A DYNAMIC CULTURE SYSTEM

By

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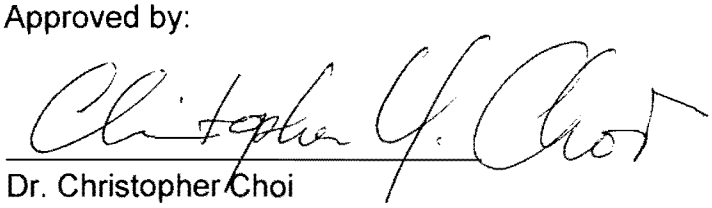

Dr. Christopher Choi
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STATEMENT BY AUTHOR

SIGNED 

(Xiao 'Lucy' Cheng)

STATEMENT OF RESPONSIBILITIES

Tulika

Tulika is responsible for setting up and programming electronic components of the system, which includes the programmable logic controller (PLC), pressure sensors, and solenoid valves.

Lucy

Lucy contributed in the research regarding the recycled media filtration system and cells' ability to survival. Lucy is responsible for determining the relevance of various media flow system components to our project; Lucy also participated in designing, acquiring parts, and assembly the media filtration system. She also participated in the testing of the system.

Jessi

Jessi participated in the computational fluid dynamics (CFD) modeling, rapid prototyping of chamber model, assembly the reservoir and the media delivery system.

John

John worked on Solidworks modeling, parts fabrication, and chamber/reservoir assembly.

Sheridan

Sheridan worked on parts fabrication, chamber/reservoir assembly, and the design of the flow control system.

Bryce

Bryce participated in setting up and programming of the electronic components, which includes the PLC, pressure sensors, and solenoid valve; Bryce is also responsible for material analysis and selection.

Final Design Report

A Dynamic Cell Culture System

From
Cultural Dynamics
Team 4371

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To
Professor Xiaoyi Wu (Sponsor)
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4/29/2010

Interdisciplinary Engineering Design Program

University of Arizona


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Abstract

The following includes information regarding the design, construction, and initial testing of A Dynamic Cell Culture System. This project has been undertaken in order to provide the Wu Lab with a tool that will allow a method to more accurately simulate human vasculature for research purposes by applying pulsatile shear, tensile, and normal pressures. This is accomplished by utilizing upper and lower Chambers between which reside tissue scaffolds (composed of silk and elastin) which are seeded with cells. A high flow-rate pump is used to pass cell culture media over the scaffolds in order to create shear. Solenoid valves are used in conjunction with pressure sensors to cause pressure to build up in one Chamber, causing both normal and tensile stresses in the scaffolds. At this time, the system has been fully constructed and has undergone only initial testing, the results of which showed that more work on sealing the Chamber and tube connections is necessary.

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A. Problem

i. Problem Statement

Tissue engineering is one of the most prominent research fields in bioengineering today. By developing lab-cultured tissues, scientists and doctors are able to restore, improve, and maintain the functions of diseased native tissues. This type of regenerative medicine has significantly reduced the complexity of organ transplantation and various surgical procedures. More specifically, cardiovascular grafting surgeries are performed more than 600,000 times annually in America and Europe. Unfortunately, effective and efficient methods of dynamically culturing tissues have not been developed. It is advantageous to create bioreactors that are capable of culturing graft tissues that are most similar to that under *in vivo* conditions, thus reducing the chance of graft rejection and other complications.

Most cell cultures are static and do not replicate the dynamic environment of living bodies. This results in cultures that do not have physical properties similar to cells that are grown *in vivo*. In order to solve this problem, bioreactors, or culture systems, have been made to apply various stresses, such as shear and tensile stresses, in the cultured cells. Current dynamic culture systems are too complicated to be easily duplicated. Also, most systems only function with scaffolds which are 100 microns thick. Scaffolds which range from 500 to 700 microns thick must be used to produce a physiologically relevant sample of cardiovascular tissue. Scaffolds thicker than 100 microns, however, inhibit cells from receiving proper nutrition in most current dynamic culture systems. A system therefore must be created to perfuse cell culture media throughout scaffolds that are 500 to 700 microns thick, apply less than 10 % longitudinal strain, and apply 0.1 to 1 Pascal shear stresses.

ii. Project Scope

This project will include constructing a dynamic culture system that applies pulsatile stresses to currently existing protein scaffolds seeded with smooth muscle and endothelial cells. The system must maintain a proper environment for cell prosperity, and will be able to operate for at least 14 days continuously without compromising culture conditions. Components of the device which come into contact with either the cell culture media or the scaffolds must be sterilizable. The design should be easy to duplicate, cost efficient, and user friendly. The protein scaffolds will be flat sheet scaffolds provided by the sponsor. The cell culture media will also be provided by the sponsor. This system will be designed and built in order to provide enough time for one or two tests before design day.

iii. Mission Statement

Through the construction of a dynamic culture system, Cultural Dynamics strives to advance the technology of tissue engineering and provide future researchers with an invaluable tool.

B. Functional Requirements

i. Supply Cells with Nutrients Throughout Scaffold

In order for the cells seeded at the center of the 500 to 700 micron thick scaffold to survive, they must receive nutrients from the cell culture media. In *in vivo* situations, vascular tissue is able to receive such nutrients through very small blood vessels such as capillaries. However, designing synthetic microvasculature is well beyond the scope of this project, so the perfusion of nutrients throughout the thickness of the scaffold must be accomplished another way.

ii. Apply Pulsatile Shear and Normal Stresses

Under *in vivo* conditions, the blood flow causes shear and normal or circumferential stresses on the walls of cardiovasculature. Due to the pulsatile nature of *in vivo* flow from the heart these induced stresses are also pulsatile. Due to the fact that this project is designed to replicate tissue grown *in vivo*, it is desirable to replicate such conditions for *in vitro* experiments. The ranges of the magnitudes of these stresses are 0.1 to 1 Pascal for shear and approximately 50 kilo-Pascals for normal stress.

iii. Apply Pulsatile Tensile Strain

In vivo vasculature also experiences longitudinal or tensile strain with blood flow. For the purposes of this experiment, strain should be applied in a pulsatile manner to mimic *in vivo* conditions. However, due to the mechanical constraints of the protein-scaffolds used, tensile strain applied must be between 2% and 10%.

iv. Maintain Constant Oxygen and Carbon Dioxide Levels

Similar to the supply of nutrients, a constant supply of oxygen as well as carbon dioxide must be supplied to the cells to keep them alive. The standard ratio of oxygen to carbon dioxide is 95% oxygen to 5% carbon dioxide. As with nutrients, oxygen and carbon dioxide will be diffused into the cell culture media. Again, it is vital to supply the entire thickness of the scaffolds with cell culture media in order to prevent cell death.

v. Maintain Constant Temperature

Human cells grow within a narrow temperature range. It is therefore vital that the temperature of the system be maintained at an ideal temperature for cell prosperity. The standard temperature of a human body is 37 degrees Celsius.

C. Constraints

i. Cost

The approximate budget for this project is $3000.00. This is an approximate budget because our sponsor has told us that more money is available if absolutely necessary.

ii. Sterilizable

In order to prevent contamination before and during experiments, all components of the system which come into contact with either the cell culture media or the protein-scaffolds themselves must be sterilizable either by heating with an autoclave or with exposure to a solvent such as alcohol. Contamination would result in the presence of cells in the scaffolds that are of different type than those intended, which would invalidate any results obtained from experiments performed on tissue samples generated from the system.

iii. Operate Continuously

The system must operate continuously for at least 14 days. It is important for the system to operate continuously due to the fact that, *in vivo*, vasculature never sees a break in stress. Further, if the system stopped, it would mean that oxygen and the other nutrients would no longer be provided to the cells. 14 days is the set goal for an experimental time since this is the length of time in a cell culture cycle.

iv. Simplicity

One of the problems with current dynamic cell culture systems is that they are very costly and hard to reproduce to complexity. Therefore, we have been instructed to keep our system as simple and easy to use as possible. This means that the system should be easy to set up and run. Additionally, the system should be reproducible.

D. Customer Requirements

i. Accommodate Provided Scaffolds

The sponsor will provide flat sheet scaffolds. The desired size of each cultured sample is 1 sq. cm. Therefore, the system must be designed to culture cells in the manner as stated above on 1 sq. cm. area of flat sheet scaffolds.

E. Ideal Final Result

An ideal design would be one that fulfills all of the functional and customer requirements and stays within the constraints of the system. The ideal system would therefore supply a 1 sq. cm. area of a flat sheet scaffold with sufficient nutrients even at a thickness of 700 microns, apply pulsatile shear stress between 0.1 and 1 Pa, apply pulsatile normal stress of approximately 50 kPa, and apply pulsatile tensile strain between 2% and 10%. It would also maintain the correct levels of oxygen (95%) and carbon dioxide(5%) in relation to each other as well as maintain the temperature of the culture at 37 degrees Celsius. All of this would be maintained for at least 14 days. The system would be built using cost efficient parts that are sterilizable by autoclave or solvent wash. The final system would be simple for a graduate student to use and easy to replicate.

F. Sponsor Information

The sponsor of this Project is Dr. Xiaoyi Wu, a professor in the University of Arizona Department of Aerospace and Mechanical Engineering. His education includes a B.Sc. in Mechanics and Mechanical Engineering from the University of Science and Technology of China, a M.Eng. in Engineering Mechanics from Tsinghua University and he received a Ph.D. from Johns Hopkins University in Mechanical Engineering. After doing research at Emory University School of Medicine and Georgia Institute of Technology for four years he joined the University of Arizona Department of Aerospace and Mechanical Engineering where he has done research for three years. Dr. Wu's research expertise includes Biomechanics, Biomaterials, Computational Biomaterial and Tissue Engineering. His current research involves growing and testing cardiovascular tissue. The current culture system that he uses is a static system which does not give him a relevant sample for testing. Therefore, he is sponsoring this project in order to obtain a system that will give him more realistic samples of cardiovascular tissue.

II. Design

Our final system design incorporates several major subsystems: the Chamber; the Media Delivery System; the Flow Control; the Reservoir; and the Environmental

Control. Each subsystem directly relates to at least one Functional Requirement and does not violate any Constraints. Figure 1: Flow of media through the system.Figure 1 displays how cell culture media will flow through the major components of the system. The media will be pumped from the Reservoir by the Peristaltic Pump into the culture Chamber where it will flow over the scaffolds supplying them with nutrients and applying the various stresses. It will then flow back into the Reservoir to replenish the media supply.

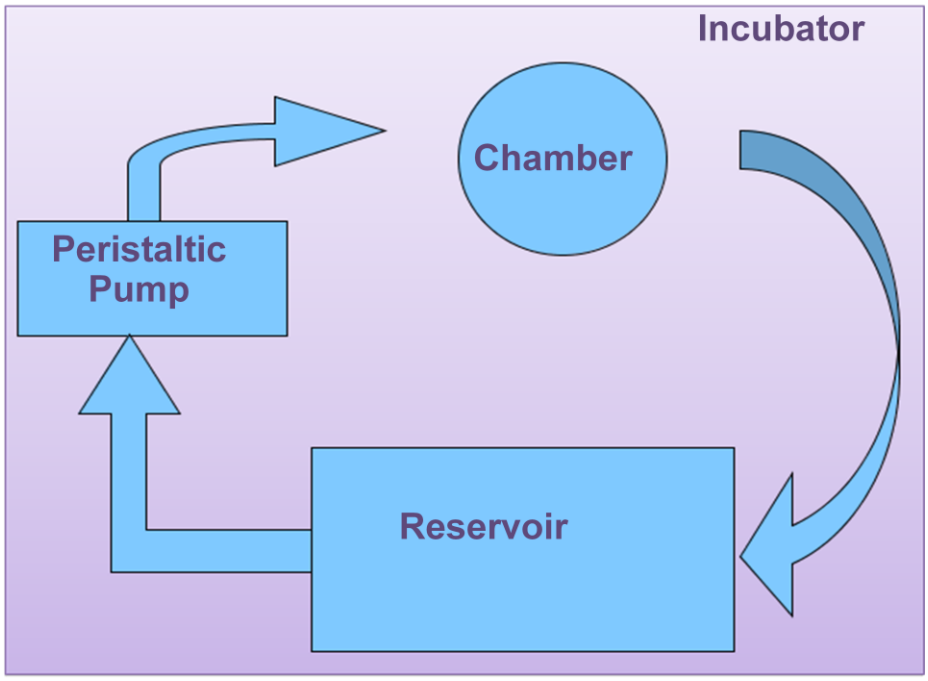

Figure 1: Flow of media through the system.

A. Chamber

i. Purpose

Our team's Dynamic Cell Culture System is meant to provide a physiologically relevant environment in which a protein scaffold seeded with smooth muscle cells and vascular endothelial cells can grow for at least two weeks. As part of this system, physiologically relevant pulsatile stresses must be applied to the scaffold and nutrients must be pressure-perfused throughout the thickness of the scaffold. The purpose of the Chamber is to hold the scaffold in place such that these actions may be performed on it. More specifically, this means that we designed a Chamber to securely hold the scaffold in place such that cell media flowing through the Chamber can flow over the scaffold, which creates the required relevant pulsatile shear stress. Additionally, the media will be pressure-perfused throughout the thickness of the scaffold, while simultaneously

supplying nutrients to the entire scaffold and creating the required pulsatile longitudinal stress.

ii. Design

In order to design a Chamber which could accomplish the above goals, our team developed three design concepts, performed a selection process in which a final concept was chosen, and detailed a final design concept. These steps are detailed below.

a. Preliminary Designs

Three different preliminary Chamber designs were originally developed. Each design presents a different way in which the scaffold could be secured in order to allow for the proper pulsatile stresses to be applied to the scaffold and to permit complete nutrient perfusion throughout the thickness of the scaffold.

i. Cylindrical

This design requires that the scaffold be delivered in a cylindrical form. Although this shape of scaffold would be more difficult to produce, it is still possible. Hence, our team developed a design concept to incorporate this possibility. In this design, the cylindrical scaffold is attached to a tube at the inlet of the Chamber and to another tube at the outlet of the Chamber as shown below in Figure 2.

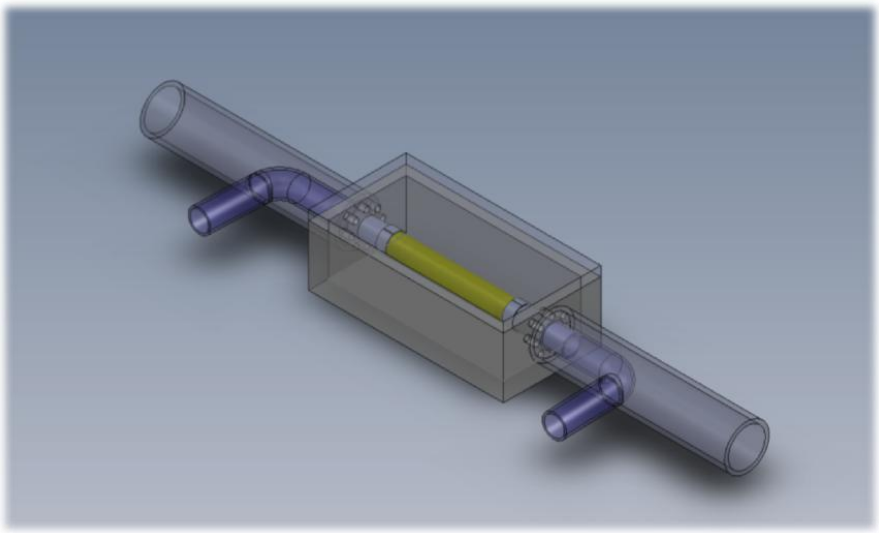

Figure 2: Chamber design for cylindrical scaffold.

Media flows through both the inside of the scaffold and on the outside. This flow would create the required shear stress for the design. The flow would also be controlled in a pulsatile manner such that pressure would build up inside the cylindrical scaffold causing it to expand radially and contract longitudinally. The resulting motion would create the required pulsatile tensile stress and the buildup of pressure within the cylindrical scaffold would be able to perfuse nutrients throughout the scaffold thickness. One of the primary advantages of this concept, besides its simplicity to analyze, is that tissue scaffolds developed using this design would experience very physiologically relevant stresses. More images of the Cylindrical Chamber design are shown below in Figures 3 and 4:

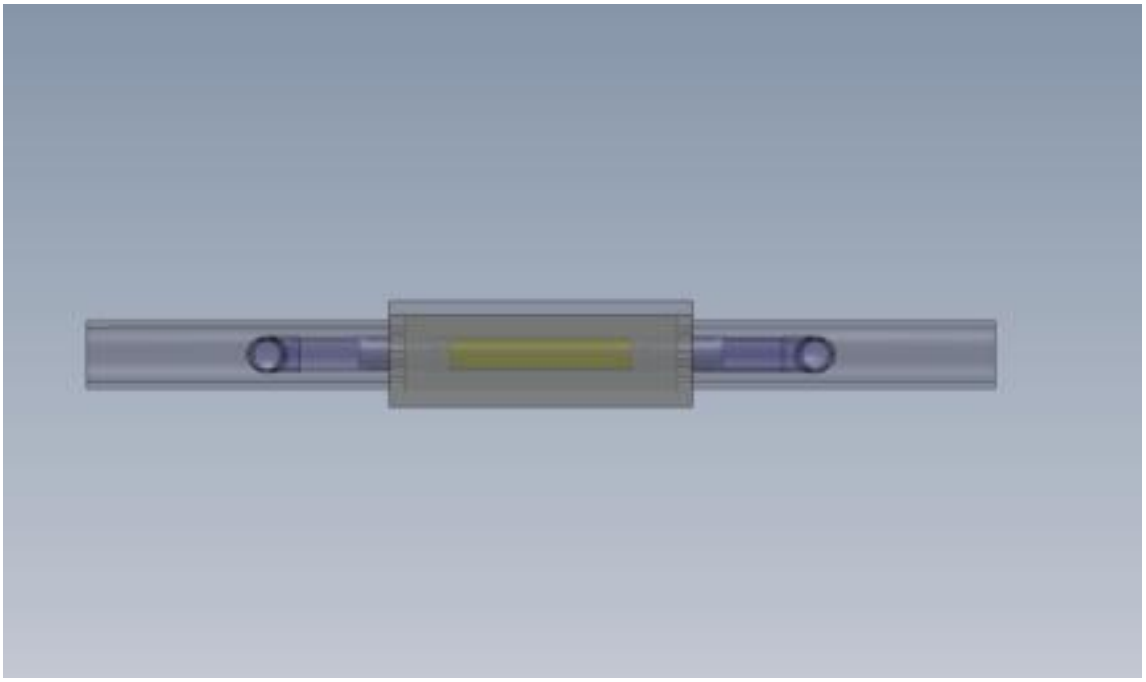

Figure 3: Side view of cylindrical design.

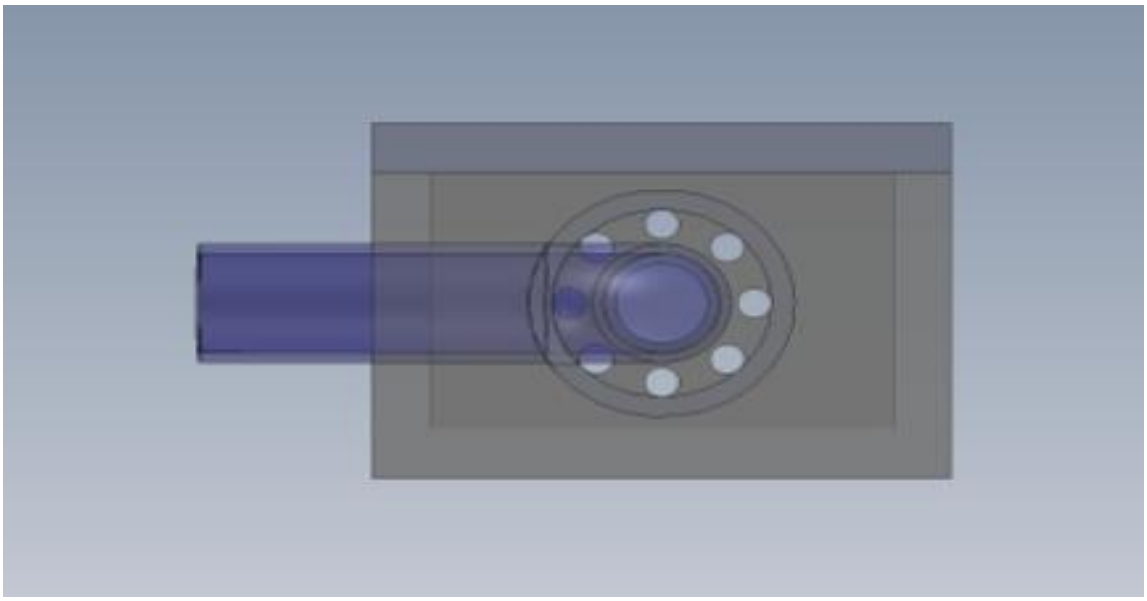

Figure 4: Cross-sectional view of cylindrical design.

ii. Flat Sheet

In this design, a scaffold in the form of a flat-sheet is used. According to our sponsor, this shape of scaffold is easy to obtain. Hence, our team developed two design concepts which incorporate this shape. The first design is shown below in Figure 5.

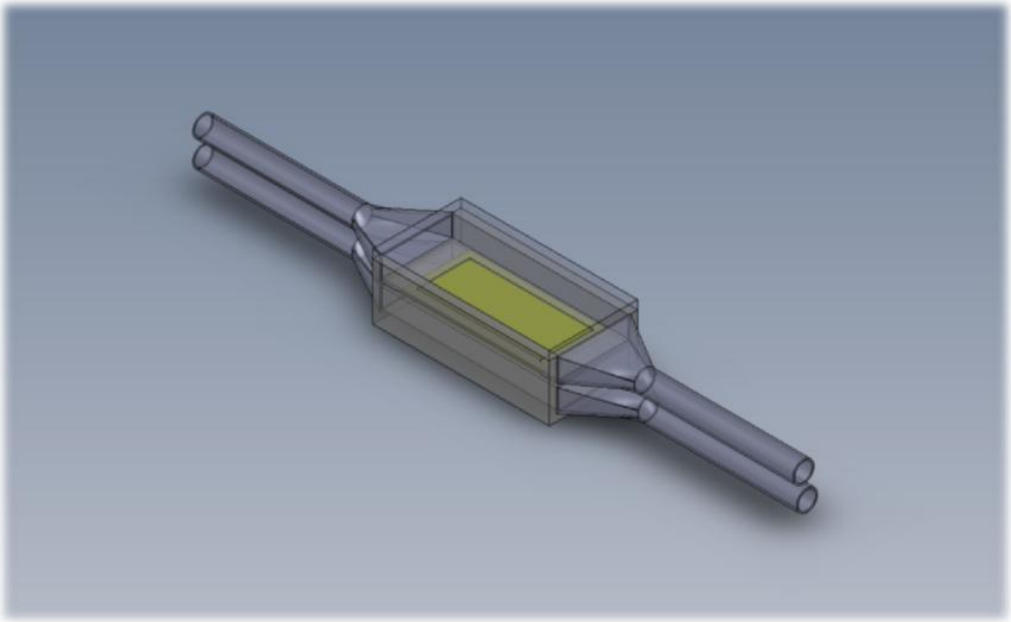

Figure 5: Chamber design for flat sheet scaffold.

As seen in the above figure, the scaffold would be clamped down by a rectangular plate and media would flow over the scaffold on both the top and the bottom in the same direction. This flow would create the required shear stress for the design. The flow would also be controlled in a pulsatile manner such that pressure would build up one side of the flat-sheet scaffold. This would cause it to bulge outwards and thereby stretch longitudinally. Flow on the other side of the scaffold would continue to flow freely. The resulting motion would create the required pulsatile tensile stress and the building up of pressure on one side of the scaffold would be able to perfuse nutrients throughout the scaffold thickness. Note that the process of building up pressure on one side of the scaffold would alternate sides between cycles. In other words, pressure would build up on one side of the scaffold during one cycle and then on the other side of the scaffold for the next cycle. One of the primary advantages of this concept is its simplicity to construct and analyze. Tissue scaffolds developed using this design would experience fairly realistic physiological stresses. More images of the Flat Sheet Chamber design are shown below in Figures 6 and 7:

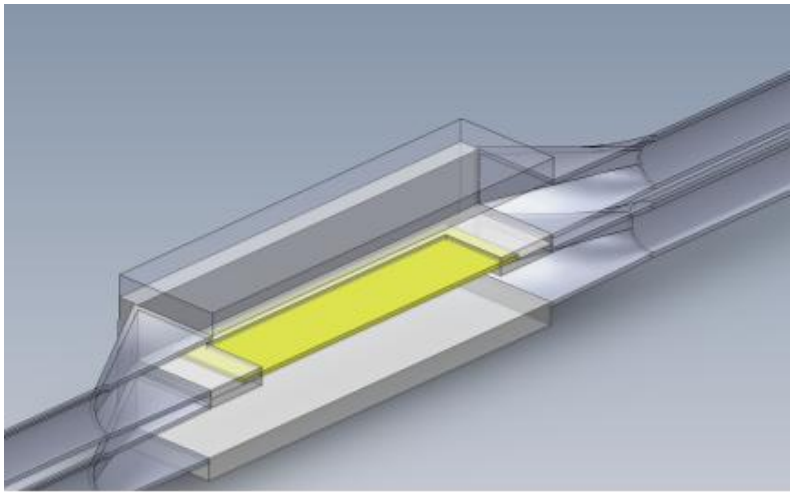

Figure 6: A cut view of the flat-sheet Chamber design.

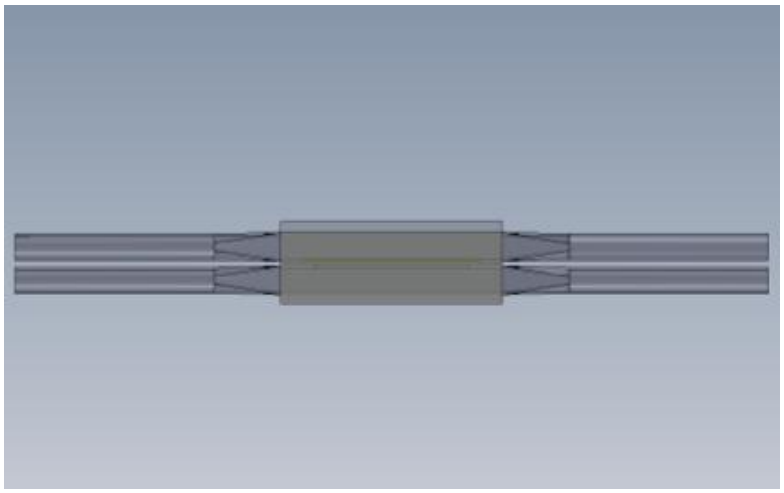

Figure 7: Side view of the flat-sheet Chamber design.

iii. Angled

The Angled Chamber design is similar to the previously discussed Flat Sheet Chamber design in that it also uses a scaffold in the form of a flat sheet. The important difference is that the scaffold is at an angle to the fluid flow which is entering the Chamber, as shown in Figure 8:

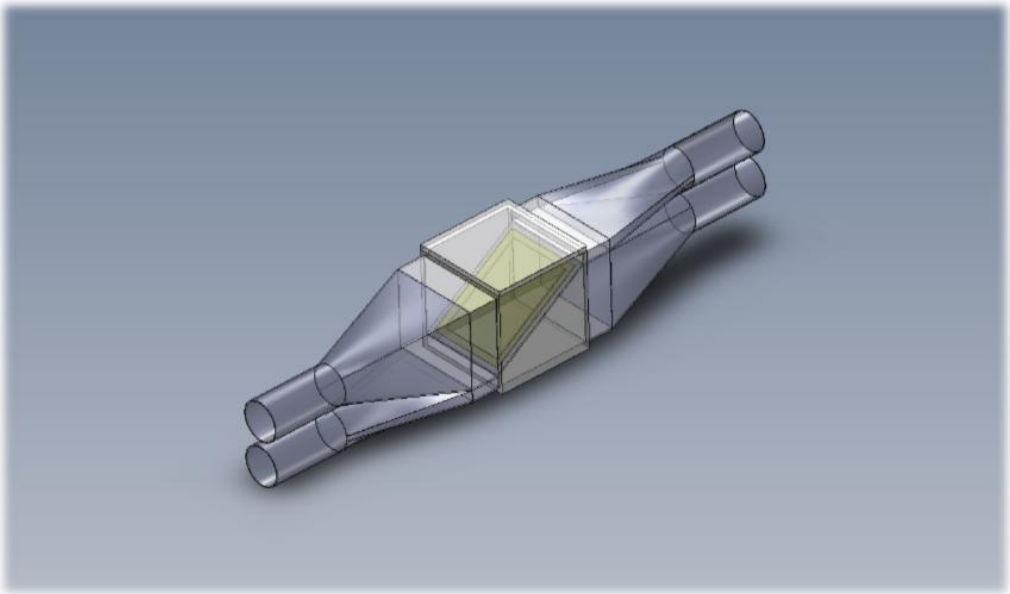

Figure 8: Chamber design holding scaffold diagonally.

As shown in the above figure, the scaffold would be clamped down by a rectangular plate and media would flow over the scaffold on both the top and the bottom in opposite directions. Media would enter the Chamber through the large duct and exit through the small narrow duct; this flow would create the required shear stress for the design. The flow would also be controlled in a pulsatile manner such that pressure would build up one side of the scaffold causing it to bulge outwards and thereby stretch longitudinally; flow on the other side of the scaffold would continue to flow freely. The resulting motion would create the required pulsatile tensile stress and the buildup of pressure on one side of the scaffold. This would be able to perfuse nutrients throughout the scaffold thickness. Note that the process of building up pressure on one side of the scaffold would alternate sides between cycles. Pressure would build on one side of the scaffold during one cycle and then on the other of the scaffold for the next cycle. One of the primary advantages of this concept is that by positioning the scaffold at an angle within the Chamber, the velocity of the media flowing through the Chamber could theoretically be used to more easily achieve complete nutrient perfusion throughout the scaffold thickness; some large disadvantages would be its difficulty of construction and its difficulty to analyze. More images of the Angled Chamber design are shown below in Figures 9 and 10:

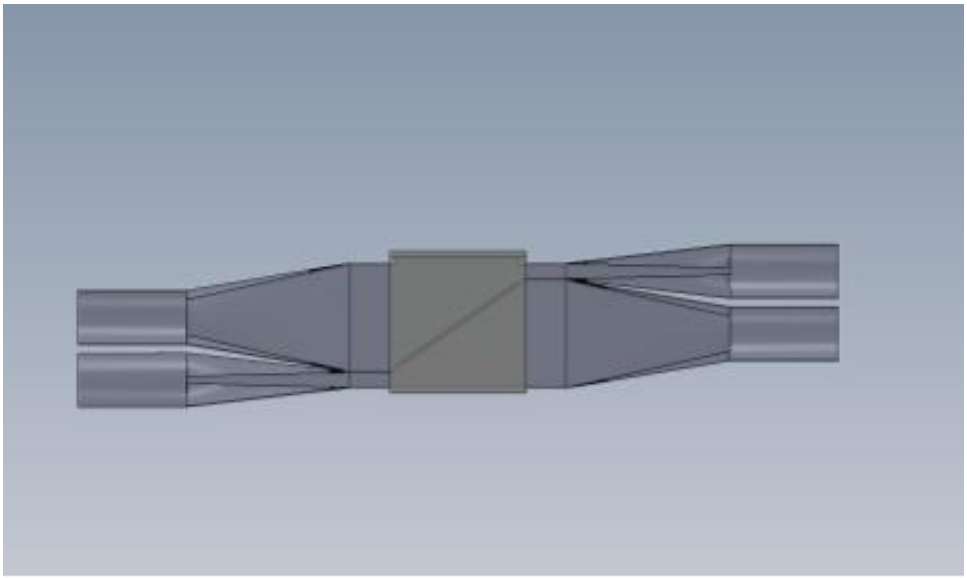

Figure 9: Side view of diagonal Chamber design.

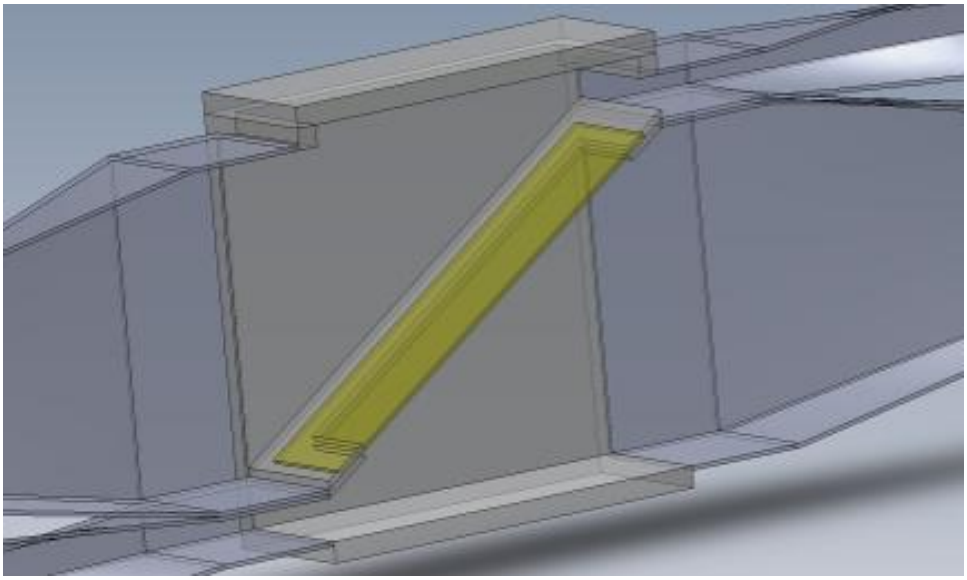

Figure 10: Detail of diagonal Chamber design.

b. Design Selection

To evaluate the three designs discussed above, a Pugh diagram was constructed which assigned weighted values to each of the properties both necessary and desired in the final Chamber design. This Pugh diagram is shown below in Table 1.

Table 1: Pugh chart for Chamber designs.

		CHAMBER			
		Ideal	Cylindrical	Flat	Diagonal
10	Pulsatile Shear Stress	50	40	40	35
9	Pulsatile Tensile Stress	45	36	27	27
6	Pulsatile Circumferential Stress	30	30	21	21
5	Cost	25	17.5	22.5	17.5
3	Accommodates Different Size Scaffold	15	10.5	6	3
3	Accommodates Different Shape Scaffold	15	3	3	3
7	Quality of Scaffold Attachment	35	24.5	28	28
8	Ease of Accessibility	40	32	40	36
10	Perfusion through 500 Micron Scaffold	50	50	40	40
9.5	Design Simplicity	47.5	33.25	47.5	19
8	Ease of Use	40	28	32	32
10	Sterilizable	50	50	50	50
10	Customer Preference	50	25	40	50
	Total	**492.5**	**379.75**	**397**	**361.5**
	Percentage	**100.0**	**77.1**	**80.6**	**73.4**

While the Cylindrical Chamber design fulfills all of the requirements of the Chamber, our sponsor expressed his preference to work with flat-sheet scaffolds rather than cylindrical scaffolds. This is reflected in the above Pugh Chart by the heavy importance assigned to customer preference. Also shown in the Pugh Chart is the fact that while there is actually not much benefit to using the Angled Chamber design, there is a much higher level of difficulty associated with both constructing and analyzing the design. Because of these reasons, and to make the system as simple as possible, the Flat Sheet Chamber concept was selected to be used in our Final Design.

c. Final Design

As mentioned above, a Flat Sheet Chamber concept was chosen to be developed into our Final Design, shown below in Figures 11, 12, and 13. One of the primary aspects which was changed when progressing from the initial to the final design is the number of scaffolds which the Chamber can accommodate. The original Chamber design was only meant to hold one tissue scaffold while the final Chamber was designed to hold up to nine tissue scaffolds. This will allow our sponsor to use the device to simultaneously culture multiple scaffolds.

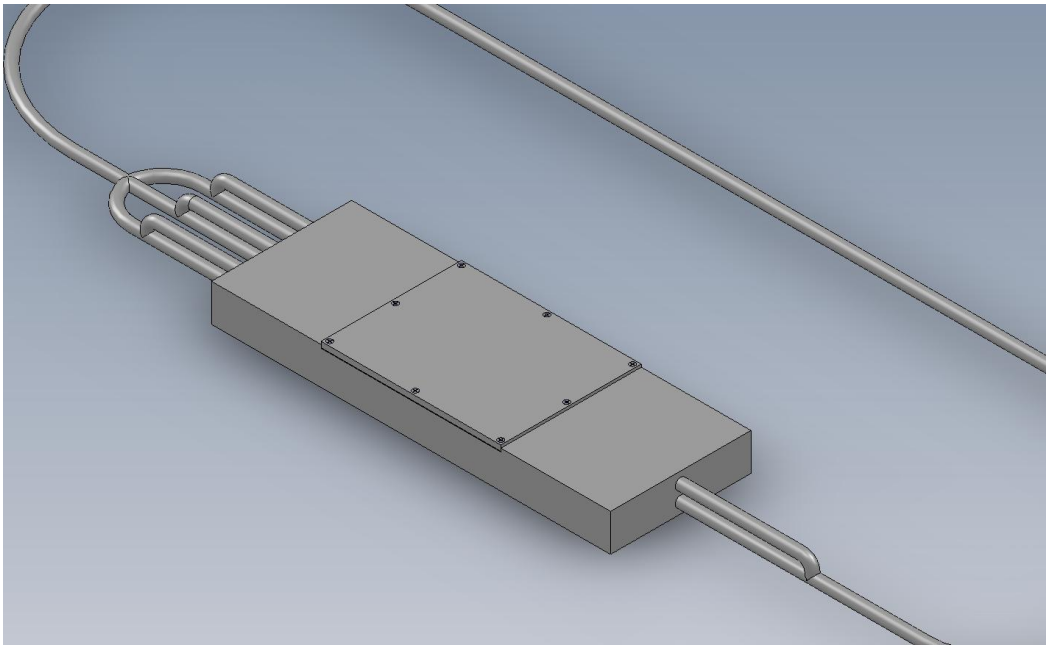

Figure 11: Final Chamber design (closed).

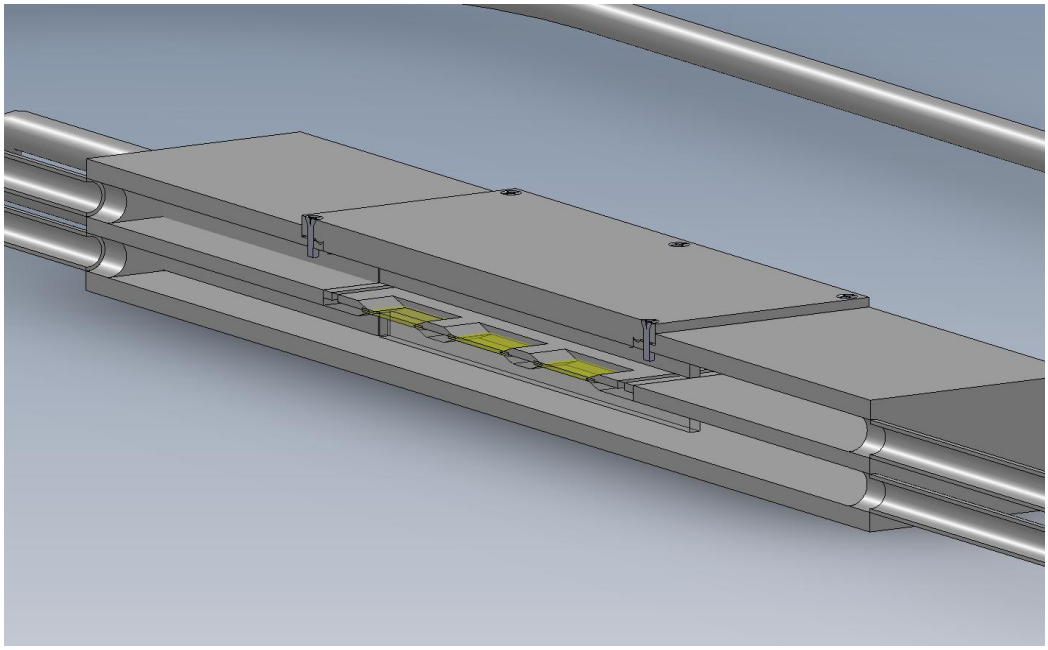

Figure 12: Final Chamber design (cut view).

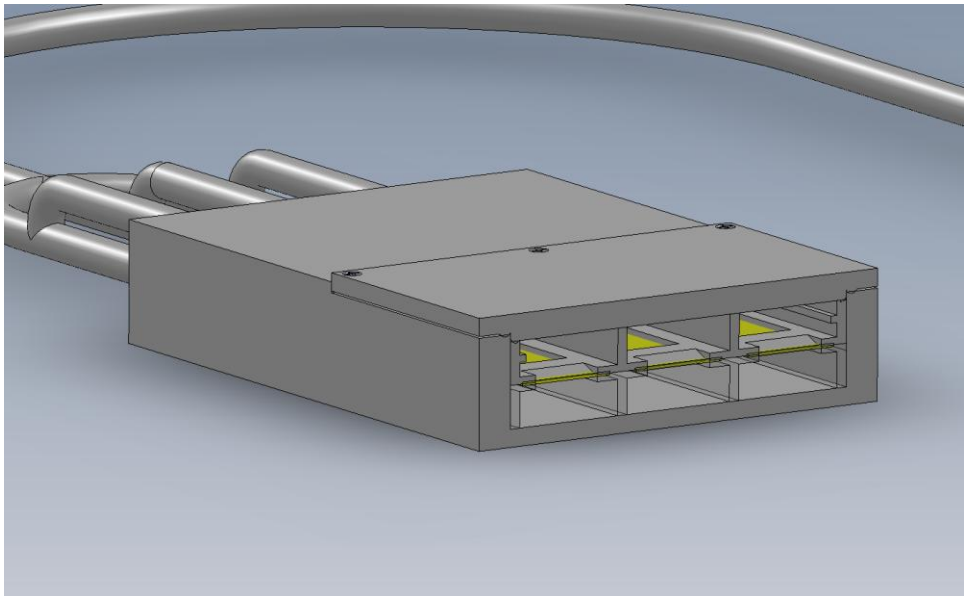

Figure 13: Cross-sectional view of final Chamber design.

In the Final Chamber Design, media enters the Chamber (in the same direction in both the top and bottom sections) via a long rectangular duct directly from a tube sealed to the Chamber wall. This allows the flow to develop before entering the section of the Chamber in which the tissue scaffolds are housed. Once developed, media then flows down the short ramp of the scaffold Securing Plate, over the tissue scaffold, and then up the ramp of the scaffold Securing Plate before continuing on to the next two scaffolds in a similar manner. Fluid will eventually exit the Chamber through an outlet tube sealed to the Chamber wall. The media flowing over the scaffolds would create the required shear stress for the design. The dimensions of the ramps of the Securing Plate were finalized through a computational fluid dynamics (CFD) analysis using Fluent. This will be discussed in detail in the Appendix.

The flow within the Chamber will be controlled in a pulsatile manner such that pressure builds up on one side of the flat-sheet scaffolds causing them to bulge outwards. This, therefore, allows for the scaffolds to stretch longitudinally while media on the other side of the scaffolds continue to flow freely. The resulting motion creates the required pulsatile tensile stress, and the build-up of pressure on one side of the scaffolds will perfuse nutrients throughout the scaffold's thickness. Note that the process of building up pressure on one side of the scaffold alternates sides between cycles. Pressure builds up on one side of the scaffold during one cycle and then on the other side of the scaffold for the next cycle. This process is discussed in more detail in Part C - Flow Control.

Shown below in Figure 14 is an exploded view of the Chamber and all of its subcomponents. To prepare the Chamber for culturing, tissue scaffolds are first placed on the lower Securing Plate. This will be done in a bio-hood outside of the main portion of the Chamber. Next, the upper Securing Plate is lowered down and matched up with

the lower Securing Plate. The two components are then attached together using four screws. This process will secure the tissue scaffolds in place and create a new subassembly. Next, this new subassembly is positioned into the main Chamber itself. Finally, the lid, which contains a rubber seal on both its upper and lower levels to prevent any loss of fluid or pressure from within the Chamber, is placed on top of the Chamber and secured using eight screws.

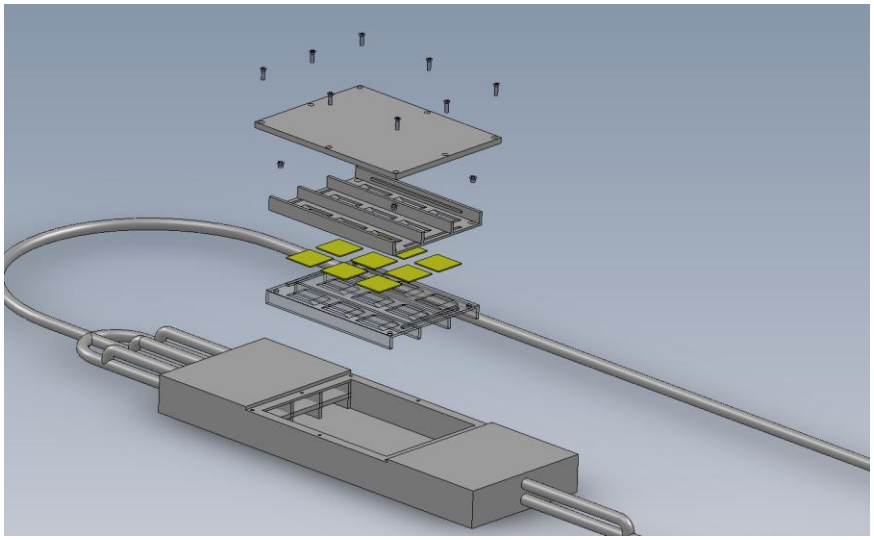

Figure 14: Exploded view of final Chamber design.

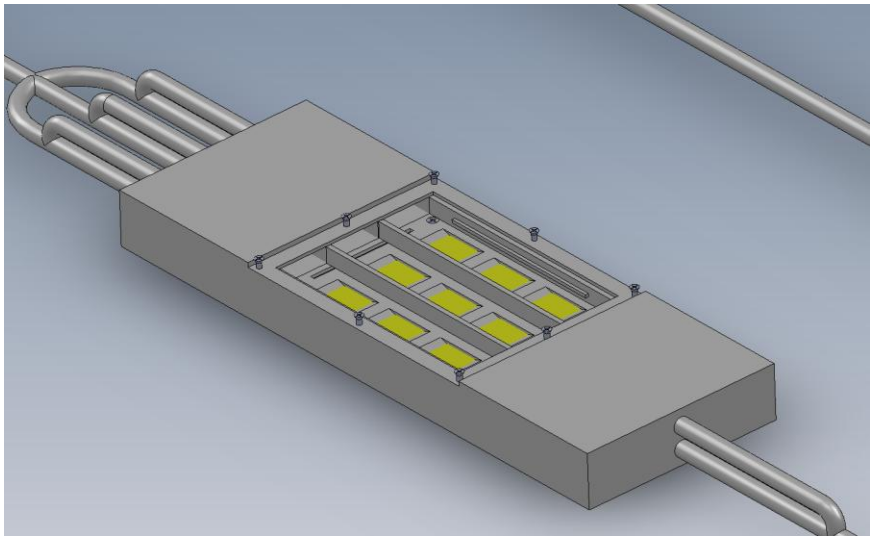

Figure 15: Inside view of final Chamber design.

To determine the pressure necessary to deflect the tissue scaffolds downwards an amount which would create a tensile strain within the scaffolds between 2 and 10%, a

finite element simulation using ABAQUS was performed. This simulation is discussed in more detail in the Appendix. From this simulation, it was determined that a pressure of 50 kilo-Pascals would have to be applied within the Chamber in order to create the appropriate strain in the scaffolds. This will create a relatively large pressure gradient within the Chamber, and the Chamber is small, thin, and must be both corrosion resistant and sterilizable. Hence, it was decided that the Chamber should be constructed out of stainless steel. Stainless steel is a readily available material and is easy to machine. Small vertical divisions (Figure 15) were added into the Securing Plates design in order to prevent any deformation from the pulsatile, cyclic build-up of pressure within the top and bottom cavities. These vertical divisions should prevent the thin Securing Plates from failing even after operating for millions of cycles. This will therefore provide a robust, sterile Chamber for the culturing of endothelial and smooth muscle cells on nine tissue scaffolds simultaneously while also holding the scaffolds in place for the desired application of stresses.

d. Second Semester Design Changes

In the Critical Design Review composed first semester, the two Securing Plates were discussed as having four vertical ribs, or channels, as shown in Figure 16. These channels were 5 mm tall. The main functions of the channels were to guide the media flowing through the Chamber and to provide structural support to the flat parts of the Securing Plates. Flow calculations relating the shear stress on the scaffolds to the flow rate of the pump and the height of the ribs were analyzed. Then, the decision was made to reduce the height of the ribs to 2 mm. This height would allow the original shear stress of 0.1 Pa to be achieved at a significantly lower flow rate.

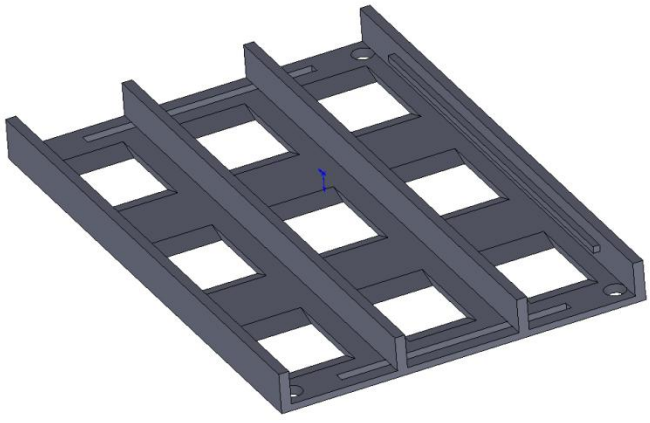

Figure 16: Original Securing Plate.

Once the methods available to machine the Securing Plates with the new rib height of 2 mm were reviewed, it was determined that a solid block of stainless steel would have to be milled down to the size of the parts. Since this process is time consuming and could create a large amount of warping in the blocks of stainless steel, it was decided that a design change was necessary. To make the fabrication of the Chamber easier, quicker, and less susceptible to errors, it was decided to "move" the vertical ribs of the Securing Plates to the Main Chamber and to the Chamber Lid as shown in Figure 17. With this change in mind, it was also decided to machine the Chamber Lid out of polycarbonate instead of stainless steel. The Securing Plates could then be machined from flat stainless steel sheets which were already at the desired 1.5 mm thickness. With these design changes in place, the outer shape of the Securing Plates and the holes in the center were easily machined, and with the new rib height of 2mm, the ribs were able to be easily added to the Chamber Lid and to the Main Chamber.

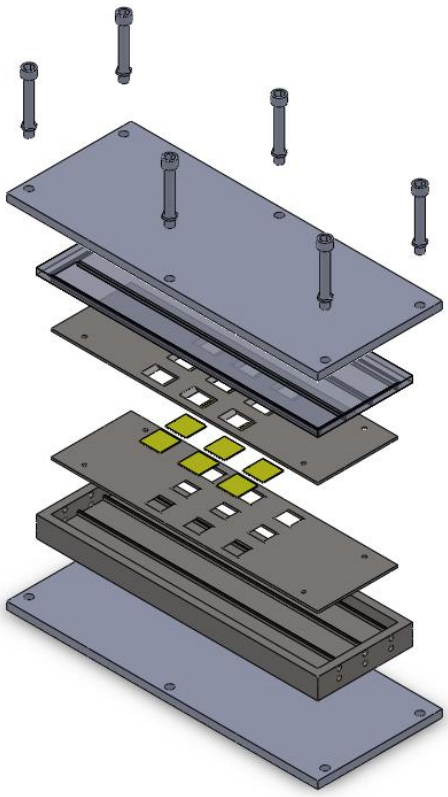

Figure 17: New Securing Plates, Main Chamber, and Chamber Lid.

Another important design change was the method in which the Chamber Lid is secured to the Main Chamber. Originally, a set of 8 screws were to go through the Chamber Lid and screw into the Main Chamber. Upon realizing that the flow rate necessary to create the desired shear stress as well as the cyclic building-up of pressure within the

Chamber could cause the Chamber Lid to warp, it was decided to “clamp” the entire system between two flat sheets of aluminum. The two sheets clamp down the Chamber Lid to the Main Chamber by employing a set of 6 bolts and nuts which run along the perimeter of the outside of the Main Chamber; this is shown in Figure 17. Using this method, the Chamber Lid does not warp under pressure and the system is capable of remaining leak-free.

B. Media Delivery System

i. Purpose

Our cell culture system will have the ability to grow both smooth muscle cells and human umbilical vascular endothelial cells on a flat sheet cell scaffold. Both cell types will require certain amounts of nutrients, carbon dioxide (CO_2), and oxygen (O_2) in order for them to thrive inside the bioreactor. The purpose of the Media Delivery System is to offer a constant supply of the needed nutrients to the cells on the scaffold in order for them to grow in the bioreactor. In addition, the Media Delivery System will provide the required shear stress to the cells through the flow velocity; our sponsor requested an applied shear stress between 0.1 and 1 Pascals. Thus, the Media Delivery System will fulfill two functional requirements: delivering media to the cells and providing shear stress to the cells attached to the cell scaffold.

ii. Pump Choices

In order to fulfill the above requirements, a pump must be incorporated to provide the system with a constant supply of fresh nutrients and a way of providing shear stress to the cells. Our team decided that one of two pumps could work with our bioreactor: a syringe pump or a peristaltic pump.

a. Syringe Pump

A syringe pump, seen in the Figure 18, works by pressing a normal syringe at a constant speed, which can be adjusted by the user, until the syringe is empty. Most syringe pumps display the exact flow rate as the syringes are being depressed, which would be helpful in later adjustments to our setup. However, the use of this type of pump will force our system to be non-circulating. This would cause the bioreactor to require more maintenance as the cells are in culture since the syringes need to be sterilized and refilled periodically. In addition, each time the syringe pump is refilled the flow has to stop. This means that the cells, especially those in the inner scaffold, would not be obtaining the necessary nutrients during that time.

b. Peristaltic Pump

A peristaltic pump, shown below in Figure 19, rotates rollers over tubing in order to push liquid through. The rollers never come in contact with the cell media. This obviously reduces the risk of contamination from the pump. The pump can run continuously throughout the two week testing period and would allow for media re-circulation. This would eliminate the need for media refills during the test. However, most inexpensive peristaltic pumps do not provide the user with a flow rate reading. This is inconvenient because the user would be required to manually measure the flow rate.

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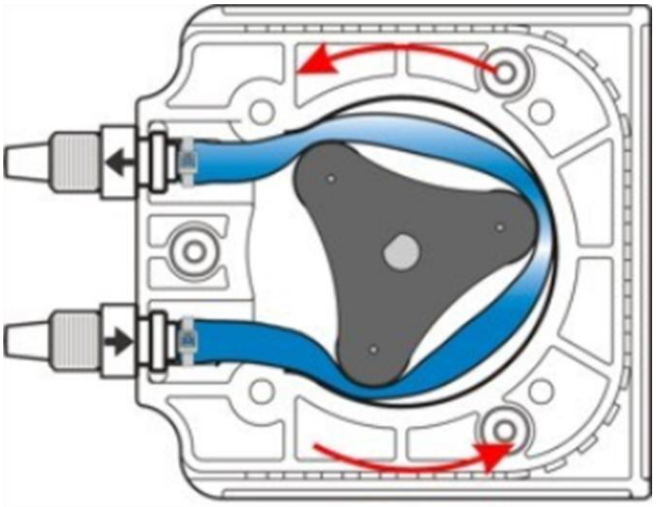
Figure 18: Syringe pump.



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white.com/images/per_pumphead.jpg

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/clarksol.com/html/prodspec
sM8000 Pump.cfm

Figure 19: Peristaltic pump.

c. Final

Through the analysis of a Pugh Chart (see Table 2), it became obvious that using a peristaltic pump would be more beneficial to our overall system design. With this pump, the user would not have to constantly re-fill syringes. A peristaltic pump would also allow for the recirculation of cell media.

Table 2: Pugh chart for pump choices.

	Ideal	Syringe	Peristaltic	Weight
Variable Flow Rate	50	50	35	10
Flow Continuity	50	20	50	10
Sterilizable	50	35	50	10
Cost	30	30	15	6
Ease of Use	35	17.5	28	7
Weighted Total	215	152.5	178	
Percentage	**100**	**70.93**	**82.79**	

iii. Design

The Media Delivery System will be comprised of a peristaltic pump, a media Reservoir, cell media, and tubing. The peristaltic pump chosen is from ClarkSol model M8000 and will provide a flow rate between 2.6 and 10.8 liters per second. The flow rate will be digitally displayed so an accurate flow rate will be known. A peristaltic pump was chosen so the pump will never directly come in contact with the cell media and will not increase contamination risks. The flow rate of the media provided by the pump will allow for the desired shear stress to be applied when the cell media has viscosity of 0.007 Pascal-seconds. The flow rate and viscosity values were generated using computational fluid dynamics with Fluent. Discussion of Fluent analysis can be seen in the Appendix.

The media Reservoir will be a glass container with a plastic, screw on lid. It will hold between 250 to 500 mL of cell media, enough to sustain the cells for a full two week testing period. Details about the media Reservoir design will be discussed under the Reservoir section.

The nutrients will be provided through a pre-mixed cell media solution containing all the nutrients both cell types will require; this solution will be provided by our sponsor. CO_2 and O_2 will also be contained in the media as it flows over the cells. Thus, the cell media will provide the cells with all proper nutritional needs.

The chosen material for the tubing is silicone, a material used often with other bioreactors. This will allow for easy sterilization, will not affect the cell media, and is soft enough for the peristaltic pump to be effective. In addition, this material will allow for the flow of gasses through the tubing wall, which assures that our media has ample amount of oxygen in it.

C. Flow Control

i. Purpose

While the Media Delivery System exists in order to ensure that there is a constant media flow throughout the entirety of the system, the Flow Control's sole purpose is to augment the flow within the Chamber**.** By doing this, we are able to induce various stresses in the cell-seeded protein scaffolds residing within the Chamber, and thus fulfill some of the primary functional requirements assigned by our sponsor. The functional requirements affected by the Flow Control are pulsatile shear stress, pulsatile tensile stress, as well as the delivery of oxygen and nutrients to cells throughout the thickness of the protein scaffold. Our sponsor has asked that we also apply a normal stress, if possible, to the scaffold; this is a lower level requirement. The shear stress should fall in the range of 0.1 to 1 Pascal, while the longitudinal strain requirement is less than 10%. In addition, the pressure required to perfuse the scaffold is approximately 50 kilo-Pascals.

We want to maintain oxygen and nutrient delivery throughout the thickness in order to ensure that cells can survive at any depth within the 500 to 700 micron thick scaffold. This is one of the most challenging functional requirements. Static systems, which allow nutrients and oxygen to diffuse through the scaffold with only hydrostatic pressure, will only support cells to a thickness of about 100 microns. The problem with this is that a 100 micron thick sample is not physiologically relevant when studying most naturally occurring cardio vasculature. However, a piece of synthetic tissue that is 500 microns thick, which is equivalent to half a millimeter, has physiological relevance. *In vivo*, tissues which grow to thicknesses even greater than our goal are sustained by the presence of capillaries and other such microvasculature throughout the volume of the tissue. This allows for an even distribution of sustenance. However, to synthetically grow or create such microvasculature is a feat yet to be accomplished, and is beyond the scope of this project. Therefore, a different method of nutrient and oxygen delivery will need to be used.

Similarly, the application of the aforementioned stresses serves to replicate *in vivo* conditions as seen in blood vessels. When the heart beats, the resulting pressure wave applies normal, shear, and tensile stresses upon the walls of the vasculature. Due to the gaps between the applied pressures of the heart, the resultant flow and induced stresses are pulsatile in nature. Thus, in order to realistically simulate *in vivo* conditions, it is desirable that the system applies similar stresses on each side of the cell-seeded protein scaffolds.

ii. Design

In order to accomplish the Functional Requirements mentioned above, a number of options were considered. If we were working with cylindrical protein scaffolds, the obvious solution would be to periodically pump cell culture media through the scaffold (along its longitudinal axis) at a pressure similar to that seen in a human aorta. However, our sponsor indicated that it would be preferable to use flat-sheet scaffolds since they are easier to produce, and therefore may be more applicable to research.

All three of our preliminary designs use a similar operational theory to accomplish the associated Functional Requirements. As can be seen in the design of the Chamber of our system, there will be two actual cavities; one above the scaffolds and one below. The flow in each cavity will be intermittent, which will create the pulsatile shear stress due to the flow over the scaffolds. Also, pressure will be built up in each cavity, again intermittently, in order to create the normal perfusion pressure, which in turn also causes a longitudinal stress and strain. By alternating a pressure build up in each cavity in this manner, we achieve a cyclic application of normal and longitudinal stresses, as well as delivering nutrients and oxygen throughout the scaffold. The three preliminary designs for this part of the system are discussed below.

a. Piston

The piston Flow Controller is unique amongst the three designs in that it does not employ a valve in its function. Rather, at some point before the inlet to each cavity, a section of the media flow has a side Chamber, or cylinder, containing a piston (see Figure 17 below). In the main section of tubing before the cavity, a constant flow is applied by the pump. This flow would fill the cylinder and continue beyond. Throughout the duration of the experiment, each piston (one for each cavity) would cyclically thrust forward, creating a spike in flow rate and pressure. Following this, the piston would slowly retract, allowing the cylinder to once again fill, and in the meantime, slightly reducing the flow rate. This method would result in a pressure versus time graph, or characteristic flow, similar to that shown in Figure 20. The pressure would rise sharply with the piston's thrust, and decrease with a gentler slope when the piston retracts. Both the upper and lower cavity would have a piston, and each would be on an opposite time scale. For example, if the cycle time was one second, then one piston would start its cycle on even seconds while the other started on odd seconds. This would create a pressure difference between the Chambers, allowing for the application of perfusion pressure and longitudinal strain.

A benefit of the piston design is that it would produce a realistic simulation of a heart given its aforementioned similarity with respect to characteristic flow. However, this design has some major flaws. One of the project constraints is that the entire system must be sterilizable so that it can be used more than once. With this Flow Control design, the piston will come into physical contact with the cell culture media. Therefore, the piston and cylinders will have to be sterilized either by heat or by contact with a solution such as bleach or alcohol. The problem with this is that the piston design includes a sealant, such as rubber, between the piston and the cylinder. This sealant may corrode and fail with either of the proposed sterilization methods, in which case the part would need to be repaired or replaced frequently. Additionally, this design is relatively expensive, and increases design complexity if the part is custom made.

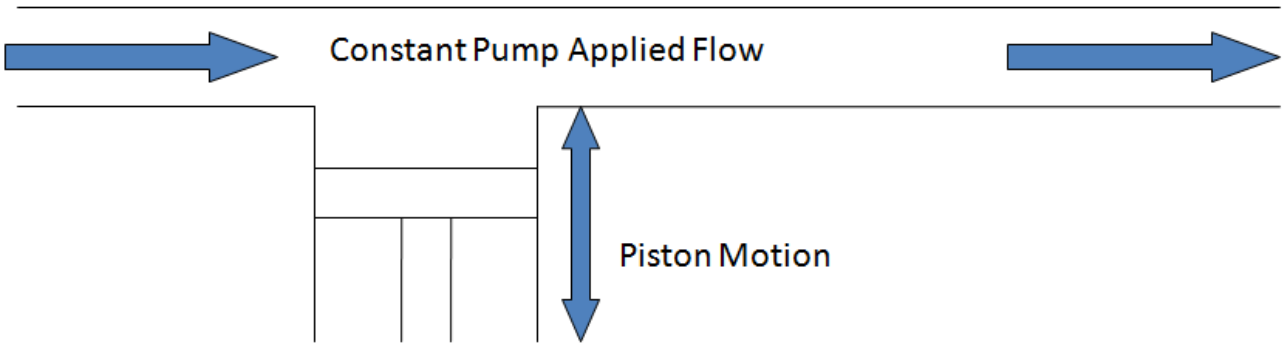


Figure 20: Pressure applied with piston.

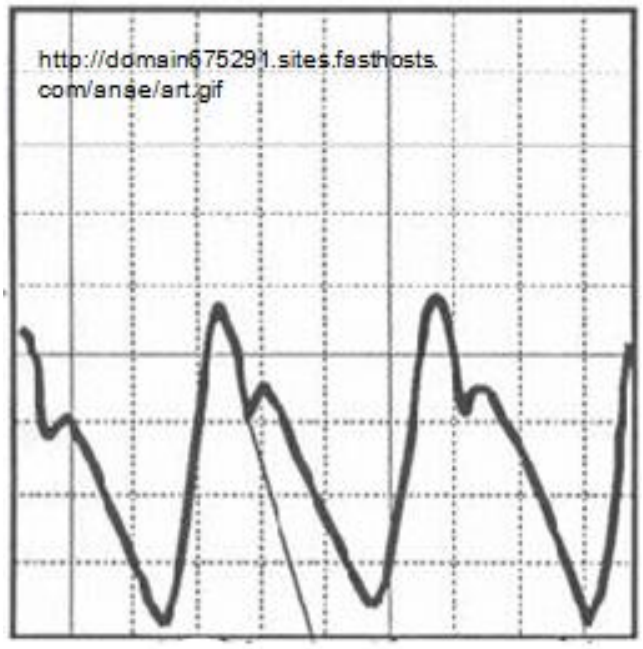


Figure 21: Characteristic wave of heart activity.

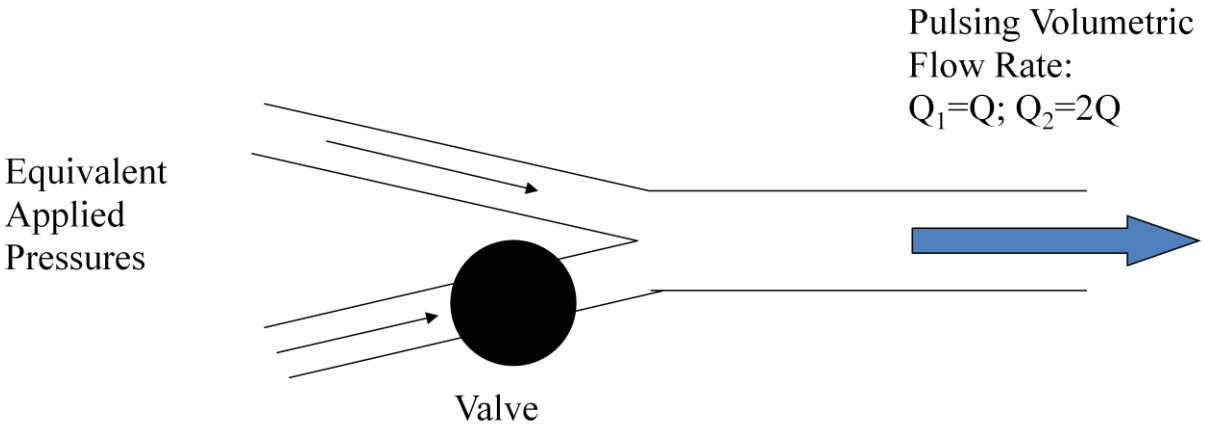


Figure 22: Preceding solenoid valve design.

b. Preceding Solenoid Valve

In our second design option, flow into each cavity is regulated by a solenoid valve placed before the cavity inlet. These solenoid valves open and close at opposite times, allowing flow in one cavity to be greater than that of the other at all times, as seen in Figure 22. This would create a pressure difference between the two cavities, applying

the normal pressure necessary to perfuse the scaffolds as well as produce longitudinal strain.

The benefits of this design include cost and design simplicity due to the availability of parts. Additionally, a characteristic flow of a square sine wave would result, which is fairly similar to that of a heart shown above in Figure 21. Also, sterilization would not be difficult due to the fact that a pinch solenoid valve can be used, which simply pinches down on the tubing. In this manner, the valve never comes into contact with the cell culture media, and hence, does not need to be sterilized.

c. Succeeding Solenoid Valve

In our third preliminary design, we use the same pinch solenoid valve from above, but it is placed after the cavity outlet, as can be seen in Figure 23 below. As with the Preceding Solenoid Valve design, when one valve is shut, the other will be open. This means that while there is media flowing through one cavity and inducing shear stress, the other cavity will have only an in-flow, allowing pressure to build up to the perfusion pressure in that cavity. When the pressure in this cavity reaches the perfusion pressure, the valves will switch states, so that now the valve with through-flow will have only an inflow. In this manner, very high normal pressures can be reached. Also, this procedure will be cyclic, so all induced stresses will again be pulsatile.

While this Flow Control design does not realistically simulate a heart's characteristic flow, it does allow for high perfusion pressures, and it satisfies all other functional requirements. Due to the fact that it would use the same materials as with the Preceding Solenoid Valve, it would not need to be sterilized and has a similarly low cost.

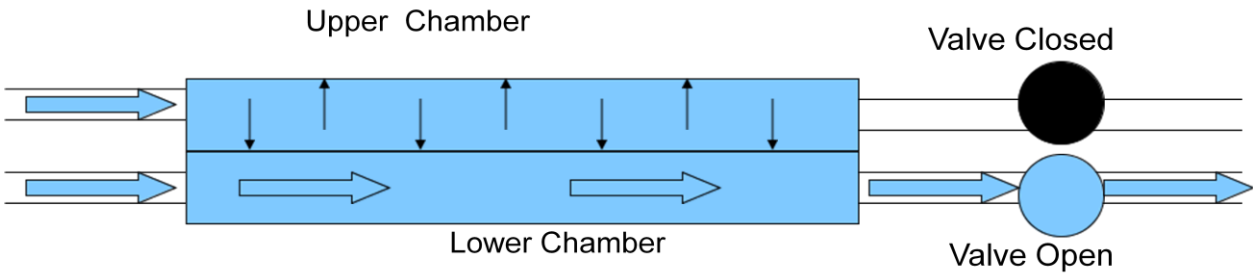


Figure 23: Succeeding solenoid valve design.

iii. Design Selection

In our original evaluation of the three above designs, we created a Pugh Chart (see Table 3 below) which weighed properties of each of the designs. As can be seen in the Pugh Chart for the Flow Control, we originally intended to go ahead with the Preceding

Solenoid Valve design. However, after the Preliminary Design Review, we realized that neither the Piston design nor the Preceding Solenoid Valve design would be able to apply a high enough normal pressure to induce a significant longitudinal strain or even perfuse the scaffold. In fact, a Succeeding Solenoid Valve would be the best option for Flow Control.

Table 3: Pugh chart for flow control.

FLOW CONTROL					
	Ideal	Preceding Solenoid valve	Piston	Following Solenoid Valve	Weight
Pulsatile Flow	50	50	50	40	10
Sterilizable	50	50	30	50	10
Ease of Use	35	31.5	31.5	31.5	7
Cost	30	24	18	24	6
Simplicity	47.5	47.5	28.5	28.5	9.5
Degree of Control	40	24	40	24	8
Simulates In Vivo	30	24	30	12	6
Weighted Total	282.5	251	228	210	
Percentage	**100**	**88.85**	**80.71**	**74.34**	

iv. Final Flow Control Design

As mentioned in the Design Selection section above, the final design for the Flow Control will use pinch solenoid valves succeeding the Chamber. For this reason, we have decided to use the Succeeding Solenoid Valve in our final design. It should be noted that in our Pugh Chart, the lowest scoring design was the Succeeding Solenoid Valve. When looking at the Pugh Chart, it is clear that the deficiencies for this design are simulating *in vivo* conditions and design simplicity. Both of these relate to the

unusual characteristic flow resulting from closing off the out-flow in the cavities. We have since reasoned that applying a pressure great enough to provide a significant longitudinal strain as well as keeping the cells in the center of the scaffold supplied with nutrients and oxygen are much higher priorities than replicating *in vivo* conditions since the former are top level functional requirements whereas the latter is not a requirement.

The theory behind how the Succeeding Solenoid Valve design works is explained in the Design section above. Necessary components for this design include: two pinch solenoid valves, both with a closed de-energized state (normally closed); a pressure sensor; a programmable logic controller (PLC); and a switch.

For the solenoid valves, the "de-energized state" is the position of the valve when no voltage is applied to it. Therefore, a normally open valve will close when voltage is applied, and a normally closed valve will open when voltage is applied. This plays a significant role in the operation of the system, which is explained below.

Figure 24 shows the operational setup for the Flow Control. It can be seen that the pressure sensor will take readings from either the top or bottom cavity. This sensor will be connected to the PLC, which in turn is connected to the switch. The switch will be responsible for signaling the valves to open or close.

When the system is used experimentally, both valves will start off closed. One of the normally closed valves will be immediately opened when the circuit is turned on. When one of the valves is closed, the pressure will increase as mass continues to flow into the confined space, increasing the pressure within that cavity. When the pressure, read by the pressure sensor, reaches a maximum which will be defined in the PLC, the PLC will cause the switch to apply a voltage to the currently de-energized valve, and to temporarily stop applying voltage to the energized valve. Hence, the valve that was closed will be opened, while the valve that was opened will be closed. This will allow pressure to build up in the cavity that was originally subject to through-flow. Once the pressure in the cavity with the sensor has decreased to a set point also defined in the PLC, the switch will stop applying voltage to the currently open valve, and the next cycle will begin.

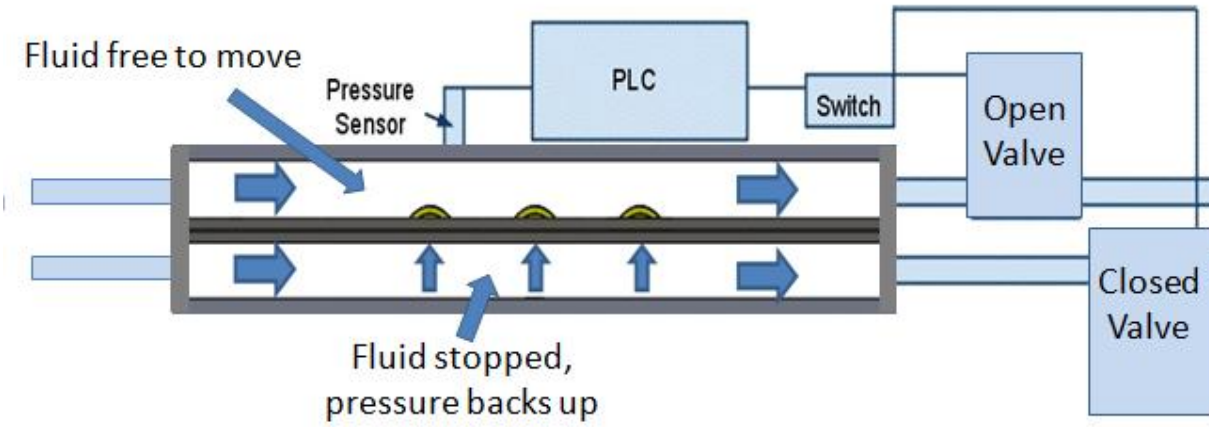


Figure 24: Operational set-up of Flow Control System.

By these means, each cavity will be subject to pulsatile shear, normal, and tensile stress. The shear stress will occur when a cavity's valve is open, while the normal and tensile stresses will be applied when the valve is closed. It can be seen that there is coupling between the normal and tensile stresses due to the fact that the tensile strain is caused by the normal stress' deformation of the scaffold. This incidence of coupling was determined to be acceptable since one of the constraints of the project is simplicity. This has limited the number of components to the system that are acceptable. If we were not constrained by cost or simplicity, we would want to keep the normal pressure from deforming the scaffold, preventing coupling, and use a motor to linearly deform the scaffolds to a predefined strain. However, for the scope of our project, the Flow Control will use through-flow in one cavity to induce shear whilst the other cavity builds up pressure to cause normal and tensile stress; the flow in each cavity will be cycled so that stress on the scaffold is symmetrical.

The components that are to be used in this system are: 2 ASCO SCH284A003 solenoid pinch valves, both normally closed; two MPXAZ6115 media resistant pressure sensors; one Arduino DEV-00666 USB board (PLC); and two Clare LCA710 switches. These components are shown in Figure 25.

Figure 25: (Clockwise) solenoid valves, PLC, relay, pressure sensor.

v. Second Semester Design Changes

The basic Flow Control design has only changed slightly since first semester. It has undergone two minor changes, both of which occurred as a result of parts. The first change was a result of the unavailability of solenoid valves. In the beginning, we had decided upon one normally open and one normally closed solenoid valve. Since we were unable to obtain one of each, we obtained two normally closed solenoid valves instead. This has only slightly changed the Flow Control circuit, in that upon powering the circuit, one valve will be turned on while the other will be turned off. This change only affected the Arduino program, which will be discussed in the Flow Control circuit portion of this report.

The second change came about during testing of the pressure sensors. One of our functional requirements was to apply a pulsatile shear stress to the cells in the system. Our sponsor had indicated a preference of applying specifically a 50 kPa pulsatile shear stress. However, upon testing the Flow Control circuit, we discovered that the pressure sensors could detect a maximum of 30 kPa. As a result, we changed our maximum pulastile shear stress of the system, and the solenoid valves switch from closed to open state at a pressure of 30 kPa instead of at a pressure of 50 kPa.

D. Environmental Control

i. Purpose

Our bioreactor is built for several purposes. Although the Chamber, the Flow Control, and the pump are essential to fulfilling the functional requirement of dynamic growth, which includes applying various stresses to the scaffolds planted with cells, the bioreactor must also be able to sustain the living cells throughout our experiments and tests. Our sponsor indicated that the time for the endothelial and smooth muscle cells to complete growth throughout the entire scaffold is approximately 14 days. The cells within the bioreactor must be provided with appropriate environmental parameters in order to thrive during this time period, which include the most crucial parameters of temperature (37° C), air (95%), and carbon dioxide CO_2 (5%). With the correct adjustment of these factors, it is also likely for bacteria to proliferate, thus, it is essential to employ a mean of preventing contamination of cells.

The Environmental Control will only be a part of our bioreactor system—it should be easy to use, sterilizable, and inexpensive, and most importantly, it should be able to operate continuously for at least 14 days. Our team proposed three major designs initially.

ii. Preliminary Designs

a. Incubator

The first design utilizes an incubator to provide the appropriate temperature and gas. An incubator is a machine involving a simple door with an open-close mechanism, and which can store bioreactors and other flasks (Figure 26) in its internal compartments. It is convenient in that it is automatic--the settings only need to be set initially and parameters are adjusted based on the magnitude detected by internal sensors. The incubator also links to a CO_2 gas tank externally so that appropriate amount of CO_2 can be injected into the incubator when the CO_2 level becomes insufficient. If this design were selected, our bioreactor can simply sit within the incubator for the 14-day culturing period. Because this machine will not come in direct contact with the bioreactor, it is unlikely for contamination to occur. As a result, it is unnecessary for our customers to

sterilize this device after each use. This machine will be provided to our team by our sponsor, so this design would not be an expense for our team.

http://www.speckanalytical.co.uk/Media/Image/product/reconditioned/Thermal%20Products/Incubators%20and%20Ovens/LEEC%20PF2%20Incubator_Large.jpg

Figure 26: Incubator.

b. Heater, Tank, and Bubbler

The second design incorporates a polycarbonate container, a bubbler, and a heater (Figure 27). The polycarbonate container would hold the nutrient media, which would be heated externally via the heater to maintain the media temperature at 37° C. There would be an inlet at the bottom of the container where gas can be injected through a porous material to provide the appropriate gas composition within the media. The porous filter between the media and bubbler both function to minimize the size of the gas bubbles. It would also be in direct contact with the media and would need to be either sterilized or exchanged for new, sterile filters after each use. These mechanisms can be set to run continuously. This design would also be relatively cheap as only a small amount of material would need to be purchased. On the other hand, there is a potential for large gas bubbles to damage our cell culture. When gas bubbles become too large, it can not only trap cells in a static condition, but it can also increase the chance of transporting bacteria to the scaffold/cell Chamber where contamination may occur, especially considering the size of the bioreactor and scaffolds.

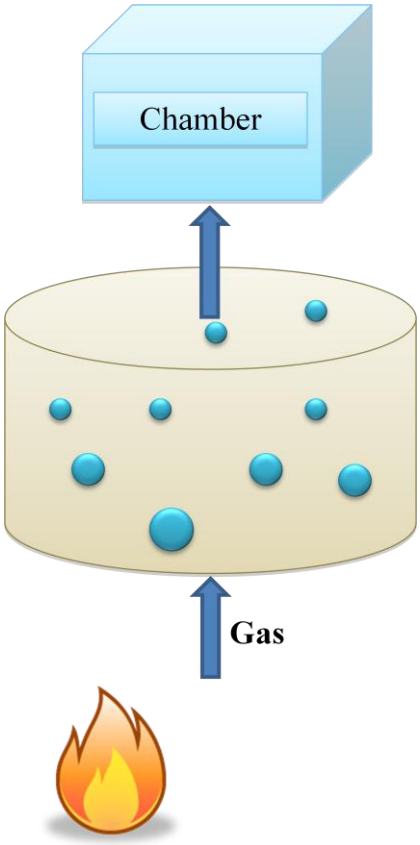


Figure 27: Chamber, bubbler, and heater.

c. Heater, Tank, and Sleeve

The last design for the Environmental Control involves a gas sleeve (Figure 28). In this design, the Reservoir of nutrient media will be heated to provide the appropriate temperature. The gas will be injected into an enclosed sleeve. This sleeve will be placed around the nutrient carrying tube. Because the nutrient carrying, silicon tube enables gas to diffuse through but not nutrient media, gas can be incorporated into the media via this pathway from the gas sleeve. The disadvantages of this design include the large expense of purchasing this specialized gas sleeve. This design is also relatively more complex and may be difficult to use. Another concern with this design is the possibility that there may not be a sufficient amount of gas to diffuse through the silicon into the media.

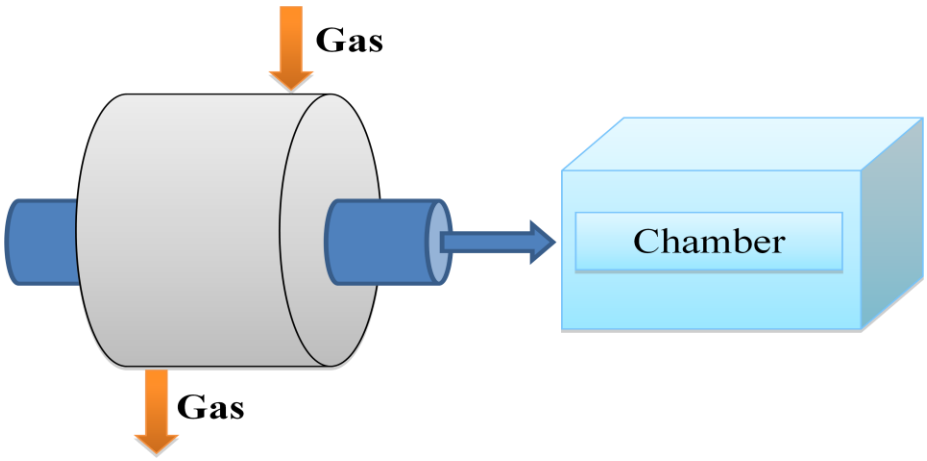


Figure 28: Gas sleeve.

iii. Design

Our team chose the incubator as the Environmental Control for our bioreactor. It can automatically provide the correct ambient parameters for the cells with the least cost. Compared to the other designs, it was most ideal. Table 4 illustrates the Pugh Chart.

Table 4: Pugh chart for Environmental Control.

ENVIRONMENTAL DESIGN					
	Ideal	Incubator	Heater/Tank /Bubbler	Heater/Tank /Sleeve	Weight
Temperature	50	50	50	50	10
O2/CO2	50	50	50	50	10
Cost	30	27	24	18	6
Ease of Use	35	24.5	35	35	7
Waste Removal	25	22.5	25	25	5
Sterilizable	50	50	40	50	10
Design Simplicity	45	45	36	27	9
Culture Safe	50	50	35	50	10
Weighted Total	335	319	295	305	
Percentage	100	95.22	88.06	91.04	

The incubator can be set to 37°C. It can then adjust to this temperature for a period of time; specifically, 14 days. The incubator also contains gas composition sensors that are able to detect the amount of CO_2 within the incubator compartment. When the amount detected shifts from the set magnitude, the CO_2 inlet is automatically opened or closed to return the level to the set point. This mechanism is also used for providing the correct composition of air within the incubator compartment. The gas within the incubator will be able to diffuse through the filter cap of our nutrient media Reservoir into the nutrient media.

Other than maintaining temperature and gas, the incubator has the ability to supply and adjust high relative humidity and to prevent contamination. An adequate amount of moisture within the compartment will prevent our nutrient Reservoir from evaporating rapidly, thus, this control could facilitate the preservation of our nutrient media and its chemical composition. The incubator is also built so that the area of potential bacterial growth is reduced. The material inside of the incubator is also selected to deflect bacterial or fungal propagation.

Our team has also incorporated another method of preventing contamination. When gas diffuses into the nutrient media within the polycarbonate Reservoir, it must pass through a filter cap. The pores of this filter are large enough for gas to diffuse through but not microorganisms, thus mitigating the probability of contamination.

Unlike the second Environmental Control design where the bubbler comes into direct contact with the cell media, the incubator never comes in contact with the cell media, so there is no need to sterilize this design after each use.

The incubator will not only fulfill the functional requirement of providing a suitable environment in which the cells can grow, it also provides several advantages such as ease of use, low cost, humidity maintenance, and contamination prevention.

iv. Second Semester Design Changes

Several changes have been made to the Environmental Control to improve our design. A stir bar has been added to the Reservoir to increase the rate of air diffusion into the cell media. Also, instead of storing the complete system within the incubator, some components have been chosen to be set outside of the incubator due to space limitations and electricity requirements.

E. Reservoir

i. Purpose

The Media Delivery System provides a mean of transporting media to the cells; however, the bioreactor also needs a method of holding the nutrient media from which the pump can obtain the media.

Because the pump will be continuously transporting media from the Reservoir to the cells, where the cells will consume the nutrient, the amount of important chemicals will be depleted eventually. Unfortunately, replacing the media involves exposing the sterile media to the external environment, which may lead to contamination. A method to reduce the number of media replacement occurrences is for the Reservoir to hold a copious amount of media that will last at least 14 days.

The media Reservoir must have an opening that exposes the media to the external environment. This pathway will enable necessary gas to diffuse through the media. However, a filter will be placed at the opening so that contaminates will not enter. Like other components of the system, the Reservoir should not damage the cells or interfere with the functional requirements in any manner. It should also be easy to use, inexpensive, and sterilizable.

ii. Design

Our team had not created multiple preliminary designs for this component because it is only required to complete the continuous function of our bioreactor system and further

designs were unnecessary. The main purpose of this component is to act as a source and sink for the cell media to flow to and from. Our team decided to fabricate a polycarbonate container that will contain a filter cap to allow gas to diffuse through (Figure 29). Because polycarbonate will be purchased to build the bioreactor Chamber, it will be less costly to use the same polycarbonate to fabricate the nutrient Reservoir. The polycarbonate is also biocompatible so it will not affect our nutrient in any manner. The transparent container surface will allow our team members to note the color change when the cell media needs to be replaced. A special micropore filter will be purchased. This filter will allow gas to diffuse through but not microorganisms, thus reducing the chance of contamination.

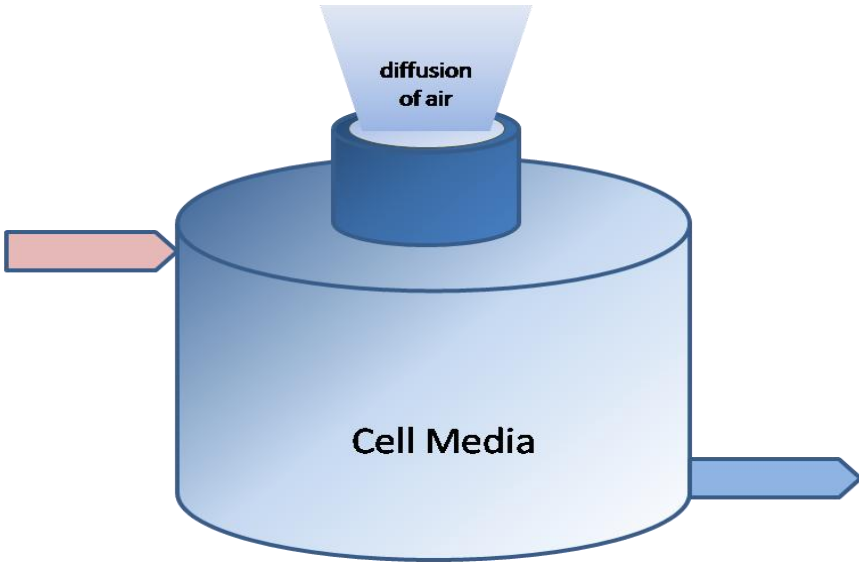


Figure 29: Polycarbonate container.

The Reservoir container will contain an inlet that is placed above the outlet. The inlet will allow recycled media to flow into the Reservoir, while the outlet is the source where media will be pumped into the bioreactor Chamber.

Our team determined that this design will be most suitable for our bioreactor system based on the budget, its simplicity, and its ease of use.

F. System Decomposition

- Chamber
  - Body
  - Upper Securing Plate
  - Lower Securing Plate
    - Securing Plate Screws (4)
  - Lid
- Lid Screws (8)
- Media Delivery
  - Peristaltic Pump
  - Silicone Tubing
    - Tube Connectors
    - Silicone Tube Sealant
- Flow Control
  - Solenoid Valves
    - Two Normally Closed Pinch Solenoid Valve
  - Programmable Logic Controller
    - Relay Switch
  - Pressure Sensor
- Reservoir
  - Body
  - Filter
- Environmental Control
  - Incubator

III. 1st Semester Conclusions

Throughout the 1st semester, we have discussed various options for components of our Bioreactor. For the System Requirements Review, we focused primarily on our Problem Statement, Project Scope, and Customer Requirements. To recapitulate, the purpose of our project is to improve upon existing cell culture technologies by applying stresses on a scaffold seeded with cells. Our design will differ from other dynamic systems in that it will be simple to both use and replicate. We hope that by developing such a system, researchers will easily grow tissue sample, similar to those grown in the body. The Preliminary Design Review included three plausible designs for each component. The components are broken down as follows: Chamber, Environment Control, Flow Control, and Media Delivery System.

The Pugh Diagrams were used to evaluate each component design in a weighted fashion through characteristics such as simplicity, cost, and sterilizability. Hence, we used four Pugh Diagrams, one for each component. After analysis of the Pugh Diagrams, we came up with our final design. The system begins with the media Reservoir, which will hold approximately 250 milliliters of cell media. This media will flow through the entire system and be recycled back into the Reservoir. The media will first flow through a peristaltic pump, which will allow for a continuous, pulsatile flow. The purpose of this is to push the fluid through the Chamber, which will be built in order to incorporate nine flat sheet scaffolds of one square centimeter in area. The Chamber will allow for placement of up to nine scaffolds. Pinch solenoid valves will be placed following the Chamber in order to control flow and build up pressure inside the Chamber. The significance of building up pressure inside the Chamber is to make sure that nutrients perfuse through the 500-700 micron thick scaffold, so that all of the cells obtain the proper amount of nutrients. The entire system will be placed inside an incubator to maintain the proper temperature of 37^{o} Celsius and proper levels of carbon dioxide and oxygen.

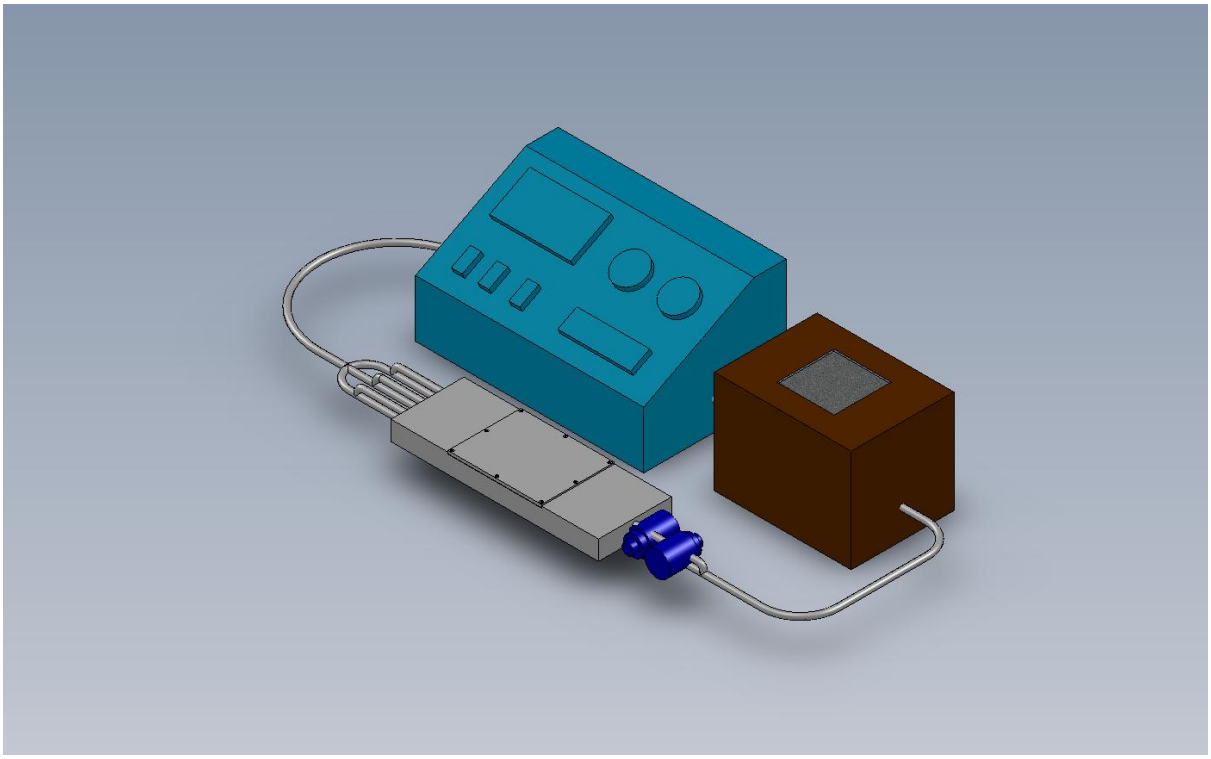


Figure 30: CAD Model of Entire system.

Although we came to a conclusion for each component in our design, there were a few contradictions we met along the way. However, we overcame these contradictions by eliminating the conflicting component designs. One example of this was deciding upon the pumps. To obtain a shear stress, the velocity of the fluid must be high. However, the higher the velocity, the more expensive the pump will be. In this case, two very important parameters, one of which is a functional requirement, conflict. Since obtaining shear stress is a functional requirement, we decided to purchase a pump that outputs a high velocity.

Despite the contradictions, our final design components are as close to the Ideal Final Result as possible. As previously mentioned, the Ideal Final Result is a system that will maintain cell growth for an indefinite period of time, apply shear, tensile, and normal stresses to the scaffold, will be made of few, readily available parts, will be sterilizable, and will cost nothing. Apart from spending no money, which is quite unrealistic, the ideality of our design is close to the Ideal Final Result. The parts we have chosen are readily available, except for parts such as the Chamber that we will be fabricating from polycarbonate. Additionally, all of the components will be sterilizable. The system revolves around the fact that stresses will be applied to the scaffold, which leads to the purpose of cell growth within the Chamber.

To organize our design process, we developed a Gantt chart. This Gantt chart includes critical dates and the estimated time to finish specific portions of the project. Our critical path includes simulating the fluid flow through the Chamber using computational fluid

dynamics with Fluent, obtaining an approximation for fluid velocity, simulation of scaffold stresses with ABAQUS, and choosing the proper materials for the Chamber and tubing. So far, we have not had any issues meeting deadlines, and hopefully we will not have any such issues in the future.

In conclusion, the Cultural Dynamics team has decided upon a final design with the four main subsystems for the Dynamic Cell Culture System. With the scope of the project in mind, our team will begin construction, assembly, and testing next semester. Keeping our Functional Requirements, Constraints, and Customer Requirements in mind, the team hopes to complete the project successfully after testing it up to three times before Design Day.

IV. System Fabrication and Construction

A. Chamber

Fabrication of the Chamber consisted of multiple operations: milling the Chamber body (main Chamber); drilling inlets and outlets in the Chamber; milling the Securing Plate; filing slopes into the Securing Plates' scaffold positions; adding rivets to one Securing Plate for ease of handling (specifically for the removal of the plate); and milling the Chamber Lid.

i. Main Chamber

Milling the Chamber body was a fairly simple pocket-milling operation. This involved using a quarter inch diameter solid carbide endmill with a one sixty-fourth inch radius. Because the geometry of the Chamber is quite simple, we were able to write the code for the Computer Numerical Controller (CNC) for the pocket mill. The operation was performed using a spindle speed of 1600 rpm and a feed rate of 150 mm/min. The endmill plunged only .125 mm (five one-thousandths of an inch) at a time to ensure a smooth finish and to preserve the endmill. Additionally, both air and oil were used to cool the endmill during the operation. A second code for the main Chamber was written to "clean up" the faces of the walls. Due to the nature of the first milling operation, some of the walls had a pattern that had a similar corrugation, so these areas were milled over for a smooth finish.

The operation to drill the inlet and outlet holes was also very simple to program. However, the difficulty here was preparing the CNC so that the holes would be drilled at precise points to ensure that the flow in the Chambers was symmetric. This was accomplished by manually measuring distances to the center-points of the first sub-Chamber and marking it. We then visually placed the drill bit at our marked point. From there, the CNC was programmed to move set distances to drill the remaining holes, so only the first point had to be marked. Again, both air and oil were used to minimize heating of the drill bit.

ii. Securing Plates

The milling operation for the Securing Plates was also written by hand. Relatively sharp corners for the holes in the plates were desired; hence a one-sixteenth of an inch diameter of solid carbide endmill was used for this operation. The spindle speed used was 1750 rpm, and the feed rate of 15 mm/min was used. Only 0.125 mm was removed per pass. Because the plates are identical, with the exception of the rivets, the same code was used for both plates. This operation simply included cutting out each of the nine square holes in which the scaffolds will reside, followed by cutting out the perimeter. In order to include slopes at each of the scaffold holes, a Dremel Rotary Tool was used to remove a significant portion of material before diamond needle files were used to create smooth finishes.

In order to make the top Securing Plate easier to remove from the Chamber, Dale Drew, the Aerospace and Mechanical Engineering Department Instrument Maker, installed rivets. Two rivets were installed, both residing in the center sub-Chamber near, but to one side, of the inlet or outlet. The proximity to the inlet/outlet keeps the rivet from significantly affecting the flow. The rivets are 2.38125 mm in diameter and approximately 2 mm tall. This allows them to be removed by hand, by tweezers, or by pliers.

iii. Chamber Lid

As with the other milling operations, the code for the Chamber Lid was written by hand rather than by using Computer Aided Manufacturing (CAM) software. Here, we again used the quarter-of-an-inch solid carbide endmill that was used in the milling of the main Chamber. In this case, a spindle speed of 1600 rpm and a feed rate of 600 mm/min were used. Because the lid now had to be manufactured to include the supporting ribs that define the upper-level sub-Chambers, the area of the milling operation included the viewing area, or the portion of the polycarbonate that is meant to be a window to visually inspect the condition of the tissue scaffolds. Because of this milling operation, the viewing area of the polycarbonate has become somewhat obscured. While it is still possible to observe the scaffolds through the viewing area, fine details cannot be seen. However, the visibility improves when the surface is wetted. Depending on time, the viewing areas may be polished to improve visibility.

iv. Sealant Application

In order to prevent any loss of pressure from the Main Chamber or any media from leaking out of the Chamber, a silicone sealant was applied to various parts of the Main

Chamber and Chamber Lid. On the Chamber Lid, a very thin layer of the silicone sealant was applied to each of the four ribs, and a thicker layer of the silicone sealant was applied around the outside of the center extrusion of the Chamber Lid. Similarly on the Main Chamber, a very thin layer of the silicone sealant was applied to each of the four ribs. In order to allow 1/16'' ID tubing to connect to the Chamber, plastic tubing connectors were externally coated with the silicone sealant, and then press fitted into the holes on the sides of the Main Chamber.

After preliminary testing, which consisted of running water through the Main Chamber with the two Securing Plates in place and the Chamber Lid clamped down, it was discovered that fluid could leak between the Chamber Lid and the Main Chamber. To solve this issue, a wide layer of a silicone sealant was applied to the Chamber Lid and two flat plates of aluminum were used to clamp the entire system together. This change completely eliminated any loss of pressure from the Main Chamber and successfully prevented any media from leaking out of the Main Chamber.

The only foreseeable issue concerning silicone sealants and the Chamber is that the silicone sealants may degrade after multiple uses. However, this only pertains to the silicone which is adhered directly to the stainless steel Main Chamber and polycarbonate Chamber Lid. If the silicone sealants at these locations degrade, they can be easily reapplied.

v. External Clamps

In order to tightly secure the Chamber Lid to the Main Chamber in an efficient manner, two flat plates of aluminum were fabricated. In these plates, six through-holes were CNC machined which would evenly surround the external perimeter of the Main Chamber. These plates secure the Chamber Lid to the Main Chamber by being bolted together with the Chamber and all of its sub-components in between using six ¼-20 hex bolts, six ¼-20 nuts, and six locking washers. An illustration of how the two flat plates of aluminum clamp together the system is shown below in Figure 31.

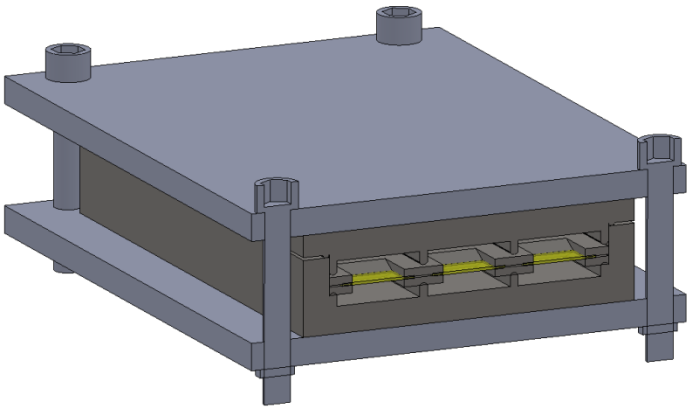


Figure 31: Aluminum clamps.

B. Flow Control Circuit

i. Integration of Electronic Components

The four main components of the Flow Control circuit are two pinch solenoid valves, the Arduino, two pressure sensors, and two relay switches. The Arduino controls and allows for integration of the entire electronic portion of the system. The Arduino is connected to the pressure sensors. The pressure sensors are connected to the rest of the bioreactor system, and are also connected to the relay switches on the circuit board. When the pressure of the media flow reaches 30 kPa, the relay that is connected to the unopened solenoid valve is activated. When the relay is activated, that particular solenoid valve is opened. The other solenoid valve is then closed due to the message of inactivation sent from the other relay.

ii. Coding of Circuit System

The Programmable Logic Controller being used in the Flow Control system is an Arduino DEV-00666 USB board (PLC). This PLC is programmed using an open source code written for Arduino PLCs. This language was used to write the Flow Control

program. The program is written in an infinite loop as follows. Initially, valve 2 is opened and valve 1 is closed. Initial pressure values are then received from both pressure sensors and assigned to specific variables P1 and P2 corresponding to valve 1 and valve 2, respectively. P1 is then compared to a determined limit of 1000 (determined in testing) in an "if" statement. If P1 is less than 1000, the program enters the "if" loop. Inside the "if" loop the pressure in the top and bottom of the Chamber is monitored and P1 and P2 are redefined with updated values until P1 is equal to 1000. As soon as P1 equals 1000, the program exits the loop. Valve 1 is then opened and valve 2 is closed. P2 is then compared to a determined limit of 1000 in an "if" statement. If P2 is less than 1000, the program enters the "if" loop. Inside the "if" loop, the pressure in the top and bottom of the Chamber is monitored, and P1 and P2 are redefined with updated values until P2 is equal to 1000. When P2 equals 1000, the program exits the "if" loop and returns to the top of the infinite loop.

C. Media Delivery System

The Media Delivery system consists of parts that connect the individual components of the design as a whole. It also ensures that nutrient flow is continuous in a closed loop. This system includes Environmental Control, the Pump, the media Reservoir, tubing, fittings, silicone sealants, and seal tapes.

i. Environmental Control Setup

It is essential for all the environmental factors to be sustained at appropriate levels for the cell to survive during incubation. Several components of our system are designed to maintain appropriate air level and temperature, and to prevent bacterial contamination of cells.

To provide a sufficient amount of air, including oxygen and carbon dioxide, the Chamber, major tubes, solenoid valves, Flow Controller, and the media Reservoir are stored in an incubator. The incubator allows the ambient temperature to remain around 37° C, while it also presents an approximate concentration of CO_2 supplied by the CO_2 tank. Because the incubator is constantly opened and closed during usage, the air inside would be in equilibrium with the air outside. Thus, the air quality within the incubator would contain about 95% oxygen and 5% carbon dioxide, which is a suitable amount for a cell to utilize. Our team has setup components that are bulky and/or require electricity outside of the incubator. As a result, our sponsor has considered creating a hole in the wall of the incubator to allow connection between major components.

Further design components have been used to maximize the amount of essential gas dissolved in the cell media and delivered to cells in the Chamber. Because the flow rate has been set at a relatively high rate, to compensate for the smaller amount of time cell media is exposed to air in the Reservoir, a syringe filter, stir bar, and silicone tubing have been adopted in the design.

The syringe filter is connected to silicone tubing, which is placed through the cell media Reservoir cap (Figure 32). It plays a key role in allowing the air concentration within the media Reservoir to equilibrate with the air concentration in the incubator. At its position on top of the cap, this low cost component allows air to pass through easily while filtering out harmful bacteria and contaminants. The air within the Reservoir diffuses into the cell media over time. A sterile, magnetic stir bar is also placed inside the cell media during cell incubation in order to enhance air diffusion throughout the media. At a low rpm (revolutions per minute), the stirring action induces forces that pull small gas bubbles into the media. Long lengths of silicone tubing have been used throughout the system because of its gas-permeable properties. Silicone can allow gas to diffuse into the cell media as cell media flows through the tubing; thus, it could increase the chance of cell survival by improving the availability of air in the media.

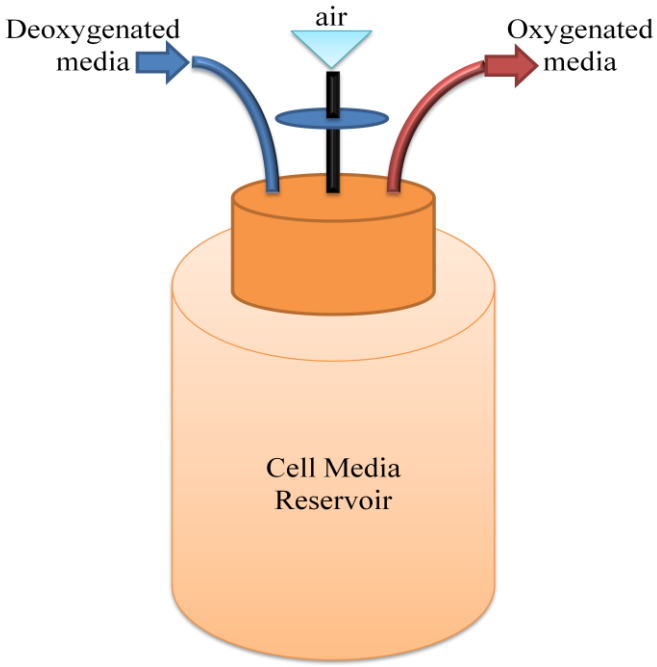


Figure 32: Media Reservoir.

ii. Pump Setup

The heart of the Media Delivery System is the pump, which acts as the source of energy that pushes the cell media nutrient throughout the system. The peristaltic pump does not come in contact with the cell media so sterilization is not necessary and bacterial contamination from the pump does not need to be considered. Our team is using the Cole Parmer peristaltic pump, which uses two heads of the same flow rate. The total flow rate the pump is set at 3.8 L/min, so each pump head supplies a flow rate of 1.9 L/min.

The pump drives cell media directly from the media Reservoir to the rest of the system. The Reservoir is a 500 mL glass container that has a plastic cap. Holes are created through the cap, so an inlet tube, an outlet tube, and a syringe filter tube can pass through the cap into the cell media. The pump has been placed outside of the incubator during testing due to its large size and power requirement.

iii. Tubing Setup

From each of the two pump heads, a 6.9 mm inner diameter (ID) tube is connected to a 1/16" ID silicone tube via fittings. The total length of tubing used between the pump and the Reservoir is 1 m. This length limit would prevent any pressure drop that is too high for the pump's capability. The silicone tubing is split into three tubes that direct the flow to each of the three sections in the top and bottom of the Chamber. To ensure that the trifurcation of the flow is relatively equivalent, small manifolds have been used at these flow-splitting points prior to the Chamber. The majority of the tubing is made of silicone to allow gas to perfuse into the cell media. From the Chamber, solenoid valves are attached to the collection tubing of the top three portions of the Chamber and bottom three portions of the Chamber. At this point, Y-fittings are used, because equivalent flow within the three tubes from each Chamber portion is unnecessary. Hence, it is more cost efficient to use Y-fittings. A set of the collection of tubing is finally set to direct flow back to the cell media Reservoir, so deoxygenated nutrient media can be replenished with oxygen and recycled through the system.

Other essential parts that are utilized include silicone sealants and seal tapes. In assembling the system, these components are important in preventing leaks and cell media and cell contamination.

D. Overall Assembly

The overall assembly involved integration of the subsystems such that they interact with each other properly, and thus achieve the desired results.

i. Subsystem Integration

System integration involved connecting the Media Delivery System tubing to the Chamber inlets and outlets, and attaching the pressure sensors and solenoid valves to the correct portions of tubing. The integration of the Media Delivery System and the Chamber required the use of silicone sealant to prevent leaks at the inlets and outlets. When attaching the solenoid valves and pressure sensors to the Media Delivery System at the outlet end of the Chamber, the solenoid valves had to be placed at a portion of the tubing after the point that branches off to the pressure sensor in order to ensure that the line to the pressure sensor is always open.

V. Verification and Validation

A. Preliminary Component Testing

Prior to assembling and testing the whole system, components and sub-systems were tested individually in order to simplify the debugging process of the full system. When testing a sub-system individually, problems are more easily diagnosed since the scope is narrowed down to a much smaller array of components.

i. Chamber

a. Sealant and Pressure Testing

To ensure that the Chamber is properly sealed and that no leaks exist, our team tested the seals between fittings and tubes, and around the Chamber. First, the team pumped air at a rate of 120 mL/min through the system. If the seals are tight and leaks are absent, the pumped air creates a pressure that can be detected by the pressure sensors. The pressure sensors signal solenoid valves to alternate between open and closed by turning them on and off via the Arduino. During the test, when air was pumped through the system, the pressure detected by the pressure sensor did not reach the expected level of 30 kPa, and the solenoid valves failed to operate. This qualitative observation demonstrated that a leak existed within the system. Furthermore, we tested the sealants within the system by pumping water through the system. As a result, we detected that water leaks through the silicone sealants between the Chamber Lid and the Chamber. No leaks were observed at the fittings when the flow rate was set to 120 ml/min.

To mitigate this problem, our team decided to reapply silicone sealants around the Chamber and the Chamber Lid. In addition, we decided to increase the amount of sealant applied. If seals are properly applied, a pressure increase can be propagated through the system that is functional to operate the solenoid valves. Further tests were conducted with new seals applied, and, to date, the system has reached a maximum flow rate of 1.1 L/min. We expect to continue testing to flow rates as high as 1.3 L/min to maximize shear stress.

ii. Flow Control Circuit

The testing and measurement of lower level functional requirements was outlined in the Verification and Validation Test Plan.

During testing, we found it useful to add an LCD to our circuit to display pressure values and switch modes. This was implemented in the program by updating the pressure values displayed every time a P1 and P2 were redefined and to display the mode of valve 1 and valve 2 by reading “off” or “on” depending on the mode of each switch. This provided a way to verify the functionality of the program as well as the other Flow Control components.

a. Pressure Sensor

The pressure sensors used in this system are MPXAZ6115AP media resistant high temperature accuracy integrated silicon pressure sensors. The output is within the range of 0.2V to 4.8V. Therefore, the output value must be converted from volts into kiloPascals. Therefore, we ran two tests: one to verify that the pressure sensors were working correctly and one to determine how to convert the output to kiloPascals.

The functionality of the pressure sensor was tested using a qualitative test with the Arduino, pressure sensor, LCD, and a syringe. The test involved applying a pressure to the pressure sensor and observing how the value on the LCD changed. The expected result was that the displayed value would increase when the pressure was applied and decrease when pressure was released. The pressure sensors worked as expected.

The conversion test was conducted using the pressure sensors, the Arduino, a syringe, various known masses and a digital multimeter. Initially, the pressure sensor was connected to the syringe with no applied pressure. The output value was read at normal, known, air pressure using the LCD and a multimeter. It was found that the Arduino code does, in fact, change the voltage value in its built-in serial read function. Therefore, the LCD displayed a value different from that of the multimeter. The pressure, LCD and multimeter values were all recorded. Then, a known mass was placed on the syringe plunger. The applied pressure was calculated using the known mass and the cross-sectional area of the syringe. This pressure value was recorded along with the value from the LCD and the multimeter. This process was repeated using five different, known masses. This gave corresponding LCD and multimeter values for each known pressure. This data was then plotted (see Figure 33). It was then used to find the upper limit of the pressure sensor and the corresponding voltage output and LCD displayed value. The calculated upper limit is used as the upper limit in the Arduino program. For the upper limit of pressure, 30kPa, the LCD value was determined to be 1000.

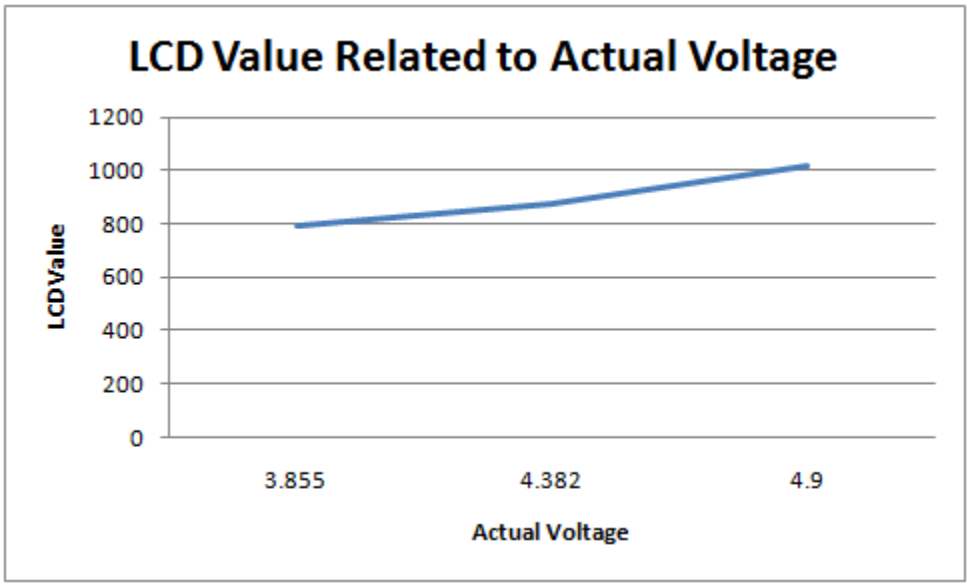


Figure 33: Voltage and pressure data.

b. Solenoid Valve

To test the solenoid valves, each valve was connected to a 12V power supply and observed. The expected result was that the valve would open when connected to the power supply. Both valves passed all functional testing.

ii. Operation of Solenoid Valves

The solenoid valves are normally closed when in a 'de-energized' state; hence, when powered, they open. When the circuit is turned on, one of the valves is set to open. The maximum pressure that the pressure sensors can detect is approximately 30 kPa. The Arduino is programmed such that when the pressure in the system reaches the 30 kPa limit, the currently open solenoid valve closes, and the other valve opens. The valves switch between opened and closed while the system is running. This allows the flow to undergo pulsatile pressure changes in order to fulfill the sponsor's functional requirements. The Flow Control system fulfills the following top level functional requirements: supplying the cells with nutrients throughout the scaffold through perfusion pressure, applying pulsatile shear and normal stress, and applying pulsatile tensile strain.

iii. Media Delivery System

a. Fluid Transport

In order to ensure that nutrients are supplied to the cells on the scaffolds, we must guarantee that fluid is being transported throughout the system properly. Thus, we must make certain that there are no fluid leakages, that the pump supplies the accurate flow rate, and that proper pressure is maintained. We will check for leakages using visual inspection of the system. A food coloring or dye may be used to make leakages more obvious. Proper flow rate will be determined by manually measuring the flow rate using a timer and a controlled volume. Finally, proper pressure within the system will be measured using the pressure sensors connected to the Arduino in the Flow Control system.

Additionally, fluid transport will be assessed by checking the cells' viability after a testing period. The cell viability test will be done using a basic live/dead dying kit that is commercially available. With the knowledge of the live/dead cell counts we will have an idea of the number of cells that are receiving proper nutrients. For example, if most cells are dead on the scaffold, then it is safe to assume that there is either not enough oxygen or not enough nutrients in the media. If the problem is with the nutrients, we will attempt to change the media more often within a particular testing period. If cells are still mostly dead, then we will assume that the media is not oxygen-enriched enough for the cells. We would then have to implement another method of oxygen infusion, such as adding an oxygen bubbler or by increasing tubing length. We will also check the number of live cells and dead cells in the cell media. If there are too many dead cells present in the cell media, the media will have to be filtered periodically during testing to ensure that the dead cells do not release toxins that will interfere with the live cells in the scaffolds.

B. Final System Testing

The full system has been tested using water and surrogate scaffolds. With the original solid-state relays, the system successfully alternates pressure build-up in the top and bottom portions of the Chambers, resulting in the application of pulsatile stresses upon the surrogate scaffolds. However, due to a circuit error, both of the solid state relays were shorted, and new relays had to be purchased and integrated into the system. These new relays originally caused some error in the operation of the Flow Control subsystem; they appeared to be drawing too much power away from the Programmable Logic Controller (PLC), which sometimes resulted in irregularities in the frequency of the opening and closing of the valves. This was especially problematic if one valve remained closed for a long period of time. If this happened at a high flow rate, pressure could build to a point where a scaffold would rupture. In this event, the valves would stop switching- one would remain open and the other would remain closed.

We found that the issue regarding the mechanical relays was caused by the use of a voltage source that was too small. Matching a new power source to the mechanical relays brought about further complications to a point where the team decided to order the original relays that worked with the Flow Control circuit. The circuit was setup with the original relays and tested as described above in order to verify proper function.

We have tested the system up to 1.3 L/min, which is sufficient to produce our desired value of shear stress. The other values of stress, normal and tensile, are achieved regardless of flow rate, as long as the pressure builds up to 30 kPa. The last that was performed on the system was an equilibrium test, in which all components were allowed to reach steady state in temperature. From this test, it is safe to assume that the system is capable of running for longer periods of time.

C. Design Requirements Validations

i. Top-Level Functional Requirements

a. Shear Stress

We do not have an experimental means of testing for the shear stress found at the locations of the scaffolds. Hence, we used the Computational Fluid Dynamics (CFD) software FLUENT to calculate the shear at the center points of the scaffolds, as well as to create diagrams depicting the shear distribution across each scaffold at various flow rates within the output range of our peristaltic pump. We found that even using a fluid with the viscosity of water, our system is capable of applying shear stresses within the target range of 0.1 to 1 Pa. The results for the Fluent simulation can be seen in the Appendix under 2nd Semester Fluent results.

b. Tensile Stress

As with the shear, we are unable to experimentally test for the tensile stress on the scaffolds. We used a Finite Elemental Analysis program called ABAQUS to test tensile stress. Here, we applied a 30 kPa pressure normal to the surface of the Securing Plates and scaffold. The material properties used in this simulation were given by our sponsor. The simulation resulted in a tensile strain of approximately 6.5%, which is within the acceptable range of 5 to 10%.

c. Normal Stress

Normal stress is the only stress which we are measuring experimentally. The pressure sensors detect when the pressure increases to the target pressure of 30kPa in the upper or lower Chamber. The Flow Control circuit then tells the respective solenoid valve to open, thus relieving the pressure built up within the Chamber.

d. Nutrient Delivery

Unfortunately, we were unable to complete any testing with actual seeded cell scaffolds. As a result, we cannot validate that the entire thickness of the scaffold will be receiving nutrients. When testing with cells does occur, the user will be able to determine whether or not the cells are getting enough nutrients based on the cell viability. A live/dead cell count is an easy indicator for the user; numerous dead cells could indicate that the cells are not receiving enough nutrients. If the user does encounter many dead cells, it may be beneficial to either increase media changing frequency or to add a stir bar to the Reservoir to provide more oxygen to the cell media. Either of these changes will increase nutrient concentrations in the cell media.

VI. Testing Results

A. Results using Surrogate Scaffold

To this date, we have only completed preliminary testing on the device using surrogate latex scaffolds from a section of a latex glove. With this contraption placed in between the Securing Plates, the device was first tested with increased air pressure within the system no fluid was used. During this test, we found that air was leaking from the system somewhere, most likely from the Chamber. In order to confirm this, we filled the system with water. When this test was performed, we found that water leaked from the Chamber Lid due to warping of the lid in sections that were not clamped down. Because of this, we re-sealed the Chamber Lid and used different types of clamps.

B. Results using Living Cells/Tissue

Currently, there have been no opportunities to test the system with seeded cell-scaffolds, so there are no results with this implementation.

VII. Conclusions and Recommendations

A. System Capability Assessment

Based on the physical system testing results and the computational results from ABAQUS and FLUENT, our system is fully capable of fulfilling all of the functional requirements. The simulations have shown that given the range of flow rates applied by our pump, the system can easily apply a physiologically relevant shear stress (0.05 - 0.1 Pa) upon the surfaces of the scaffolds. Furthermore, by applying a 30 kPa normal stress, a tensile strain of approximately 6.5% is achieved, which falls within the required range of 5 - 10%. We also know that the system can be run continuously without complication.

Although the system has not yet been tested with living cell-seeded scaffolds, all components of the system which come into contact with either the cell culture media or the scaffolds are sterilizable by either autoclave (heating to 120 degrees Celsius) or by exposure to ethanol. Additionally, in order to ensure that oxygen diffuses into cell culture media at a sufficient rate, we have included a filter in our Reservoir and implemented the use of silicone tubing which allows for the diffusion of gases through the tubing surface. We are, therefore, confident that the system can successfully perform its intended purpose.

B. Areas for Improvement

We recommend that, in its current state, the system not be run at a flow rate greater than 1.3 L/min. Through our testing process, we have found that pressure at high flow rates can cause the seals on tubing, especially the seals at the inlets to the Chamber and at the points of compression from large tube diameter to small diameter, to rupture. If seals do rupture, silicone must be reapplied in order for the system to be usable again. The silicone should be allowed to cure for at least 24 hours prior to attempting to run the system again. It may also be a good idea to apply multiple layers of the silicone sealant at all of the seals such that these areas are completely encased in silicone. Similarly, encasing the side faces of the main Chamber in silicone may also keep the inlet and outlet seals from rupturing.

The pressure sensors used in the current system only detect up to 30 kPa and therefore are being used at their upper limit. Pressure sensors rated for higher pressures should be implemented for any desired pressure build up higher than 30kPa. Some research was done to look into this possibility. It was found that the MPX2200DP pressure sensor would fulfill any pressure requirements higher than 30 kPa. The MPX2200DP is a differential pressure sensor that measures the change in pressure relative to ambient within the range of 0-200 kPa. However, slight modifications would need to be made to the Flow Control circuit in implementing this pressure sensor because it has a supply

voltage of 10V instead of the 5V supply voltage of the current sensor used. This change would allow for a much wider range of pressures to be applied to the scaffolds.

C. Bill of Materials.

The final cost of our system is just under budget at $2996.62. Details of purchases can be seen below in the Bill of Materials.

Table 5

Components:	**Cost ($):**
Solid State Relays (Allied Elec)	25.39
Silicone Sealant (VWR)	110.96
Arduino (sparkfun)	38.38
Pressure sensors (mouser)	44.41
Stainless Steel (onlinemetals)	123.8
lexan/polycarbonate (eplastics/ridout)	90.76
Pump system + 2nd head (Cole-Parmer)	1570.48
Manifolds	200.09
U.S. Plastics (PVDF couplers, etc.)	73.13
Tygon Tubing (Cole Parmer)	97.09
Solenoid Valves (Aztech)	198.5
Machining Tools (Industrial Tool and Supply)	141.87
Additional Flow Control Components	43.27
Additional Machining Tools (Industrial Tool & Supply)	57.23
Files and Clamps (Harbor Freight)	14.58
Cork/Ruber Gasket (Autozone)	5.39
Bolts, Nuts, and Lock Washers (ACE)	5.25
New Electronic Components (Mouser)	$113.67
Purchases from Elliot's Electronics	42.37
Expenditure to Date ($):	$2,996.62

VIII. Acknowledgements

Thanks to Dr. Wu, Fred Highton, Hyuck Jin Kwon, David You, Dale Drew, and Joe Hartley for their help and support throughout the course of the project.

IX. References

R. W. Fox and A. T. McDonald, *Introduction to Fluid Mechanics*, 6th Ed., Wiley, New York, 2003.

X. Appendix

A. Questions asked during presentation

What is the price range you found for peristaltic pumps?

Price ranges vary from a couple hundred dollars to a few thousand of dollars. Mainly, price is dependent on the flow rate that is needed. A low flow rate peristaltic pump many only be a few hundred dollars. However, if a larger flow rate is desired the price dramatically increases.

Do you have to worry about regulating the gas from the gas tanks?

No, we will not have to worry about regulating the gas from the gas tanks because the incubator will do that for us. The air inside the incubator will already be adjusted as we need it.

Does the failure of one scaffold ruin all of the scaffolds? Do you need to make provisions for such an occurrence?

The failure of one scaffold would not ruin all of the scaffolds, but would ruin testing if gone unnoticed since proper stresses would not be applied to the remaining scaffolds in that portion of the Chamber for the period of time the failure is unnoticed. The failure of one scaffold would cause the pressure difference between the upper and lower Chamber to cease to exist in that portion of the Chamber. This would in turn cause no perfusion pressure or strain to be applied to the remaining two scaffolds. These two scaffolds would still be intact and surface cells on those scaffolds would still live, as nutrients would still be provided to them. However, it is possible that cells in the inner portion of the scaffold would not survive as there would be no perfusion pressure being applied to force nutrients into the inner scaffold.
Splitting the main Chamber into three portions partially mitigates this problem. Because there are three scaffolds in each individual portion, only scaffolds in one section would

be affected if there were a failure. Scaffolds in the other two portions would be unaffected.

Did you make any design changes because of the results of your FMEA?

Yes, we changed our Chamber design because of our FMEA. One of our main concerns in our FMEA was the holding plate, which was originally going to be machined out of polycarbonate, would crack. In order to avoid this, we decided to machine it out of metal. However, this brought up the concern of the metal bending while we applied pressure in one of the Chambers. So, we decided to break the Chamber into three portions. Thus, the walls of each portion will provide more support for the metal plate.

Have you determined entrance effects due to your inlets?

Yes, we have used computational fluid dynamics to determine effects of inlets, shear stress, and the velocity profile of our system in the Chamber.

Do you need to worry about creating bubbles from media agitation?

Yes, bubbles would negatively affect the cells if they were immobilized onto the scaffold. The area under the bubble would not receive nutrients and the cells would most likely die. In order to minimize this, the outlet in our Reservoir will be at the bottom of the container, while the inlet will be closer to the top.

Have you checked on the material compatibility of your system?

Yes, all materials used in the system can be sterilized and are used in other bioreactor systems or medical devices.

How do you supply power to the system in the incubator?

The incubator is designed to accommodate objects that need a power supply. There is an opening for a cord to be placed through one of the walls such that power can be supplied inside the incubator.

Would it be better to use a dynamic pump instead of a peristaltic pump?

It may be easier to provide a faster flow rate if a dynamic pump is used. However, a dynamic pump would be more difficult to sterilize, as it would come in contact with the cell media. Also, we are concerned that a dynamic pump would introduce bubbles to the system. Thus, we believe a peristaltic pump is a better choice given our circumstances.

Have you looked into keeping air contamination out?

Yes, all assembly will be completed under a flow hood after all parts are sterilized either with ethanol or autoclaved. Cell media will be filtered before it is added to the system. If media needs to be replaced during testing, it will be done under the flow hood as well.

B. 1st Semester ABAQUS Simulations

As mentioned before, one of our functional requirements is to induce a tensile strain of less than 10% in the protein scaffolds. Since, in our final design, we are using the deflection due to normal pressure to produce the tensile strain, we need a way to measure the strain caused by the perfusion pressure. Originally, we had intended to measure the deflection optically. However, multiple factors dissuaded us from this course of action. For example, measuring the deflection from a horizontal position in the Chamber is not possible because the scaffold Securing Plates will obstruct the view of the scaffolds. Therefore, the only view possible is from the vertical. But, even from this vantage point, there will be about half a centimeter of cell culture media between the camera and the specimen, as well as the lid of the Chamber. Additionally, the strains we will be inducing will be equivalent to less than a millimeter of longitudinal deflection, which would be obscured to some extent due to the concavity of the specimen when it is strained.

Therefore, we have used the finite elemental analysis (FEA) program ABAQUS to simulate deflection of a scaffold when subjected to a 50 kilo-Pascal pressure. The results of this test can be seen below in Figure 34 and Figure 35. For the material properties, we used values given to us by our sponsor, with the modulus of elasticity being 10 mega-Pascals and Poisson's ratio being 0.4. In addition, we simulated the scaffold Securing Plates and the interactions between them and the scaffold itself. For the Securing Plates, we used values for a much stiffer material; the value for the modulus of elasticity is 200 giga-Pascals and the Poisson's ratio is .35. These properties are similar to those of steel. As you can see in the images below, the maximum tensile strain with the aforementioned values is approximately 5.25 %, with the central portion straining to 3.5 and 4.0 %.

We have spoken with our sponsor, and ideally, he would like to see physical confirmation of our findings using the system itself. However, he has stated that the ABAQUS values can be used to validate our method of applying tensile strain.

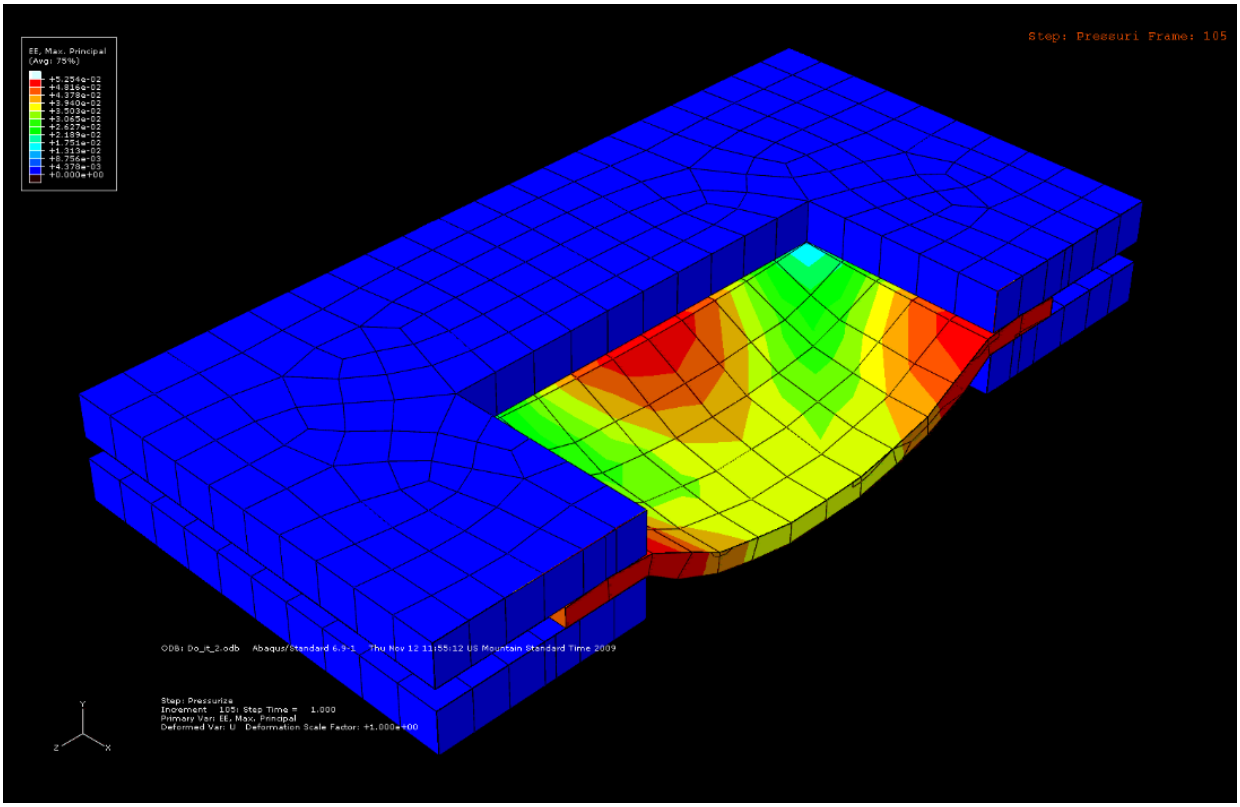


Figure 34: ABAQUS simulation showing downward deflection.

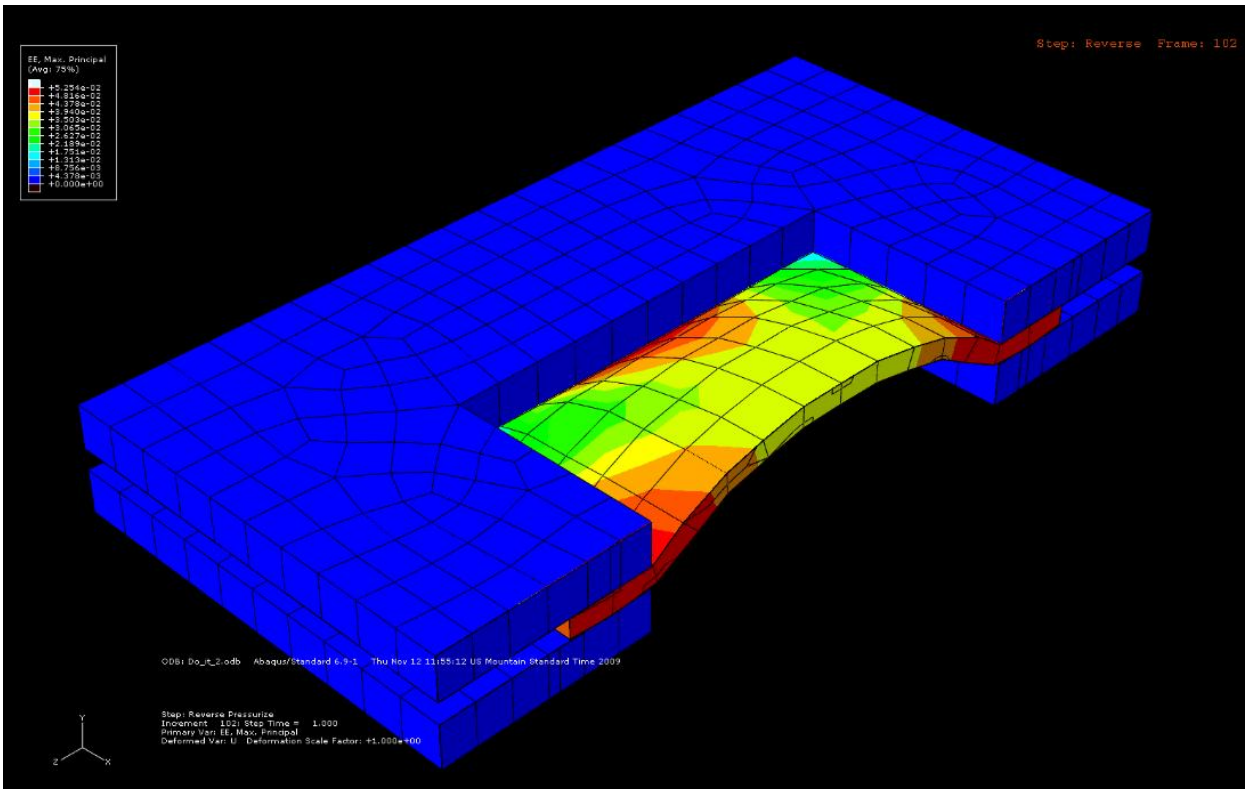


Figure 35: ABAQUS simulation showing upward deflection.

C. 1st Semester Fluent Simulations

Fluent is a computational fluid dynamics program that uses the finite volume method to create a wide variety of models. In our case, we used Fluent to find the ideal viscosity, the flow rate, and to check the Chamber dimensions. We modeled one section of the Chamber in Gambit, a program that allows the user to set up a specific geometry for analysis in Fluent. The model was drawn in 3D. The mesh is shown in Figure 30. In the model, the entrance and exit lengths are 5 centimeters, the tube diameter for the entrance and exit is 3 millimeters in diameter, each scaffold is 1 centimeter apart from the preceding one, the Chamber height is 5 millimeters, the depth is 16 millimeters, the slope of the plate just before and after the scaffold is 1/3. For the simulation displayed below, we used an inlet velocity of 1 meter/second and a viscosity of 0.007 kilogram/meter*second. Before running this 3D model, we ran a few 2D models to get an idea of the characteristics of the fluid, for which the data is not shown. From this 2D model, we found that a 1/3 slope preceding and proceeding the scaffold would be sufficient to allow for there to be a velocity over the scaffold. In the 3D model, the entrance and exit lengths are each 5 centimeters, the tube diameter for the entrance and exit is 3 millimeters in diameter, each scaffold is 1 centimeter apart from the preceding one, the Chamber height is 5 millimeters, the depth is 16 millimeters, and the slope of the plate just before and after the scaffold is 1/3.

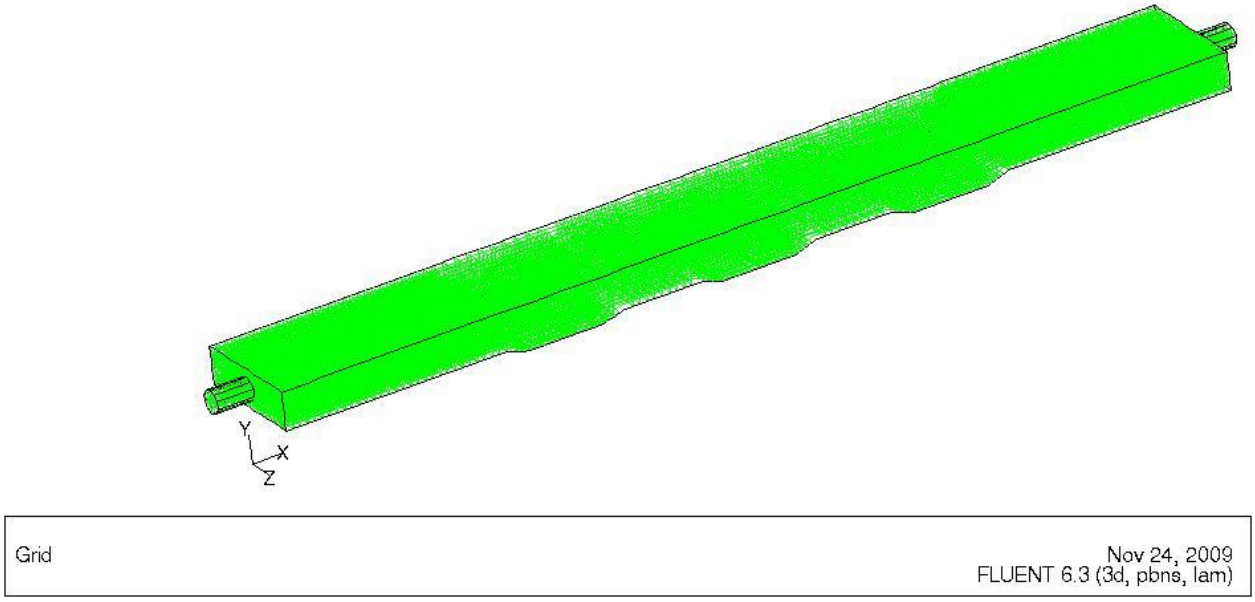


Figure 36: 1st semester Fluent mesh.

For analysis purposes, we selected the mid-line of the Chamber to view velocity and shear stress values. As seen in Figure 31, this results in velocities between 0 and 0.5 meters/second occurring in the Chamber. Figure 32 shows a close up of the velocity

profile of the scaffold. Velocities decrease greatly within this area, with the values being extremely close to zero.

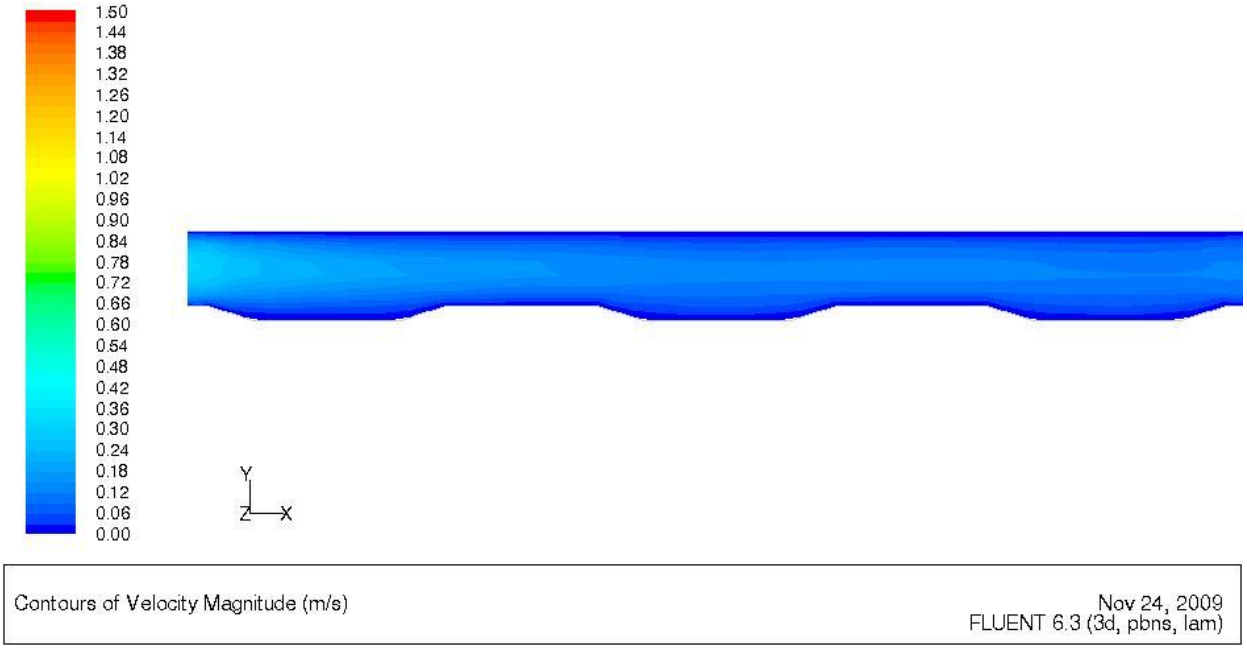


Figure 37: 1st semester Fluent velocity distribution.

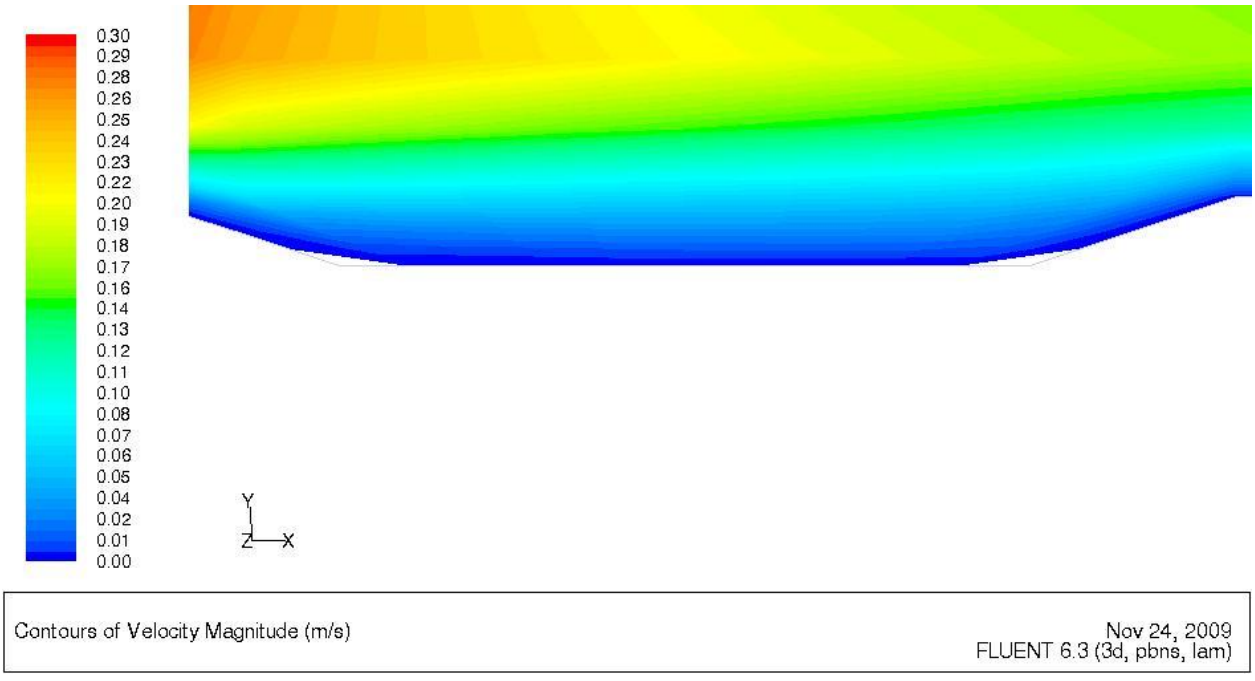


Figure 38: 1st semester Fluent velocity distribution (close up)

Fluent was also used in order to calculate an approximate shear stress on the scaffolds. Shown in Figure 33 is the shear stress distribution in the entire Chamber. As it appears, most of the shear stress is between 0 and 1 Pascal. Figure 34 shows a zoomed in shot of the shear stress on the scaffold. The shear stress on the scaffold will be between 0.1 and 0.25 Pascal, which is on the lower end of our desired range. In the future, we may attempt to increase our inlet velocity or viscosity such that we can test up to our limit of 1 Pascal.

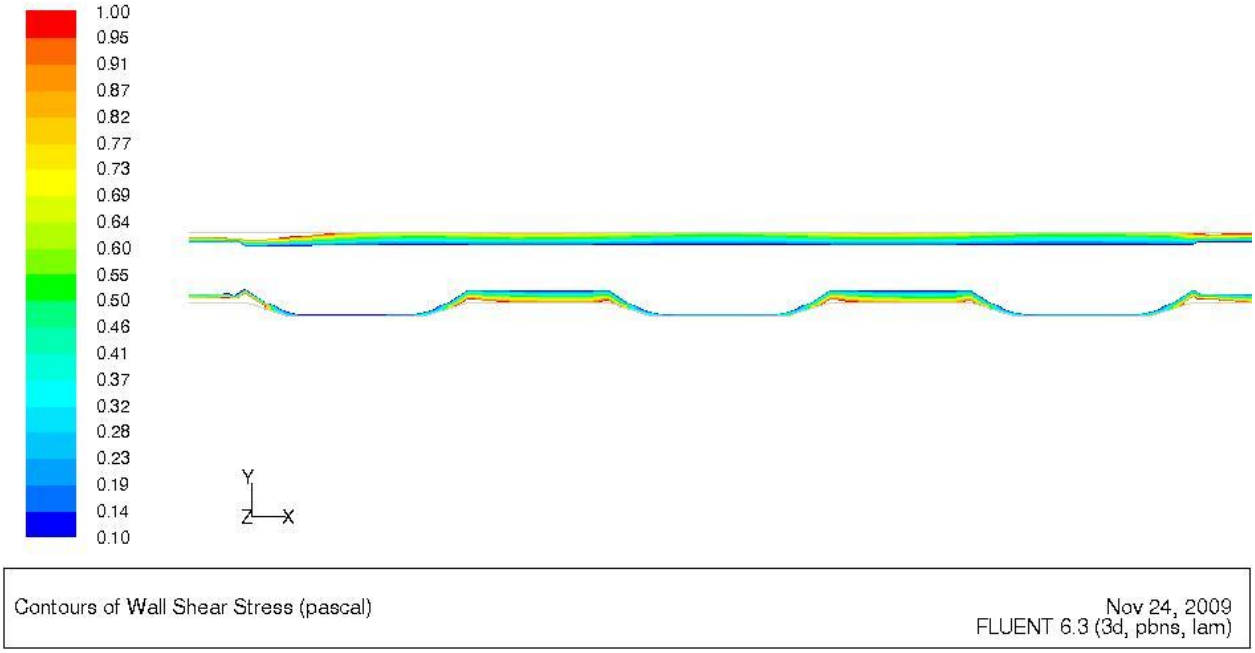


Figure 39: 1st semester Fluent shear stress.

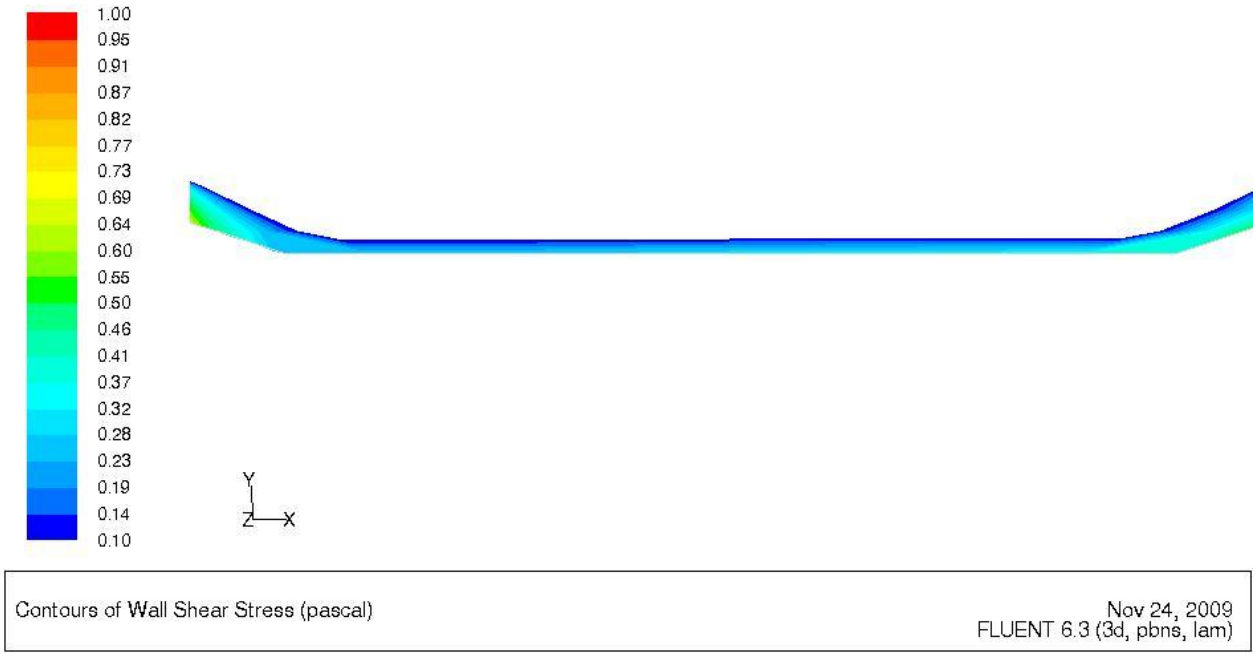


Figure 40: 1st semester shear stress (close up).

D. 2nd Semester Fluent Results

As mentioned previously, Fluent is a computational fluid dynamics program that uses the finite volume method to create a wide variety of models. Second semester, we used Fluent to test our actual Chamber design in order to find the ideal flow rate for our required amount of shear stress. Again, we modeled one section of our Chamber in Gambit, a program that allows the user to set up a specific geometry for analysis in Fluent. The model and simulation was done in 3D. Below is a chart of flow rates tested and the shear stress at the center point of each scaffold (scaffolds are numbered left to right). Figure 35 shows an oblique view of our 3D model, the rest of the figures (Figures 36-40) are shown as if looking directly down into one Chamber. We believe that our ideal flow rate to be 1.3 L/min as it gives the best shear stress distribution closest to own needed value.

Table 6

Flow Rate (L/min)		**Center Point Shear Stress (Pa)**		
Total	**Sub Chanmber**	**1st Scaffold**	**2nd Scaffold**	**3rd Scaffold**
0.43	0.072	0.0728856	0.06130028	0.0461025
0.86	0.144	0.0860131	0.05981569	0.05696823
1.30	0.216	0.1125317	0.05440777	0.04749779
1.73	0.288	0.1102661	0.07122335	0.09430136
3.40	0.56592	1.1290758	0.42001337	0.41033402
MAXIMUM				

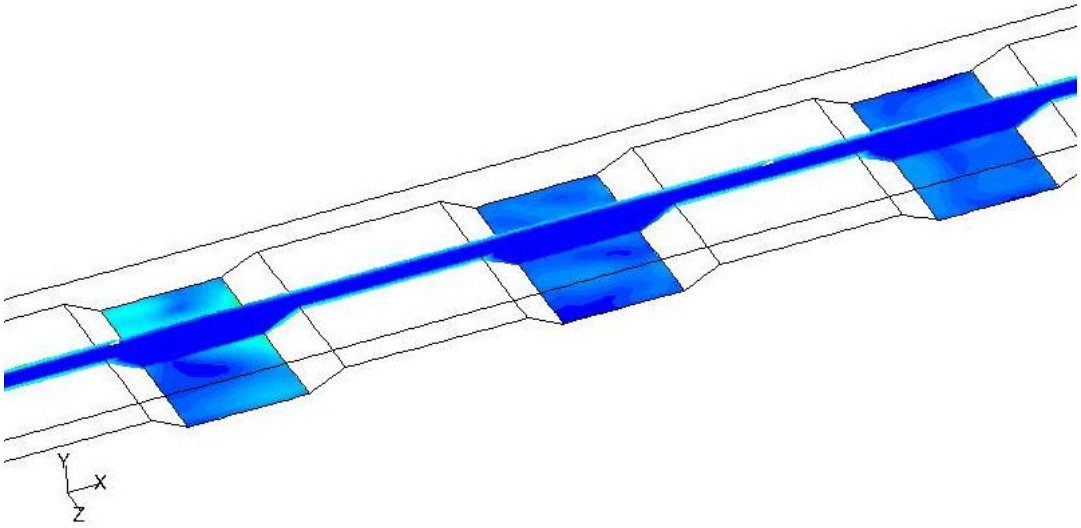


Figure 41: 2nd semester Fluent simulation of wall shear stress; oblique view, 1.30 L/min.

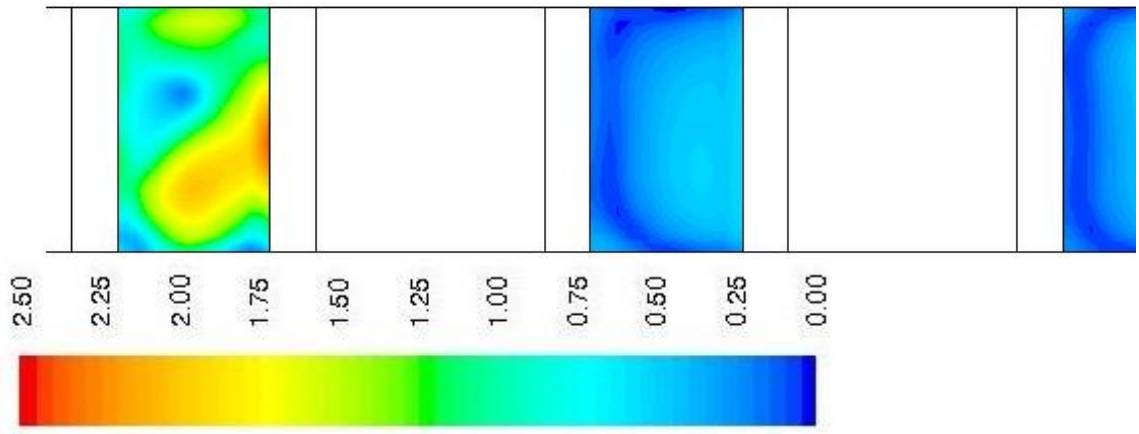


Figure 42: 2nd semester Fluent simulation of wall shear stress, 3.4 L/min.

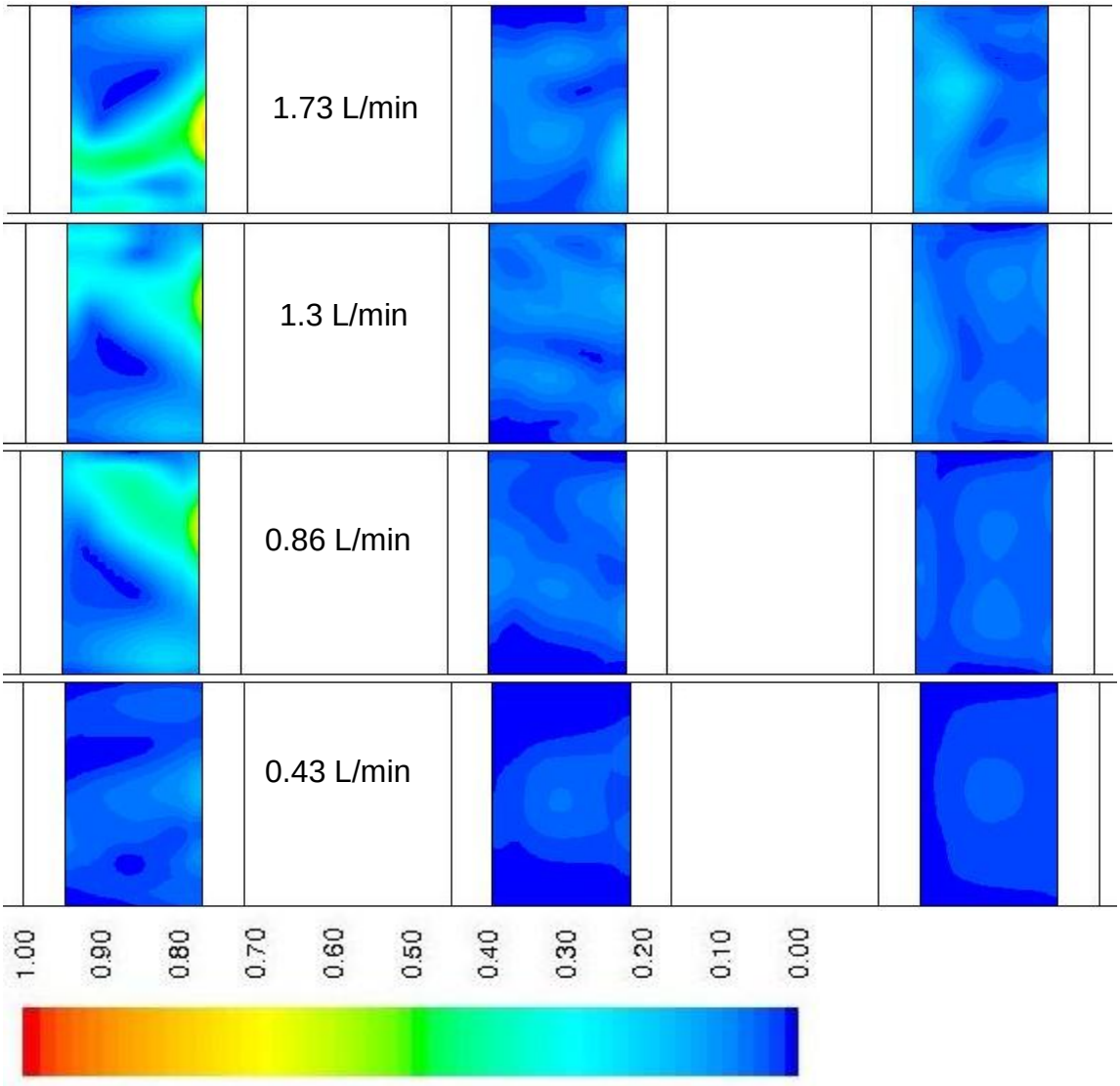


Figure 43: 2nd semester Fluent simulations of wall shear stress.

E. 2nd Semester ABAQUS Simulation

The ABAQUS simulation performed in the second semester was very similar to the one performed in the first semester. In the simulation, a model of a tissue scaffold of dimensions 14mm x 14mm x 0.5mm was created with a Young's Modulus of 3 MPa and a Poison's Ratio of 0.4; these material properties were the average of the most recent range provided to us by our sponsor, Dr. Wu. This scaffold model was then centered between two, rigid metal plates, each of which featured a 10mm by 10mm square hole.

With the 4 vertical edges of the scaffold constrained to not move in any direction, a normal, downwards pressure was applied. The magnitude of this pressure was varied until the desired strain of approximately 6% was achieved at 30,000 Pa. A cross-sectional view of the deformed model is shown below. The contours represent the principal strain that occurs in the model in a single direction only; a similar contour plot can be generated for the principal strain that occurs in the perpendicular direction.

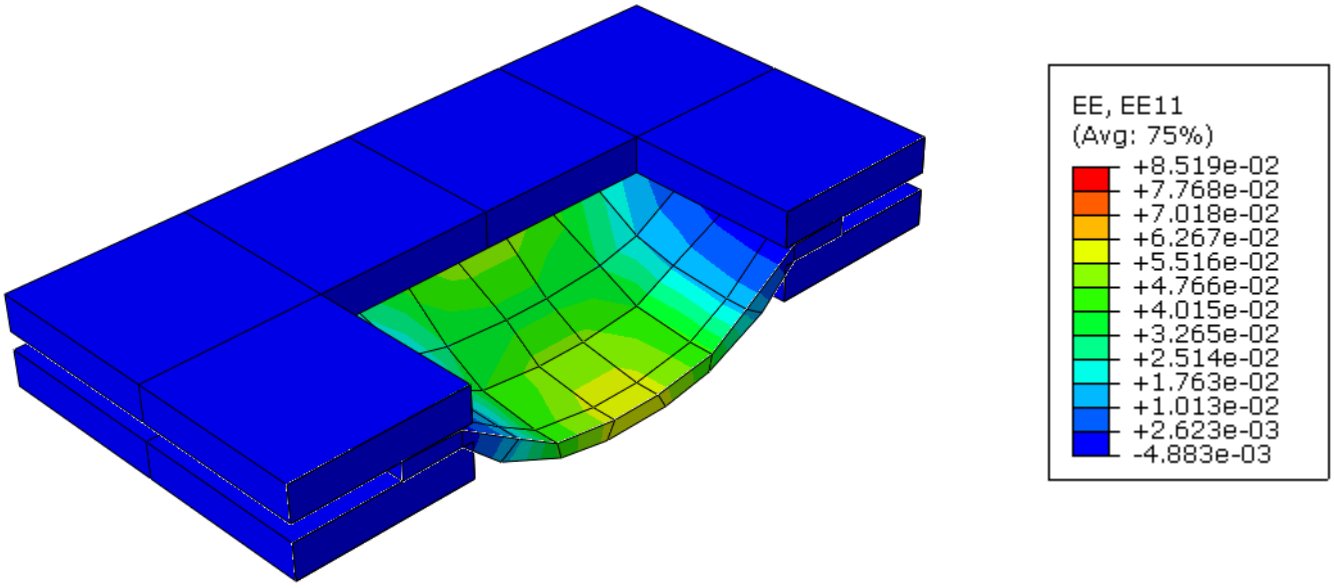


Figure 44: 2nd semester ABAQUS simulation; downward deflection.

F. Failure Modes and Effects Analysis (FMEA)

Tables containing the FMEA are attached.

Team 4371 - A Dynamic Cell Culture System						
Design FMEA Summary						
Subsystem	**Component**	**Function**	**Failure Mode**	**Consequence**	**Cause**	**Mitigation**
Reservoir	Structure	Holds bulk of media	Cracking	Leaking/ contamination	Impact loading	Inspect before running experiment/material selection
					Cyclic heating	Inspect before running experiment/material selection
					Contact with sharp object (stress concentration)	Inspect before running experiment
		Location of air flow and diffusion into media	Allows entrance of contaminants	Contaminated media/ Cell death	Poor sealing	Inspect before running experiment, monitor throghout
					Pore size is too large	Obtain correct filter
					Physical degradation	Inspect prior to use and monitor through experiment, replace filter if necessary
			No air flow into reservoir	No oxygen delivery to cells/ cell death	Clogging	Inspect prior to use and monitor through experiment, replace filter if necessary
Reservoir	Filter	Allows for air flow while maintaining sterile reservoir environment	Allows entrance of contaminants	Contaminated media/ Cell death	Poor sealing	Inspect before running experiment, monitor throghout
					Pore size is too large	Obtain correct filter
					Physical degradation	Inspect prior to use and monitor through experiment, replace filter if necessary
			No air flow into reservoir	No oxygen delivery to cells/ cell death	Clogging	Inspect prior to use and monitor through experiment, replace filter if necessary

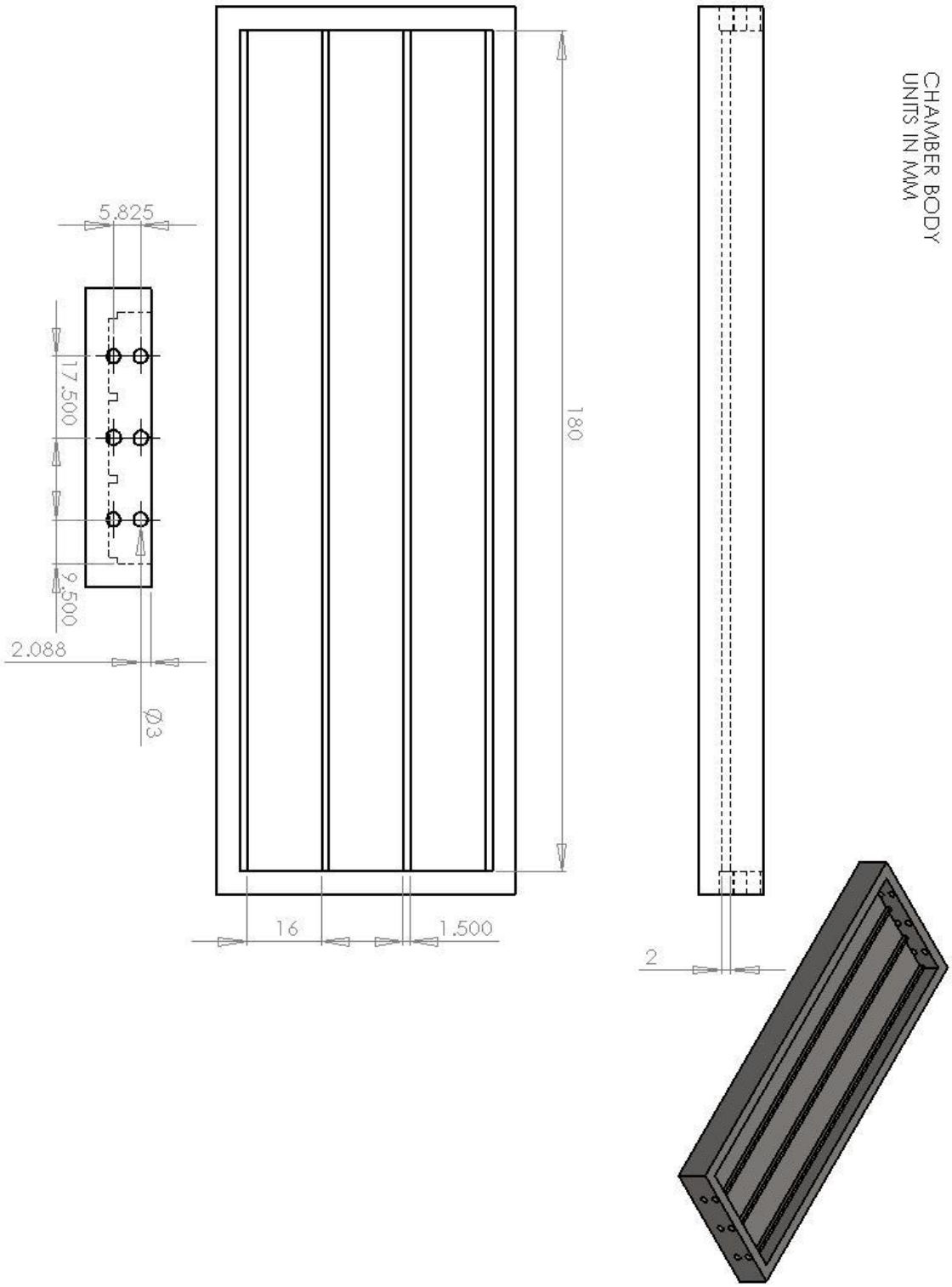
Subsystem	Component	Function	Failure Mode	Consequence	Cause	Mitigation
Media Delivery System	Tubes	Allow for transport of media from one component to another	Cracking/ degradation	Leaking/ contamination	Cydic stress due to pinch valves	Inspect before running experiment, material selection
			Clogging	Improper flow rate or no flow	Obstruction	Inspection before experiment and daily throughout experiment's duration
Media Delivery System	Tube Sealant	Prevent Leaking/ contamination at joints between tubing and components	Cracking/ degradation	Leaking/ contamination	Material Degredation	Proper material selection, inspection before use, replacement of part if necessary
			Improper sealing	Leaking/ contamination	Improper placement of	Proper application or reapplication of sealant to tube and part and regular
Media Delivery System	Tube Connectors (Bifurcations)	Allows for single flow to be broken into multiple flows or visa versa	Flow divided incorrectly	Undesired flow rates	Improper setup	Set up properly
			Improper sealing	Leaking/ contamination	Gaps in sealant	Proper application or reapplication of sealant to tube and part and regular examination of equipment
			Cracking/ degradation	Leaking/ contamination	Defective part, impact	Inspect before running experiment, avoid damaging, replace if necessary
Media Delivery System	Peristaltic Pump	Applies media flow rate	Applies incorrect flow rate	Application of improper shear stress on samples	Improper calibration	Check calibration regularly
			No flow	No nutrient supply or shear stress	Mechanical failure/breakdown of device	Test prior to experiment and monitor through duration
					Loss of power supply	Test equipment prior to experiment; emergency power system if available; monitor through duration

Subsystem	Component	Function	Failure Mode	Consequence	Cause	Mitigation
Chamber	Structure	Allows for media flow over scaffolds	Cracking/ degradation	Leaking/ contamination	Impact loading	Inspect before running experiment/material selection
					Cyclic heating	Inspect before running experiment/material selection
					Contact with sharp object (stress concentration)	Inspect before running experiment
Chamber	Lid	Allows Access to inside of chamber	Cracking/ degradation	Leaking/ contamination	Impact loading	Inspect before running experiment/material selection
					Cyclic heating	Inspect before running experiment/material selection
					Contact with sharp object (stress concentration)	Inspect before running experiment
Chamber	Structural Sealant	Prevents Leaking/ contamination at lid seams	Cracking/ degradation	Leaking/ contamination	Material Degredation	Proper Material Selection
			Improper sealing	Leaking/ contamination	Improper placement of sealant	Proper application or reapplication of sealant to tube and part and regular examination of equipment
Chamber	Scaffold Securing Plates	Hold scaffolds	Insufficient clamping force	Sample Unstable	Plastic warped or machined incorrectly	Inspect part before use and machine with care
Chamber	Scaffold	Contains cells/replicates tissue	Physical failure (bursting/tearing)	Sample unusable	Excessive perfusion pressure	Inspect equipment prior to running experiment and monitor throughout
				Clogging tubes	Broken scaffold comes free and enters tubing	Inspect equipment prior to running experiment and monitor throughout

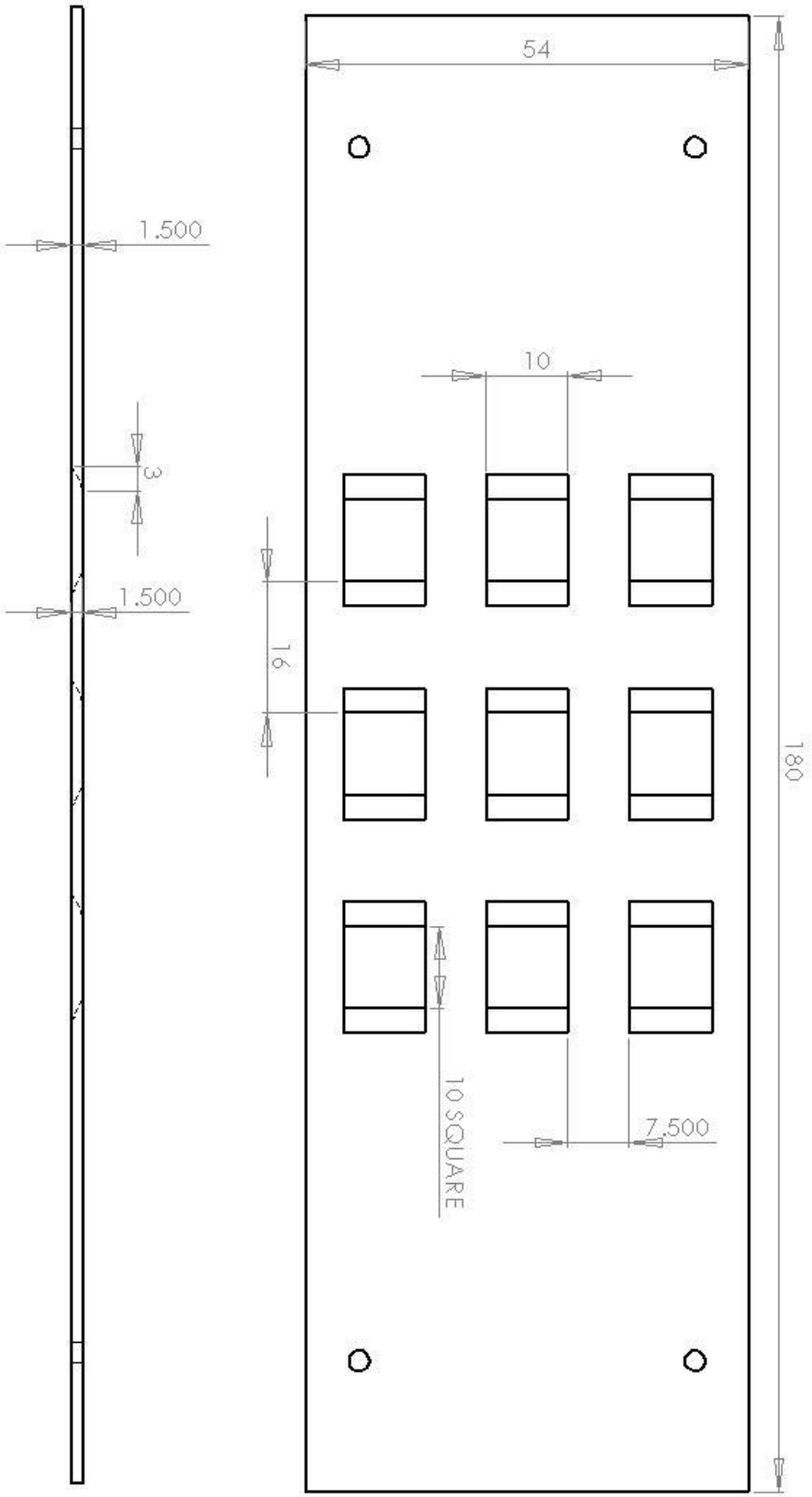
Subsystem	Component	Function	Failure Mode	Consequence	Cause	Mitigation
Chamber	Screws	Apply necessary force for sealing connections of removable parts	Improper sealing	Leaking/ contamination	Inadequate sealing force	Properly torque screws/Inspect before running experiment
				Inaccurate stresses	Loss of pressure	Properly torque screws/Inspect before running experiment
				Cell death	Non-sterile environment	Properly torque screws/Inspect before running experiment
		Apply necessary force for damping samples	Insufficient damping force	Samples unstable	Inadequate force to hold samples in place	Properly torque screws/Inspect before running experiment
Flow Control System	Solenoid Valves	Controls flow rate	Valve stuck open	Inadequate perfusion pressure; no pulsatile flow	Valve malfunction	Inspect equipment prior to running experiment and monitor throughout
					PLC malfunction	Inspect equipment prior to running experiment and monitor throughout
					Switch malfunction	Inspect equipment prior to running experiment and monitor throughout
					Loss of power supply	Test equipment prior to experiment; emergency power system if available; monitor through duration
			Valve stuck closed	Perfusion pressure surpasses scaffold structural limit	Valve malfunction	Inspect equipment prior to running experiment and monitor throughout
					PLC malfunction	Inspect equipment prior to running experiment and monitor throughout
					Switch malfunction	Inspect equipment prior to running experiment and monitor throughout
					Loss of power supply	Test equipment prior to experiment; emergency power system if available; monitor through duration

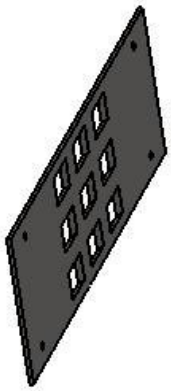
Subsystem	Component	Function	Failure Mode	Consequence	Cause	Mitigation
Flow Control System	Pressure Sensor	Relays current chamber pressure to PLC	Detects pressure incorrectly	Solenoid valve malfunction	Pressure sensor malfunction	Test equipment prior to experiment; Inspect equipment prior to running experiment and monitor throughout
			Does not detect pressure	Solenoid valve malfunction	Pressure sensor malfunction	Test equipment prior to experiment; Inspect equipment prior to running
					Loss of power supply	Test equipment prior to experiment; emergency power system if available; monitor throughout
Flow Control System	Programmable Logic Controller (PLC)	Interprets information from pressure sensor	Interprets information incorrectly	Solenoid Valve Malfunction	PLC malfunction	Inspect equipment prior to running experiment and monitor throughout
					Loss of power supply	Test equipment prior to experiment; emergency power system if available;
Flow Control System	Switch	Turns valves on or off	Does not signal valve open/close	Solenoid valve malfunction	Switch malfunction	Inspect equipment prior to running experiment and monitor throughout
					Loss of power supply	Test equipment prior to experiment; emergency power system if available;
Flow Control System	Battery for PLC	Supplies PLC with power	Does not supply PLC with power	PLC power loss	End of battery life (loss of charge)	Inspect prior to experiment and monitor throughout
Environmental Control	Incubator	Maintains proper temperature	Improper temperature	Cell death	Incubator malfunction	Inspect before experiment and monitor throughout
					Loss of power supply	Test equipment prior to experiment; emergency power system if available; monitor through duration
		Maintains proper Air/CO2 balance	Improper Air/CO2 balance	Cell death	Empty air tanks	Inspect pressure tank levels prior to experiment and monitor throughout
					Loss of power supply	Test equipment prior to experiment; emergency power system if available; monitor through duration

G. Technical Drawings



SECURING PLATE
UNITS IN MM





CHAMBER LID
UNITS IN MM