix

Design Concept 1: Pump moves fluid repeatedly over threads sitting in a PDMS mold that is contained within a gas permeable cell culture plate that has been altered to accommodate the pump.

Design Concept 2: Threads hung from a rod in a large cell culture flask and submerged into cell suspension within the flask. A magnetic stirrer is used to create a gentle flow throughout incubation.

Design Concept 3: Threads are hand loaded into conical tubes with fluid and are rotated on varying axis during the incubation period.

Design Concept 4: Threads are hung from a rod in a box with a reservoir of fluid above. The fluid slowly drips down the treads over a period of time and the fluid is collected at the bottom for disposal.

Design Concept 5: Design Concept 5: Using a dual-syringe pump, media can be pushed and pulled over removable piece with fibrin microthreads places in parallel or series.

	Design 1	Design 2	Design 3	Design 4	Design 5
Safe	Yes	Yes	Yes	Yes	Yes
Easy	Yes	No	No	Yes	Yes
Consistent	Yes	Yes	No	Yes	Yes
Resource Efficient	No	No	Yes	Yes	Yes/No
Time Efficient	Yes	Yes	No	No	Yes
Effective	No	Yes	Yes	No	Yes

Appendix F: Metrics on Objectives

Objectives	Excellent - 1		Good - 2		Average - 3		Poor - 4	
Safe	**Cells**	**User**	**Cells**	**User**	**Cells**	**User**	**Cells**	**User**
	> 95% viability	No exposed moving parts, sharp edges, or wires	> 90% viability	Some exposed moving part. No exposed sharp edges or wires.	> 85% viability	Some exposed moving parts and sarp edges. No exposed wires.	< 85% viability	Very Dangerous
Easy	reproducible process minimal training automated process		reproducible process some learning curve semi-automated process		variations in process significant learning curve manual process		poor user interface extensive training manual process	
Resource Efficient	small footprint minimal waste low energy use		small footprint some waste low energy use		small footprint considerable waste low energy use		large footprint excessive waste energy inefficient	
Time Efficient	< 12 hours		< 18 hours		< 24 hours		> 24 hours	
Consistent (SEM)	± 10%		± 12%		± 15%		> ± 15%	
Effective	>16,000 Cells/cm >33% efficiency		>13,000 Cells/cm >25% efficiency		>10,000 Cells/cm >20% efficiency		<10,000 Cells/cm <20% efficiency	

Appendix G: Protocols for Cell Culture

Media Preparation Protocol

Materials

- Lonza DMEM (Dulbecco's Modified Eagle's Medium)
- PAA Labs FBS (Fetal Bovine Serum)
- GIBCO® Pen Strep (Penicillin-Streptomycin Solution)
- L-Glutamine
- Drummond® Pipette Aid
- Sterile VWR™ Serological Pipettes 10ml
- Sterile VWR® 55mL CENTRIFUGE TUBES WITH SCREWCAPS (conical tubes)
- ISOTEMP 210 Fisher Scientific (Water bath)
- Thermo Forma Class II A/B3 Biosafety Cabinet
- 70% Ethanol spray bottle
- Haier® Refrigerator with -20°C Freezer

Reference

1. *Protocol for general aseptic technique*
2. *Protocol for thawing and plating*

Preparation

1. General practice points:
    1.1 After each use of a micropipette tip, discard the tip into a biohazard bag
    1.2 Each subsequent use of a micropipette requires a new tip.
2. Place DMEM, FBS, and Pen Strep containers in Water bath.
3. Once containers have reached the temperature of the water bath, remove them from the Water bath.
4. Spray the containers with 70% Ethanol.
5. Wipe the containers down with Kimwipe and respray with 70% Ethanol.
6. Steriliy place DMEM, FBS, and Pen Strep containers at back of sterile Biosafety cabinet.
7. Using a sterile serological pipette, take up 60mL of DMEM and dispense it into an empty sterile 55mL conical tube.
8. Using a different serological pipette, take up 5mL of Pen Strep and dispense it into the DMEM bottle.
9. Using a different serological pipette, take up 5mL of L-Glutamine and dispense it into the DMEM bottle.
10. Using a different serological pipette, take up 50mL of FBS and dispense it into the DMEM bottle.
11. Close containers.
12. Place the DMEM bottle and conical tube with DMEM medium in to refrigerator.
13. Dispose of biohazardous materials properly (example used pipettes and pipette tips).

Protocol for Feeding Human Mesenchymal Stem Cells

Preparation

1. Wash your hands when you enter the lab. General lab safety measure.
2. Put media in the water bath.

Verifying Cells are Healthy

3. Take cell culture flask out of the incubator. Take care not to tilt the flask, the media should not enter the neck of the flask.
4. Inspect the media visually:
    a. Color: - should be dark pinkish red. If yellow / yellowish orange – immediate action – change media / discard cells.
    b. Transparency: Cells in good health show transparent, clear media. If cloudy → sign of contamination / aging / dying cell culture.
5. Microscopic examination: Examine the cell culture flask under an inverted microscope; first under low magnification (usually 4x) and then under medium magnification (usually 10x). Things to look for:
    a. Floating cells (dead or unhealthy), cellular debris, bacteria or fungi, other unidentifiable debris → signs of unhealthy culture. Immediate action necessary:
        i. If contaminated – suction out all media into waste, spray the inside of the flask with alcohol, suction the alcohol, and then discard in biohazard.
        ii. If unhealthy, but not contaminated – change media with fresh, warm culture media. Examine again after 24 hours and feed with fresh media again.
    b. If cells are nicely spread out (adhered), look for vacuoles within the cells. Presence of too many vacuoles is an indication that the media needs to be changed.
    c. Confluence of culture → Look at atleast 5 locations within the flask, usually four corners and the center. Calculate confluence and note it down. For 80 – 100 % confluence – passage the cells. For cultures older than 14 days – passage the cells, irrespective of confluence. For the rest, replace old media with fresh media (which is referred to as "feeding the cells.")

Preparing the Hood

6. Take cell culture cart from lab bench area to cell culture room, along with 1000 µL micropipette / pipette aid (depending upon flask size), corresponding sterile pipette tips and other items as necessary.
7. Put gloves on, spray hands with alcohol. (do this every time something unclean/not sterile is touched)
8. Spray the inside of the laminar flow hood (working surface and bottom third of the side walls) and wipe down. Make sure to clean the vacuum line also, by spraying both the outside and inside of the tube with the vacuum turned on.
9. Spray and wipe all objects that you intend to take inside the laminar flow hood. Be careful not to spray the cap of the flasks the cells are in.
10. Attach the Pasteur pipette on the vacuum tube and place it in a manner that the tip doesn't touch any object while you work in the hood. Set up all other items in the hood.

11. Bring warm media from the water bath and the cells from the incubator. WIPE DOWN MEDIA AND CELL CULTURE FLASKS THOROUGHLY WITH ALCOHOL, as the water bath and incubator are common sources of contamination.

Feeding

12. Unscrew the flask cap and place it in a way that its inner side doesn't touch anything while you're working on the flask. Tilt the flask so as to accumulate the media in one corner of the closed end of the flask (opposite the open end through which you insert the Pasteur pipette).
13. Insert the Pasteur pipette slowly taking care not to touch any inner walls of the flask. Suction the media out from the cell culture flask taking care not to touch the bottom surface, where the cells are attached.
14. Add required volume of fresh, sterile, warmed media with a micropipette/pipette. Take care not to touch any part of the flask (especially inner surface of the neck) with the pipette tip while dispensing media. If the tip touches any surface accidentally, discard the tip and use a fresh one. It's important not to contaminate the stock solution of media, therefore use only a fresh, sterile tip to aspirate media from its storage bottle.
15. Cap the cell culture flask immediately after media is added screw the cap on tight. Return the cell culture flask to the incubator. (As a general rule, try keeping the cells out of the incubator for as short a duration as possible: mammalian cells like to be in a 37^{o}C-environment – that of the incubator).

Clean-up

16. Cap all bottles (media, sterile PBS, other) tightly, remove all objects from the laminar flow hood. Spray the inside of the laminar flow hood with alcohol, and wipe down. Be sure to clean the vacuum line as well.
17. Switch off the light of the laminar flow hood. (DO NOT turn on the UV light unless explicitly instructed to do so).
18. Return all items to their designated storage places.
19. Update Cell Culture inventory / inform your mentor.

Proper Volumes of Media

T-75: 10-12ml

T-25: 4ml

Protocol for Passaging Cells (Generic)

1. Place media, trypsin in water bath at 37C.
2. Remove T-75 flask and verify cell viability and confluence with scope. Place in bio-safety cabinet.
3. Remove cap and aspirate media off cells with sterile Pasteur pipettes.
4. Add 5ml of trypsin to flask.
5. Put flask back in incubator and let sit for 3 min.
6. Remove flask and confirm cell detachment with scope. (Detached cells will float freely and appear round)
7. Add 5ml of 10% FBS in DMEM (or MSCGM) to T-75 flask. (This deactivates the trypsin)
8. Pipette contents of tube in 10ml pipette and place in a 15ml conical tube.
9. Centrifuge the 15ml conical tube for 5min @ 1000rpm making sure to balance the centrifuge.
10. Being sure to spray down the 15ml conical tube, reintroduce it into the sterile field and aspirate off the supernatant being sure not to disturb the cell pellet.
11. Resuspend the pellet in desired amount of media. (Varies between 0.5ml to 10ml based on pellet size)
12. Triturate the solution with a 1000ul pipette to ensure the solution is homogenous.
13. Remove 30ul of cell suspension and add it to the 30ul of trypan blue stain.
14. Load 10ul of the cell+trypan blue mixture in each side of the hemocytometer.
15. Count enough boxes to achieve a count of 100 cells of greater. Once you begin counting a box you must count the whole box.
16. Use this formula to determine the cell density.

$$\frac{\#\ of\ cells\ counted}{\#\ of\ boxes\ counted} * 2 * 10{,}000 * \#\ of ml = total\ cell\ count$$

17. Either seed 500,000 cells per T-75 flask, with 10-12ml of media, or use cells for other intended purpose. Recommended seeding density for hMSCs ≈7000 cells per cm^2

Appendix H: Fibrin Microthread Suture Protocols

Fibrin Thread Bundling Protocol

Materials

- Laboratory tap
- Two sets of dissection forceps, serrated tips
- 8.5x11inch plastic sheet
- Bundling rack
- 50mL beaker
- 1mL pipette
- Distilled water
- Latex gloves

Bundling

1. Adhere a (~1x1cm) piece of laboratory tap to the top-center of an 8.5x11inch plastic sheet.
2. Remove the dried threads from the racks by grasping the ends with tweezers.
3. Slightly lift up an edge of the tape and press it down on one end of a thread.

   Note: The thread should adhere to the tape and not the plastic, allowing for the tape to be lifted up again.

4. Repeat step 3 until 12 threads are adhered to the piece of tape, making sure that they are positioned together as close as possible.

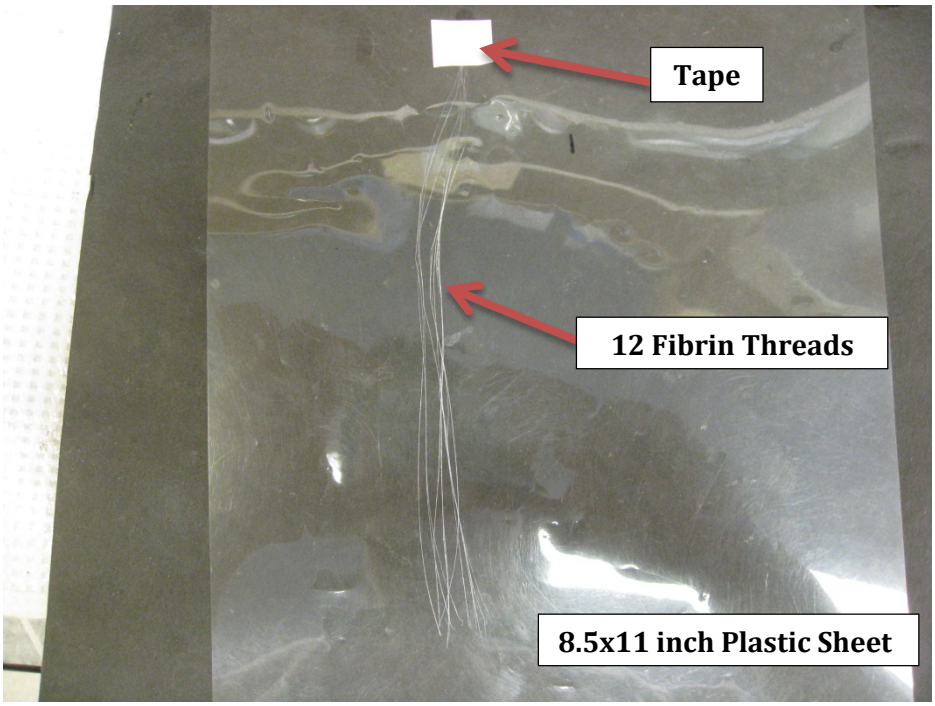


Figure 4: Taped Threads

5. After 12 threads are attached to the tape, fold it onto itself and clamp it to the top of the bundling rack so that the threads are dangling above an empty beaker.
6. Using a pipette carefully drop DI water down the length of the threads, insuring that they become fully hydrated and adhere together.(Volume DI)
7. Once fully hydrated, grasp the bottom end of the thread bundle with your thumb and forefinger and slowly perform ~20 twists (or X Per cm) in the same direction.

   Note: While twisting, insure that the entire length of the thread bundle is twisted, and that it does not become dry. If necessary, twist from the center of the bundle, and rehydrate.
8. Ensure the thread bundle is straight and clamp the loose end to the bottom of the bundling rack.
9. Allow the thread bundle to dry under minimal tension for 30 minutes.

   Note: After securing both ends of the bundle to the rack, it's length should be a minimum of 16cm.

Fibrin Thread Needle Attachment Protocol

Materials

- 200mL DI water
- Latex Gloves
- Large petri dish
- Securos Stainless Steel 3/8ths circle tapered surgical needles (003372)
- Hypodermic needle tips (SIZE)
- Two sets of dissection forceps, serrated tips
- Iris scissors
- 8.5x11inch plastic sheet
- 1mL pipette
- Slide clamps (x16)
- 3cm sections of laboratory tubing (1.98mm ID x 3.18mm OD)
- Latex gloves

Needle Attachment

1. Fill a large petri dish with DI water.
2. Use tweezers to remove the thread bundles from the bundling rack and place them on the plastic sheet.
3. Sever ½ cm from each end of the thread bundle with iris scissors.
4. Using a ruler and iris scissors, divide the 16cm thread bundle into 4cm sections.
5. Thread the 3/8ths circle tapered needles with the 4cm sections and then place them on the bottom of the petri dish filled with DI water.
6. Allow the threaded needles to soak in the DI water for ~2min.
7. Remove the threaded needles individually from the bath and place them on the plastic sheet.
8. With each bundle position the needle such that it is located at the midpoint of the thread bundle.
9. Using tweezers, lift the needle off the plastic sheet and grasp the two loose ends of the thread bundle with your thumb and forefinger.
10. Gently squeeze and twist the ends ~10 times to form a uniform suture.
    a. As necessary moisten thread bundle using a pipette and DI water.
11. Place the suture on the plastic sheet and let dry under ambient conditions for ~5min. Repeat for each threaded needle.
12. Using forceps lift up a dried suture and carefully slide a 3cm section of laboratory tubing over the end opposite of the needle.
    NOTE: Insure that half of the needle is within the tube. (IMAGE)
13. Holding the suture and tubing in one hand, position a slide clamp around the needle and tubing in order to fix them together.
14. Carefully insert a hypodermic needle tip into the needle end of the tubing so that it bypasses the clamp.
    Note: Insure that the needle does no puncture the tubing.
15. Repeat for each suture.

Diameter Measurement on a Dry Fibrin Microthread

Materials

- One Dried Fibrin Microthread
- Glass slide
- Dissection forceps, serrated tips
- Two sets of dissection forceps, serrated tips
- Scissors
- Laboratory tape
- Coverslip
- Leica OMLB2 Microscope
- 'Threaddiameter' Matlab Program
- Latex gloves

Slide Preparation

1. Using tweezers, remove a dried fibrin microthread from the drying rack by lifting up the ends, and place it on a sterile lab bench.
2. Identify the areas of the thread that you wish to image, and then use the Iris scissors to cut ~1cm segments of the desired sections.
3. Position all the sections horizontally on a glass slide and then place a coverslip on top of them.
4. Use 4 small rectangular pieces of laboratory tap on the four corners of the coverslip to secure it to the slide. (PHOTO)

Imaging

1. In the microscopy suite, sign in the log book (name, date, start time).
2. Turn on the Leica OMLB2 Microscope; log on to the computer, open the LAS V3 program on the desktop.
3. Position the slide on the stage, and ready the 5x objective.
4. Use the course adjustment and mechanical stage adjustment knobs to find and focus the microscope on a fibrin microthread section.

   Note: If you are having difficulty obtaining clear images of the thread, adjust the exposure knob.
5. Once focused, switch to 20x magnification and use the fine adjustment knob and slightly increase the exposure to further focus the microscope until a clear image of the diameter of the thread can be seen.
6. Slide the shutter to "PHOTO" in order to display the image on the LAS program.
   a. If the image is unclear on the LAS V3 program, then under the "Aquire" tab, adjust the exposure settings to enhance the quality of the image.
7. Once an image is displayed on the LAS V3 program that clearly depicts the diameter of the thread section, click the "Capture" button to save the image to the "My Documents" folder.
8. Relocate the ".tiff" image file to gaudettelab (\\research.wpi.edu)/Vitathreads/John6 Vitathreads.
9. Repeat steps 3-7 for each thread section.

Diameter Measurement

1. In the "Vitathreads" folder located in the "gaudettelab (\\research.wpi.edu)" folder, open the Matlab program labeled "threaddiameter".
2. *fx>>*

Appendix I: Hoechst Staining Procedure

1. Rinse PBS 5 min
2. Fix 4% paraformaldehyde 10 min
3. Rinse PBS 2 min 3x
4. Permeabolize with 0.25% Triton X-100 10 min
5. Rinse PBS 2 min 3x
6. Hoechst dye 1:6000 in PBS 5 min
    a. 0.5µL Hoechst in 3mL PBS
7. Rinse PBS 2 min 3x
8. Coverslip