Design of a Bioreactor for Tissue Engineered Vascular Grafts

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Abstract

The current standard of treatment for the failure of small diameter (<5 mm ID) blood vessels is bypass surgery using biological or synthetic grafts. However, these grafts have limitations, such as the secondary wound site caused by autologous sources. Tissue engineered vascular grafts (TEVGs) are a promising alternative because they could be non-immunogenic, vasoactive, customizable, and have an off-the-shelf availability. Previous studies have shown that a bioreactor can condition TEVGs to meet these ideals. A bioreactor is a device that simulates an *in vivo* environment to improve the structure and function of engineered tissues. TEVGs should be cultured in a dynamic environment that provides luminal laminar flow. These conditions facilitate nourishment of the cells and prevent occlusion of the graft. In this study, we designed, manufactured, and validated a novel bioreactor system for TEVGs. The bioreactor design successfully provided luminal laminar flow, incorporated an isolated flow loop, prevented leakage, remained sterile, limited mounting time, accommodated different sized samples, was easy to machine and cost effective.

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Chapter 1: Introduction

When a blood vessel becomes diseased or obstructed, it cannot transport blood through the circulatory system properly. The medical standard for replacing small diameter (<5 mm inner diameter) blood vessels is to use a bypass graft (Nerem & Seliktar, 2002). In the United States, about 500,000 coronary artery bypass graft surgeries are performed annually (Rosamond *et al.*, 2008). Bypass graft surgery is a surgical procedure in which blood flow is rerouted around the damaged vessel, generally performed using an autologous graft - a transplanted vein or artery from the body of the patient (Sales *et al.*, 2005). Medical professionals also use grafts in patients with kidney failure, who often need dialysis treatment to remove waste and excess water from the blood. A surgeon joins an artery and a vein with a tubular graft, which creates an artificial high-flow vessel for dialysis (Kawecka, 2008). Dialysis grafts are generally needed for patients with smaller diameter blood vessels because they are the least accessible and require a higher volumetric flow rate (Dardik *et al.*, 1996).

Unfortunately, suitable autografts are not available in one-third of patients due to previous surgeries and other preexisting vascular conditions (Beamish, Kottke-Marchant, & Marchant, 2010). Autologous grafts also create a secondary wound site, consequently increasing the patient's recovery period (Engbers-Buijtenhuijs *et al.*, 2006). Finally, the mechanical properties of the autologous grafts may be different from the vascular tissue of the affected area due to structural differences between veins and arteries (He *et al.*, 2010).

In order to address the limitations of autografts, researchers are currently investigating tissue engineered vascular grafts (TEVGs) as an alternative. TEVGs would eliminate the secondary wound site created by autografts, more accurately mimic native tissue, and provide "off-the-shelf" availability to surgeons. Researchers in Professor Marsha Rolle's laboratory at Worcester Polytechnic Institute (WPI) are developing methods to create TEVGs, but these samples show lower suture retention and burst pressure strengths compared to natural blood vessels (Doshi, 2009). By culturing TEVGs in a bioreactor that simulates the *in vivo* environment, they would more accurately mimic native vascular tissue (Bjork & Tranquillo, 2009).

The goal of our project was to design a novel bioreactor to provide luminal laminar flow and a constant shear stress to the TEVG. Luminal flow will improve nutrient and waste transportation and shear stress will ensure uniform luminal cell growth (Engbers-Buijtenhuijs *et al.*, 2006). Constant shear stress is a result of an organized flow pattern without the mixing of fluid layers, also known as laminar flow (Traub & Berk, 1998). In addition to luminal laminar flow, the bioreactor must have isolated flow loops, remain sterile, limit mounting time, be easy to machine, and accommodate different sized samples.

Currently, the laboratory is using a bioreactor designed in 2011 that does not currently meet these objectives.

We began this project by conducting a literature review on vascular physiology, tissue engineering, biofluid mechanics, and the current state of TEVG and bioreactor technology. Using an initial client statement, client interviews, and the literature review, we developed a set of design criteria. We organized these criteria into constraints, objectives, functions, and specifications. After ranking the criteria by importance, we developed conceptual design alternatives. We created preliminary prototypes of the alternative that best addressed the criteria. After several iterations of the initial prototype, the device evolved into the final design presented in this report. We developed experiments that addressed each of the design criteria to determine the success of the device. The results of the experimental testing were then used to make final recommendations to the design. In this project, we created a functional bioreactor that could provide luminal laminar flow of media to a TEVG created in the Rolle laboratory.

Chapter 2: Literature Review

This chapter presents information that is necessary to understand the need and purpose of our project. It discusses the physiology of vascular tissue, the importance of TEVGs, the culturing methods, the media flow requirements, and the different types of bioreactors for TEVGs currently available.

2.1: Vascular Physiology

The main function of the cardiovascular system is the transportation of nutrients, gases, hormones, waste products and other biological compounds to and from all cells in the human body. This is especially important in maintaining homeostasis (Berne & Sperelakis, 1979). The cardiovascular system is composed of the heart, the lungs, and blood vessels. The blood is oxygenated in the lungs and circulated systemically through the rest of the body, providing the oxygenated blood to the cells (Raines, Noordergraaf, & Baan, 1978). Blood is transported via three types of vessels: arteries, veins, and capillaries.

Arteries and veins are composed of three layers: the tunica intima, tunica media, and tunica adventitia, shown in Figure 1. The innermost and thinnest layer is the tunica intima, located on the lumen of the blood vessel (Fox, 2001). It consists of a single layer of endothelial cells surrounded by a thin layer of elastic tissue called the internal elastic lamina (Ryan, 1988). The middle layer is the tunica media, which is composed of SMCs and elastin. The media layer is responsible for the vasodilation and vasoconstriction of the blood vessels (Mohrman, 2003). This layer is thicker in arteries than veins because the pressure of the blood flow is higher in arteries (Camilleri, 1989). The tunica adventitia is the outermost layer and is made mainly of collagen and fibroblasts. This serves to anchor and stabilize the vessel to the surrounding tissue (Thrillet, 2008). Because veins do not have a supportive media layer due to low blood pressure, veins require stability from the adventitia layer, and therefore it is thicker in veins (McDonald, 1979). Developing a TEVG that models the structure of native tissue would allow the TEVG to mimic native mechanical properties.

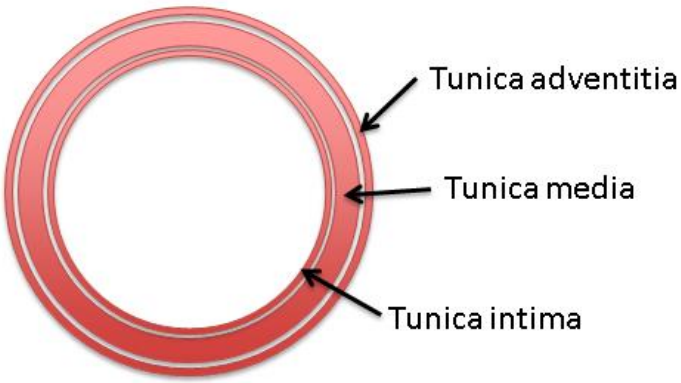

Figure 1: Blood vessel structure

2.2: Importance of Tissue Engineered Vascular Grafts

The heart pumps about 2,000 gallons of oxygenated, nutrient-rich blood throughout the body each day (Khan, Farah, & Domb, 2012). Plaque build-up, known as atherosclerosis, in the coronary arteries can cause restricted blood flow to the heart and can lead to coronary artery disease (CAD), a major cause of death in the United States (Thompson *et. al.*, 2002). The non-linear geometry of the coronary arteries causes increased incidences of atherosclerosis (Chiu & Chien, 2011). There are multiple methods of restoring blood flow in the veins and arteries including bypass graft surgery, or the rerouting of blood around a damaged vessel. This is one of the most common procedures used clinically. (Khan *et al*, 2012)

Coronary artery bypass graft (CABG) surgery uses an artery or vein from another source to bypass an affected artery. In the United States alone there are approximately half a million CABG surgeries performed each year (Rosamond *et. al.*, 2008). The saphenous vein in the leg, mammary artery in the chest, and the radial artery in the arm are the conventional sources for grafts. However, these sources are limited because patients have a limited number of potential blood vessels for use in grafts and the grafts may not have the same mechanical properties as the vessel that is being bypassed (Subramanian & Abhilash, 2009).These surgeries also create a secondary wound site which can prolong the length of recovery time. Consequently, these limitations create a need for the development of TEVGs. Advantages of using TEVGs include the ability to be customized to the patient's needs, the possibility of reducing the immunogenic response, and the capacity to provide an off-the-shelf availability to surgeons. Unlike synthetic grafts, TEVGs can have a biological component that will allow the graft to repair itself and exhibit contractile properties (Pankajakshan & Agrawal, 2010).

2.3: Current State of Culturing Tissue Engineered Vascular Grafts

TEVGs can be created in a laboratory setting using various methods. The method for culturing TEVGs varies by laboratory, and different processes have yielded differing results in culture time, suture retention testing, and burst pressure testing (Doshi, 2009 & L'Heureux *et al.* 1998). Suture retention and burst pressure testing are important indicators of mechanical properties. Suture retention strength is the amount of tensile load that is applied on a suture through a material at the point of failure. Burst pressure strength is the maximum pressure the graft can withstand before rupturing. The benchmark for this testing is natural blood vessels. Exhibiting the same mechanical properties as a native blood vessel is the most important objective when culturing TEVGs (Li, L'Heureux, & Elisseeff, 2011). In this section, we will discuss two methods of culturing TEVGs: the tissue rolling method and direct cell seeding method. We chose to discuss these two methods because the tissue rolling method grafts are currently being used in clinical trials and the direct cell seeding method is currently used in the Rolle laboratory.

2.3.1: Tissue Rolling Method

One method used to create TEVGs is known as the tissue rolling method in which SMCs are cultured in a sheet, rolled onto a mandrel, and cultured into a tube. The SMC layers fuse together to form a single layer. This SMC layer can be covered with a fibroblast layer using the same method. The SMC layer of the TEVG mimics the media layer of natural tissue, and the fibroblast layer mimics the adventitia layer. This process is illustrated in Figure 2. (L'Heureux *et al.*, 1998)

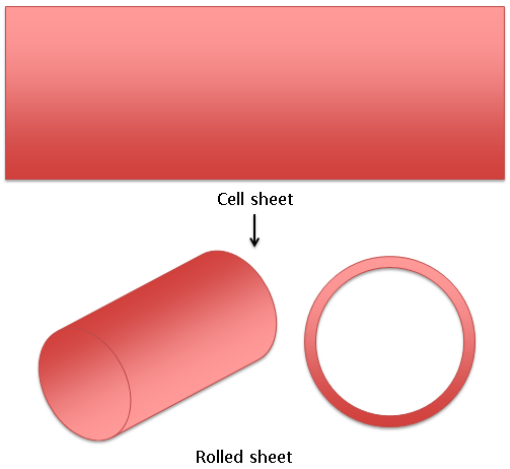

Figure 2: Tissue rolling method

Results from burst pressure testing and suture retention testing show that TEVGs cultured using the tissue rolling method have similar mechanical properties to natural tissue. The mean burst pressure strength is 3517 mmHg for a 5.5 mm inner diameter graft created using the rolling method (L'Heureux *et al.*, 1998). The culture time for the tissues is usually three months (L'Heureux *et al.*, 2007). This diminishes the availability of the TEVG and increases production costs. In addition, for TEVGs cultured using the tissue rolling method, the mechanical strength comes from the fibroblast adventitia layer, not the media layer, as in natural tissue (L'Heureux *et al.*, 1998).

2.3.2: Direct Cell Seeding Method

Researchers in Prof. Rolle's laboratory are developing an alternative method, in which SMCs are seeded directly on to a silicone mandrel. Silicone support rings are placed on a well plate and silicone tubes are placed over the rings. A cell suspension of SMCs is then pipetted into the center of each support ring. The plates are inverted and cultured in an incubator for 30 minutes. They are then removed from the incubator, turned upright, and the cellular suspension is aspirated. The wells are filled with media and cultured for fourteen days to allow the cells to proliferate and deposit an extracellular matrix. This process is illustrated in Figure 3. (Doshi, 2009)

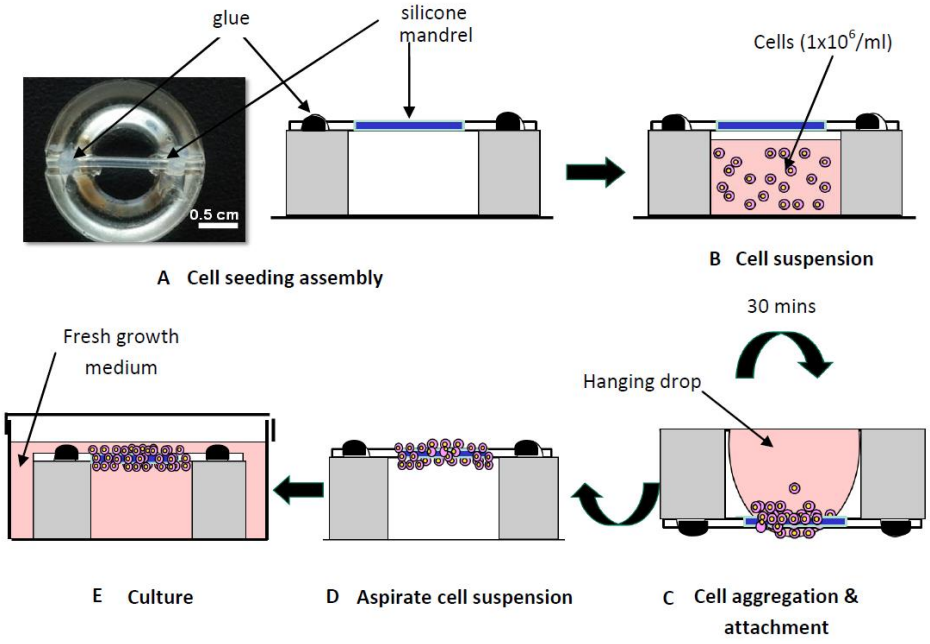

Figure 3: Direct cell seeding method (Doshi, 2009)

This method successfully creates TEVGs in a shorter period, although they are not as strong as those created by the tissue rolling method. One limitation of the direct cell seeding method is the diffusion limit. When the TEVGs reach a thickness of 250 µm, nutrients can no longer diffuse through the outer surface to the lumen, causing necrosis of the luminal tissue (Doshi, 2009). The TEVGs usually reach this point at about 12 days in culture. The diffusion limit restricts the amount of time the TEVGs can be cultured, which negatively affects the mechanical properties of the graft. The burst pressure strength of TEVGs cultured using the direct cell seeding method is about 255 mmHg, a degree of magnitude lower than that of natural blood vessels, discussed further in the next section (Doshi, 2009).

Previous studies have shown that a bioreactor can be used to condition grafts to imitate native tissue (Bjork & Tranquillo, 2009). A bioreactor is a device that simulates an *in vivo* environment. For vascular tissue, flow of cell culture media through the lumen simulates blood flow through natural vessels. A basic schematic of a bioreactor is shown in Figure 4. It incorporates a pump that moves media through the lumen of the TEVG and a reservoir of media that provides nourishment to the outer surface of the graft. The fluid dynamics of the media are important to consider in the development of a bioreactor for TEVGs.

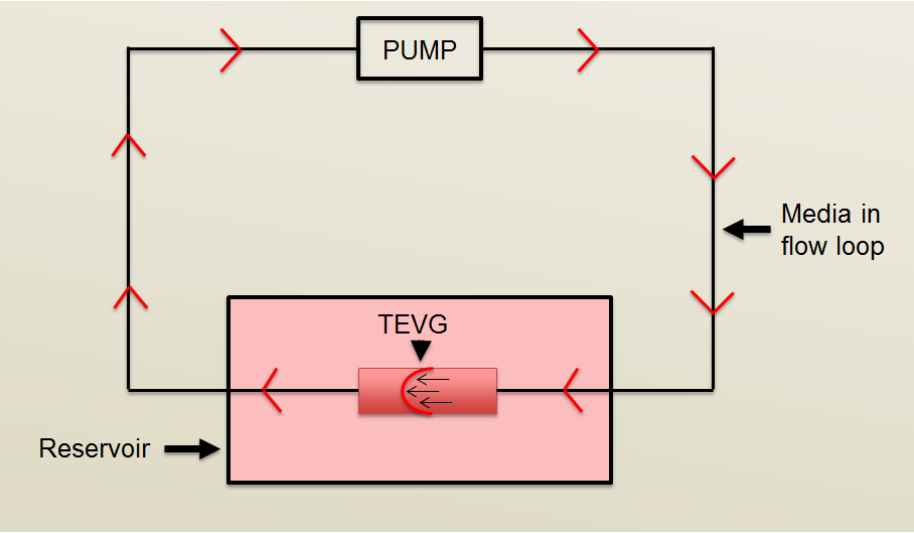

Figure 4: Basic bioreactor system

2.4: Flow and Shear Stress

A laminar luminal flow bioreactor is needed to continue the growth and development of three-dimensional TEVGs. Laminar flow is the movement of a fluid without the mixing of layers, and ideally forms a parabolic flow profile as shown in Figure 5. Laminar flow is responsible for imparting constant shear stresses against the wall of the container it is flowing in, in this case the lumen of the TEVG. The

wall of the TEVG needs this constant shear stress in order to mimic the *in vivo* environment (Brooks, Lelkes, & Rubanyi, 2002).

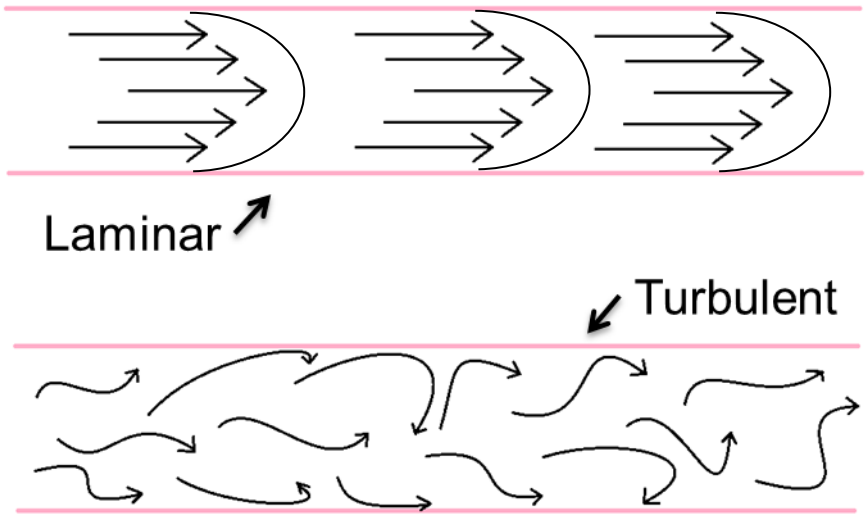

Figure 5: Laminar and turbulent flow

Shear stresses caused by laminar flow are essential for the growth and development of vascular tissue (Traub & Berk, 1998). A constant shear stress is needed for the survival and orientation of endothelial cells that line the lumen of the blood vessel (dela Paz *et al.*, 2012). In a study done by Nathaniel dela Paz *et al.* at Harvard Medical School, it was shown that a constant shear stress decreased apoptosis in human umbilical vein endothelial cells (HUVECs) by increasing the production of vascular endothelial growth factor (VEGF) in arterial cells. This protein has many functions in the cardiovascular system, including angiogenesis (dela Paz *et al.*, 2012). Since a constant shear stress is necessary for the growth and development of TEVGs, laminar flow is required in our bioreactor.

The shear stress imparted on the wall of an idealized tube by the movement of a viscous fluid can be calculated using Equation 1. Turbulent flow causes inconsistent shear stresses on the lumen, which can lead to varying wall thicknesses along the length of the vessel (Brooks, Lelkes, & Rubanyi, 2002). This turbulence can also cause destruction of the endothelial layer and eventually lead to vascular diseases such as atherosclerosis (Zhixin *et al.*, 2011). Turbulence is usually not found in straight blood vessels *in vivo*, but can be found at bends in vascular architecture, as seen in coronary arteries (Traub & Berk, 1998). The TEVG can be damaged or have a varying wall thickness if turbulent flow is found in the bioreactor's flow loop. Shear stress on vascular tissue *in vivo* ranges from 10-40 dynes/cm^2 (Resnick *et al.*, 2003). Using a known shear stress, the velocity of a fluid through a tube can be calculated.

Equation 1: Calculation for shear stress: τ is shear stress, μ is dynamic viscosity of the fluid, *U* is mean velocity of the fluid, and *D* is the inner diameter of the pipe.

$$\tau = \frac{8\mu U}{D}$$

Because we are designing a bioreactor system for a known shear stress in the TEVG, the dependent variable in Equation 1 is velocity (U). Using the inner diameter of the TEVG and the calculated velocity, we can calculate the flow rate of the entire system using Equation 2. The flow rate will remain constant no matter what size tube the fluid is flowing in order to have the desired velocity in the TEVG.

Equation 2: Flow rate calculation: U is velocity of fluid, and A is cross sectional area of the tube in which fluid flows

$$Q = UA$$

The flow profile in our bioreactor can be determined by calculating the Reynolds number of the fluid flowing through the TEVG. The Reynolds number is a dimensionless metric that can be calculated to determine whether the flow of a viscous fluid in a tube is laminar or turbulent. If the Reynolds number is less than 2100, then the flow is laminar (Waite & Fine, 2007). If we assume the tube is rigid with a constant diameter and the fluid flow rate is constant, we can calculate the Reynolds number using Equation 3. The limitations of this assumption are discussed in Chapter 6.

Equation 3: Reynolds number where: ρ is the density of the fluid, U is the velocity of the fluid, μ is the dynamic viscosity of the fluid, and D is the inner diameter of the tube.

$$Re = \frac{\rho UD}{\mu}$$

Another important consideration for laminar flow is entrance length. Entrance length is the length of tube that is necessary for the development of laminar flow. The development profile is illustrated in Figure **6**. The calculation for entrance length is shown in Equation 4.

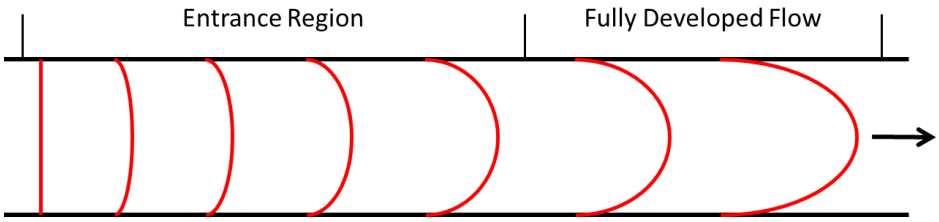

Figure 6: Development of laminar flow

Equation 4: Entrance length, where L_e is entrance length, Re is Reynolds number in the tube, and D is inner diameter of the tube.

$$L_e = 0.06 \times Re \times D$$

We found that there was a lack of published data regarding the effect of shear stress on three-dimensional constructs purely derived from SMCs. Many articles reported on the effect of shear stress on ECs because only ECs are exposed to flow *in vivo*. In order to predict the effect a laminar flow bioreactor would have on the TEVGs, we considered the effect of shear stress on two-dimensional SMC sheets to predict the effect on three-dimensional grafts.

In 2006, Wang *et al.* reported that shear stress could induce EC transdifferentiation of mouse SMCs. Mouse SMCs were cultured to 80% confluence and exposed to laminar fluid shear stress via a parallel plate flow chamber. The SMCs were exposed to a steady shear stress of 15 dynes/cm^2 for 6, 24, and 48 hours. At each time point, Wang *et al.* extracted RNA, which they reverse transcribed into cDNA. They used PCR to assess mRNA levels of murine platelet-endothelial cell adhesion molecule-1, von Willebrand factor, and VE-cadherin. These markers were chosen because they are expressed by ECs (Wang *et al.*, 2006). They also used PCR to test for SMC markers, specifically alpha-smooth muscle actin, calponin-1, and smooth muscle myosin heavy chain. The results of this experiment showed that after exposure to a laminar fluid shear stress for 24 and 48 hours, SMC exhibited increased EC markers and decreased SMC markers, including alpha-smooth muscle actin. They also observed increased cell alignment in the direction of the flow after 24 and 48-hour flow exposure period compared to the statically cultured SMCs. Wang *et al.* concluded that shear stress might promote endothelial cell transdifferentiation from SMCs. This finding was corroborated by a study conducted in 2010, which indicated that when SMCs were exposed to shear stress of 14 dynes/cm^2 for 24 hours, had decreased transcription of endothelin, a protein associated with constriction of blood vessels (Ekstrand, 2010). This is logical because if the endothelial layer of a blood vessel were damaged *in vivo*, the hemodynamic environment would alter the gene expression of SMCs to repair the damage. (Wang *et al.*, 2006)

Fitzgerald *et al.* reported in 2007 that laminar shear stress stimulates vascular smooth muscle cell apoptosis. Bovine aortic SMCs were cultured in a sheet and exposed to 11 dynes/cm^2 for 24 hours. These dynamically cultured SMCs were compared to SMCs exposed to static media. They found that the SMCs exposed to shear stress had 38% fewer cells, a 15-fold increase in TUNEL staining, a 3-fold increase in caspase-3, and a 67% decrease in Akt phosphorylation. TUNEL is a method that detects DNA fragmentation due to apoptosis. Caspase-3 is a member of a protease family that plays an essential role in the execution phase of apoptosis. Akt is a protein kinase that inhibits apoptosis. The results of this experiment indicate that SMCs exposed to shear stress have decreased cell proliferation and increased apoptosis. Considering *in vivo* applications of this discovery, increased SMC apoptosis would prevent stenosis of the blood vessel by limiting SMC proliferation. (Fitzgerald *et al.*, 2007)

Although this research is based on two-dimensional SMC samples, we anticipate similar results on three-dimensional grafts. We hypothesize that a luminal laminar flow bioreactor providing shear stress would decrease levels of alpha-smooth muscle actin, align SMCs in the direction of flow, and prevent luminal occlusion.

2.5: Current Technology

Presently, there many different types of bioreactor designs used in research settings. Many of these designs are custom made and tailored to the specific nature of the experiments being conducted. As such, bioreactor designs can vary from laboratory to laboratory. Bioreactors can be used to culture either two-dimensional or three-dimensional samples. This chapter will focus on three-dimensional bioreactor designs; two-dimensional bioreactors are beyond the scope of this report.

2.5.1: Cerulli *et al.* Bioreactor

Prof. Rolle's laboratory currently uses a laminar luminal flow bioreactor to aid in culturing TEVGs. This bioreactor incorporated mounts for the TEVG that were located on the underside of the lid, shown in Figure 7. The graft, depicted in red, was sutured between these two needle mounts. One arm (A) was adjustable, allowing the user to easily suture the sample to both needle mounts and adjust them accordingly. The mobile arm was kept in place with a magnet, while a track allowed for stability, labeled B in Figure 8. Media flowed into the bioreactor through the inlet on the left. It then went through the lumen on the graft and emptied into the reservoir after passing through the mobile arm A. Media flowed from the reservoir through the outlet on the right, back to the pump. A photograph of the bioreactor's lid is shown in Figure 9.

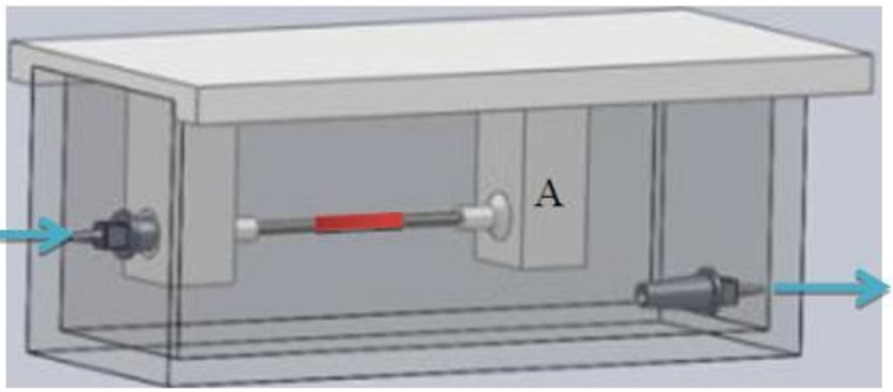

Figure 7: Previous bioreactor design, isotropic view with mobile arm (A)

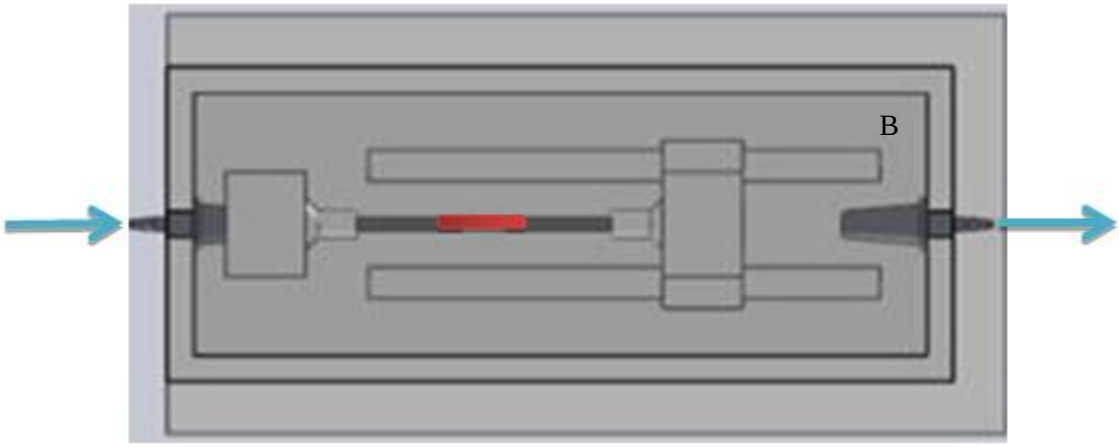

Figure 8: Previous bioreactor, underside of lid with track (B)

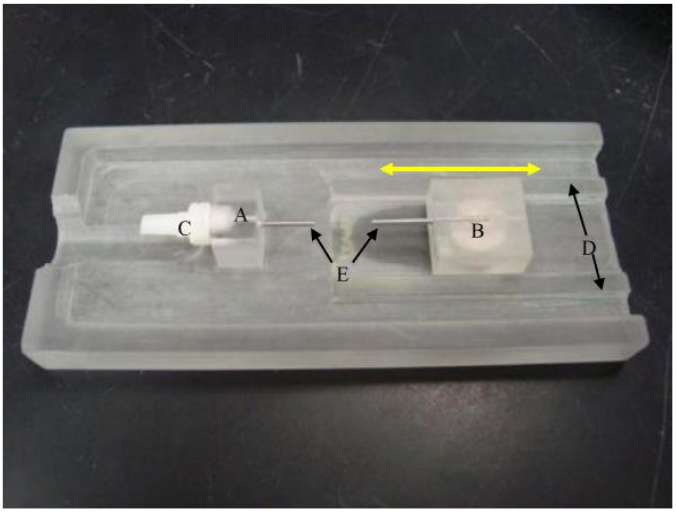

Figure 9: Cerulli *et al.* lid, which includes the stationary mount (A), the removable mount (B), luer connector (C), track for removable mount (D), and the needle mounts (E)

The novel design of the Cerulli *et al.* bioreactor allowed it to accommodate different sized samples and made mounting the TEVG easier for the user. However, this design is not without limitations. The Cerulli *et al.* bioreactor does not have isolated flow loops. This means the media that passes through lumen mixes with the media in the reservoir. This restricts the user to using the same type of cell culture media for the lumen of the TEVG as well as the exterior. Additionally, the mobile arm of the bioreactor is susceptible to unwanted movement during the culture period. Although the design uses a set of tracks to prevent motion of the magnetized mobile arm, they are sometimes ineffective. This causes the misalignment between the mobile arm and the fixed arm. Misalignment disrupts laminar flow and causes turbulence and uneven shear stress. The inlet and outlet ports to the reservoir are below the media level. Because the connection points are not completely sealed, leakage occurred inside the incubator, shown in Figure 10. A gap between the lid and the reservoir, denoted with the arrow, was designed to let the user connect the silicone tubing to the inlet port of the luminal flow loop. This gap allows air to contact the reservoir of the bioreactor directly, increasing the chance of contamination. Media leaks from the gap when the bioreactor is moved from the biosafety cabinet into the incubator.

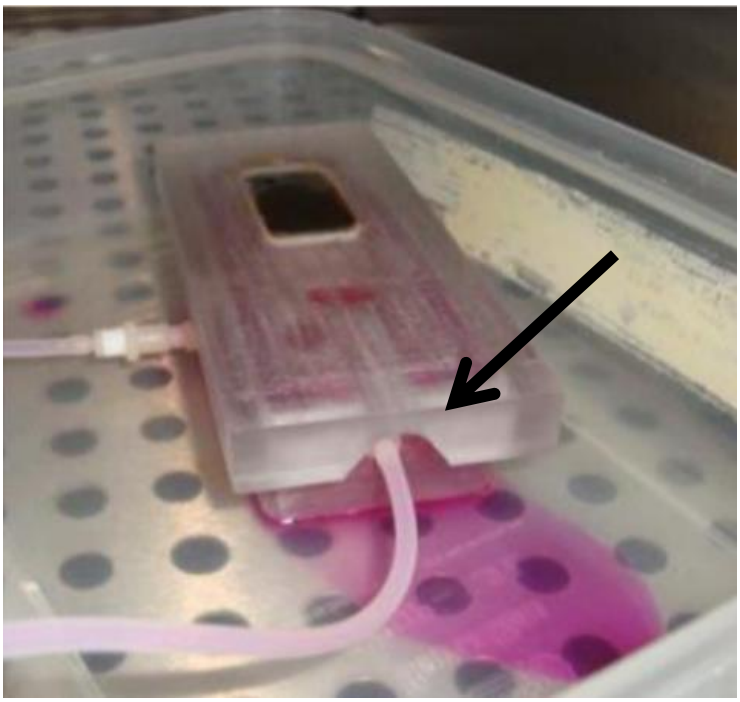

Figure 10: Cerulli *et al.* bioreactor leakage

2.5.2: Banes *et al.* Bioreactor

Professor Albert Banes from the University of North Carolina holds the patent for a unique bioreactor design, which includes a novel mounting device and can be adjusted to accommodate a wide range of sample sizes. We considered the mounting device of this design as an alternative to using sutures to mount the TEVG to the luminal flow loop of the bioreactor because of the potential to reduce the mounting time of each sample. Reducing the mounting time is critical because the TEVG is not in cell culture media during the mounting process and cell death can occur if the TEVG is without cell media for too long. (Banes, Wang, Qi, & Kheradpir, 2004)

The mounting device, shown in Figure 11 and Figure 12, has three main components. In Figure 12, the attachment site for the TEVG is labeled A. The TEVG is slid over this cylindrically shaped component and over the small grooves beyond the tip, which secure the TEVG in place. Each mount has four arms that fix the TEVG in place. Each arm can be set to one of two possible positions: open and closed. In the open position, the arm extends away from the mounting device to provide the operator with enough room to mount the sample. In the closed position, a small curved area of the arm contacts the sample and applies pressure, labeled B. These arms are positioned perpendicular to each other so that they contact the sample radially every 90^0. Constant uniform pressure is applied to the sample via an O-ring, labeled C. The O-ring can be positioned such that it fits into a grooved slot on each of the arms; the grooved slot is labeled D. The O-ring prevents arms from disengaging the sample during the experiment. To remove the sample, the O-ring is slid out of the groove, the arms are extended to the open position, and TEVG is carefully slid off the mount. This mounting mechanism is mirrored on the other end of the bioreactor. (Banes, Wang, Qi, & Kheradpir, 2004)

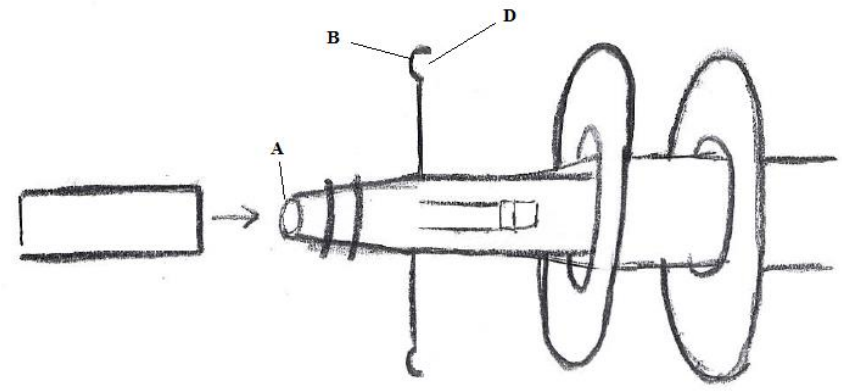

Figure 11: Banes mount open

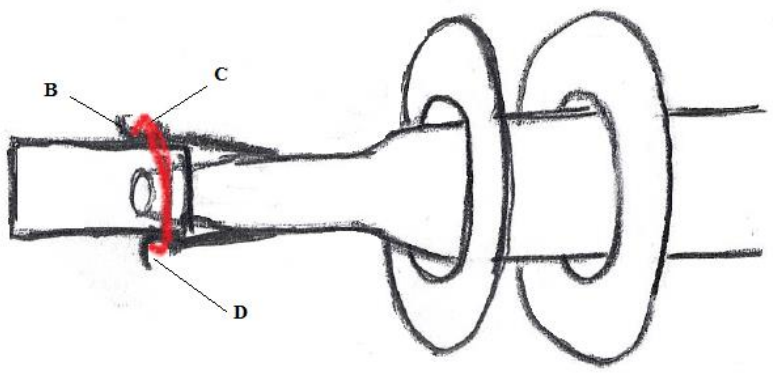

Figure 12: Banes mount closed

The main disadvantage of this mounting mechanism is that it destroys the cells at the ends of the sample. The pressure required to fix the TEVG to the mount crushes the cells at that location. This would require that these sections be removed before the results can be analyzed. Another possible complication with this design is that the TEVG may tear if the tissue becomes caught in the grooves on the mount as its being removed. Because of the size and complexity of the mechanism, it could be difficult and costly to machine. (Banes, Wang, Qi, & Kheradpir, 2004)

The other unique feature of this design is the mechanism for adjusting the distance between the two mounts to accommodate different sized samples. The gear mechanism with a knob allows the operator to set the distance between the mounts manually, shown in Figure 13. When the knob, labeled A, is turned by the operator, it simultaneously moves both ends of the mounting device. The sample is housed in a media-filled reservoir, labeled B. The reservoir is the main limiting factor in the accommodation of different sized TEVGs in the bioreactor. (Banes, Wang, Qi, & Kheradpir, 2004)

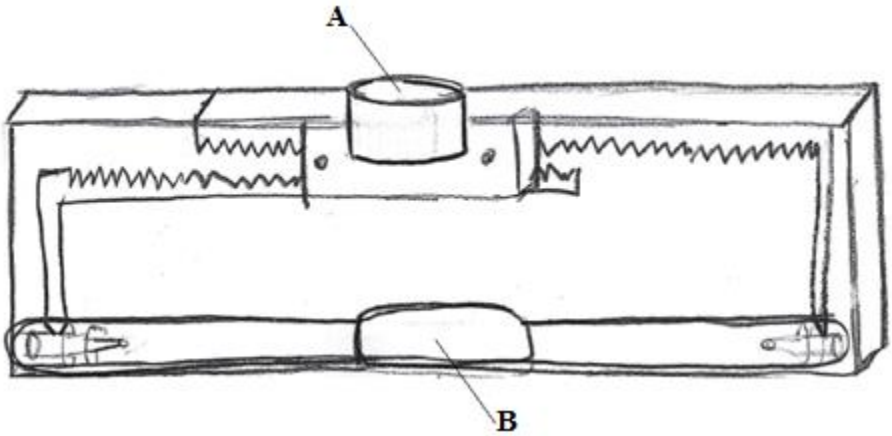

Figure 13: Banes adjustable mount mechanism: knob (A) and reservoir (B)

A limitation of this design is that the distance between the mounts can only be adjusted in discrete intervals. This could be a problem if a sample requires a distance that is between two intervals. However, as long as the intervals are kept small enough this issue can be avoided in most cases. Another limitation of this bioreactor is the complexity of the small parts. This increases the possibility that errors could be made during manufacturing. Additionally, the size of the TEVG is limited by the length of the well, shown in Figure 13, labeled B. This device also has a large footprint, which would affect how many TEVGs can be cultured in an incubator at the same time. (Banes, Wang, Qi, & Kheradpir, 2004)

2.5.3: Williams & Wick Bioreactor

Chrysanthi Williams from Georgia Institute of Technology published a design for a modular bioreactor. This bioreactor, unlike many other designs, was intended to culture several samples per trial. The entire bioreactor is made from glass, which allows for durability during autoclaving. The Williams & Wick bioreactor design has two main components: the individual modules and the head plate where each module is attached in parallel. The module has both luminal flow and exterior flow. Each module is cylindrically shaped with mounts penetrating into the interior of the cylinder. The module does not have adjustable mounts as in the Banes *et al.* design. The ends of the sample are mounted using sutures. Modules can be connected to one another via interlocking head plates. An O-ring is used to compress each module together, forming a tight seal. The media flows through the lumen top down while the exterior flow goes from left to right. This design enables the incubation of several samples at one time. The number of samples that could be placed in parallel is limited by the number of available modules, the size of the incubator, and available cell culture media. (Williams & Wick, 2004)

Suturing the TEVG to the mounts is difficult in the Williams & Wick design because the mounts are fixed in place. It also requires the researcher to produce consistently sized tissue samples. If the samples are too small, they could not be sutured to the mounts. If they were too big, then too much of the TEVG would be covering the mount. The tissue that overlapped on the mount would most likely be unusable. It is also possible the sample is simply too large to fit onto the module mounts.

The Williams & Wick bioreactor is made from hand blown glass. While this increases the production cost of the bioreactor, it does provide several advantages. The glass will not deform after multiple autoclaving cycles (Williams & Wick, 2004). The durability of this design makes it a good investment for long-term use. It also has very few ways in which it can fail because it has no moving parts. If the device were to fail, it would most likely be due to leakage because of incorrectly fabricated parts. If the modules do not line up exactly, there is a high probability that leakage from the exterior flow loop will occur.

The limitations of autologous and synthetic grafts used to treat diseases, such as atherosclerosis, create a need for TEVGs. By reviewing the literature, we were able to develop a greater understanding of the importance of tissue engineering and the biofluid mechanics necessary in a bioreactor system. We also researched other novel bioreactor designs that addressed a similar need to that created by the TEVGs cultured in the Rolle laboratory. We then developed our project strategy to design a bioreactor that provides luminal laminar flow to a TEVG.

Chapter 3: Project Strategy

The first step in designing the bioreactor was organizing the client's needs into constraints, objectives, functions, and specifications. The constraints were the strict limits that the bioreactor design must have met in order to be successful. The objectives identified the desired attributes of the bioreactor design. Lastly, the functions were the actions the bioreactor must perform and the specifications are the quantitative requirements related to the functions. We determined these design criteria using the client statement, client and user interviews, and literature research. The following chapter describes each of the constraints, objectives, functions, and specifications of the bioreactor.

3.1: Initial Client Statement

When we first started the project, the client provided us with the following statement:

"Bioreactors have been shown to improve the structure and function of engineered tissues by providing conditions that simulate the in vivo *environment in which the tissue normally exists. In addition, bioreactors can facilitate seeding, organization and culture of cells to support tissue growth and maturation. For tissue engineered blood vessels, bioreactors that provide flow of cell culture medium through the center (lumen) of the blood vessel allow seeding of endothelial cells, nutrient diffusion to cells near the lumen of the vessel, and application of shear stress to endothelial cells lining the vessel. In addition, bioreactors must provide appropriate mechanical stimulation to facilitate the culture and development of tissue-engineered blood vessels that match the structural, physiological, and mechanical properties of normal vascular tissue. Previous MQP teams have designed and tested two individual bioreactor systems - one that provides luminal flow, and on that imparts cyclic mechanical distension. The goal of this project is to create a novel bioreactor system that incorporates both luminal flow and appropriate mechanical conditioning for vascular tissue engineering. The bioreactor must securely anchor the tissue sample without damage or leaking. Ideally, the bioreactor should be inexpensive and easy to manufacture, such that multiple samples can be cultured in a single experiment."*

3.2: Current Device – Cerulli *et al.* Bioreactor

The previous MQP group had successfully designed a bioreactor for three-dimensional TEVG constructs cultured using the direct cell seeding method. The previous design was sterilizable, provided

luminal flow to the TEVGs, and incorporated an adjustable mount to increase ease of mounting. Although it is functional, there were problems with the device that needed to be addressed. The previous bioreactor design had many different parts and connections, causing a large volume of media leakage and compromised sterility. Post-manufacturing modifications, namely the shortened needle mounts, did not allow enough entrance length to allow laminar flow to develop. In addition, the laminar flow bolus test performed on the device was inconclusive. Considering the advantages and disadvantages of the previous design, we created another iteration of a laminar luminal flow bioreactor for TEVGs.

3.3: Design Constraints

In order to design a bioreactor that satisfied the needs of our client, we first needed to identify and meet all of our constraints. The constraints of the design were that is provide luminal flow, securely mount the tissue sample, permit gas exchange, fit inside incubator, be sterilizable, be within a $496 budget, and be completed within the 28-week time frame. The users of the bioreactor are Prof. Rolle, Zoe Reidinger, and WPI students working in the Rolle laboratory.

Providing luminal flow through the TEVG was required for the development of viable tissue samples. Luminal flow is important because it prevents necrosis in the inner surface of the TEVG by allowing for the transportation of nutrients and waste to and from the lumen (Engbers-Buijtenhuijs *et al.*, 2006).

In addition to the flow constraints, the bioreactor also needed to meet operational constraints. First, the mounting mechanism needed to be able to attach the TEVG securely in such a way that would permit media to flow through the lumen and provide media to the exterior surface. We also determined that the bioreactor must securely mount the sample without causing extensive damage to the tissue. However, our client, Zoe Reidinger informed us that minor necrosis at the very ends of the tissue sample was acceptable because this portion can be removed. Because the TEVGs must be cultured under incubator conditions, the dimensions of the bioreactor cannot exceed those of the incubator.

To sterilize the device, the materials used needed to be disposable or able to be sterilized in some manner. Autoclaving was the chosen method for sterilization because it is the preferred method in the Rolle lab. This also eliminated any additional costs associated with other methods of sterilization.

The design of the device was to cost $496 or less, since WPI was funding the project at $124 per team member. We selected materials carefully to ensure that the budget was not exceeded. Moreover, there were only twenty-eight weeks to design, build, and test a bioreactor to prove the concept of the design. We worked in a timely manner to complete the assigned tasks. This limited design period created a tight schedule for developing and selecting a design, manufacturing and testing the prototype, manufacturing and testing the final device, and constructing the final report.

3.4: Design Objectives

Through our client meetings with Prof. Rolle and Zoe Reidinger and a review of the current literature, we were able to identify seven main objectives for the device. All objectives are shown in Table 1.

Table 1: Objectives

Objective
Laminar Flow
Prevent Leakage
Isolated Flow Loop
Remain Sterile
Limit Mounting Time
Easy to Machine
Accommodate Different Samples
Cost Effective

Laminar flow provides a constant shear stress to the lumen of the TEVG. Laminar flow is necessary to simulate the *in vivo* environment of vascular tissue (Brooks, Lelkes, & Rubanyi, 2002). The constant shear stress prevents cellular occlusion of the graft (Wang *et al.*, 2006).

An isolated luminal flow loop was an objective of our design because it reduced the risk of cross-contamination in the bioreactor. It would allow for an increase in the types of experiments that could be conducted on the TEVG because different media could be used internally and externally.

In order to achieve accurate results from the experimental research conducted using the bioreactor, the device must be sterile and not leak. Leaking wastes cell culture media and can cause contamination. The loss of media increases the operational cost and can cause the sample to become dehydrated. The presence of pathogens, such as bacteria, could affect the development of the TEVG (Inaba *et al.*, 2009). It is important to eliminate pathogen presence to ensure that histological results are due to the dynamic flow in the bioreactor. By eliminating leakage, we reduced the amount of media used and ensured sterility. The bioreactor needed to remain sterile during the duration of a culturing experiment in order to reduce risk of contamination. Contamination increases the presence of pathogens, which can damage the TEVG.

Streamlining the mounting process would increase the efficiency of the experiments using the bioreactor. This is because each sample is mounted outside of a media bath and the TEVG could dehydrate if exposed to the air for an extended period. With a shorter mounting process, the overall time needed to run an experiment would be reduced.

In order to address ease of manufacturing, we created a simple design. Simplicity ensured that during manufacturing, we could prevent as many machining errors as possible. Due to the small scale of the TEVG and the subsequent small scale of the bioreactor, even small errors in machining could render the bioreactor unusable. In a further attempt to limit machining errors, we considered using commercially available parts as alternatives to machined parts. We believed that by reducing the amount of material and media used during manufacturing and operation, it would also reduce the overall cost of the device. Achievement of the manufacturing objective would reduce the chances of leakage and contamination due to manufacturing errors.

The mounting mechanism should also be able to accommodate different sized samples. This would increase productivity and allow the bioreactor to accommodate samples of different dimensions along with the inherent variance amongst the samples themselves.

Finally, we decided that ideally the bioreactor would cost less than or equal to the design currently being used to culture TEVGs. This would prevent an increase in experimental costs for Prof. Rolle's laboratory and prevent any reduction in the number of TEVGs that could be cultured concurrently.

We evaluated the objectives using a pairwise comparison chart, as shown in Appendix A. The ranked objectives are shown in Table 2. We ranked these objectives based on a literature review and personal communication with our client and users.

Table 2: Ranked objectives (descending importance)

1.	Laminar Flow
2.	Prevent Leakage
3.	Remain Sterile
4.	Limit Mounting Time
5.	Isolated Flow Loop
6.	Easy to Machine
7.	Accommodate Different Sized Samples
8.	Cost Effective

3.5: Functions and Specifications

The functions of the bioreactor described the actions the bioreactor must perform on the specific TEVGs fabricated in the Rolle lab. The specifications were the metrics we had to achieve in order to achieve each function. The functions and specifications are summarized in Table 3. The four functions of the device were to provide laminar flow, apply shear stress to lumen, accommodate TEVGs cultured in Prof. Rolle's laboratory, and fit in the laboratory incubator.

Table 3: Functions and specifications

Function	**Specification**
Provide laminar flow	Reynolds number < 2100 Entrance length
Apply shear stress to lumen	Shear stress = 10 dynes/cm^2
Accommodate samples cultured in the Rolle laboratory	12-14 day old rat aortic SMC-derived TEVGs 10 mm in length and 1.2 mm in diameter
Fit inside laboratory incubator	Overall dimensions must be less than 3 ft. by 3 ft. by 3 ft.

From the client statement, we determined the required shear stress on the walls of the TEVG was 10 dynes/cm^2 (1 Pa) for a 10 mm long and 1.2 mm inner diameter tissue sample. We estimated the viscosity (μ) to be equal to that of water. Using these values, we calculated the desired average velocity using Equation 5. Since the bioreactor system could incorporate tubes of varying diameters, we calculated the desired flow rate of the entire system based on the specifications of the TEVG. The flow rate would be controlled by the pump.

Equation 5: Velocity calculation from shear stress in TEVG, where τ is shear stress, μ is dynamic viscosity of the fluid, U is mean velocity of the fluid, and D is the inner diameter of the pipe

$$\tau = \frac{8\mu U}{D} : U = \frac{8\mu\tau}{D} = \frac{8(6.92 \times 10^{-4}\,\mathrm{Pa \cdot s})(1\,\mathrm{Pa})}{(1.2 \times 10^{-3}\mathrm{m})} = 0.217\ \frac{\mathrm{m}}{\mathrm{s}}$$

$$Q = UA = (0.217\frac{\mathrm{m}}{\mathrm{s}})(\pi)(0.6 \cdot 10^{-3}\mathrm{m})^2 = 14.7\frac{\mathrm{mL}}{\mathrm{min}}$$

With a known velocity, we calculated Reynolds number in the tissue tube using Equation 6. Laminar flow is characterized by a Reynolds number less than 2100 (Waite & Fine, 2007). We estimated the media viscosity (μ) and density (ρ) to be equal to the values of water.

Equation 6: Reynolds number calculation in TEVG, where ρ is the density of the fluid, U is the velocity of the fluid, μ is the dynamic viscosity of the fluid, and D is the inner diameter of the tube

$$\mathrm{Re} = \frac{\rho U D}{\mu} = \frac{(1000\ \frac{\mathrm{kg}}{\mathrm{m}^3})(0.217\ \frac{\mathrm{m}}{\mathrm{s}})(1.2 \times 10^{-3}\mathrm{m})}{6.92 \times 10^{-4}\mathrm{Pa} \cdot \mathrm{s}} = 376$$

After confirming that the flow would be laminar in the TEVG for the desired shear stress, we tested the settings on the Fischer Scientific Peristaltic Pump provided to us to determine which setting allowed a flow of 14.7 mL/min. An image of the pump is show in Figure 14. The control knob is located in the lower left corner of the pump and the slow/fast switch is located next to the power switch. We timed how long each setting took to fill a 10 mL graduated cylinder. We performed the test twice times for each pump setting. The data are shown in Table 4.

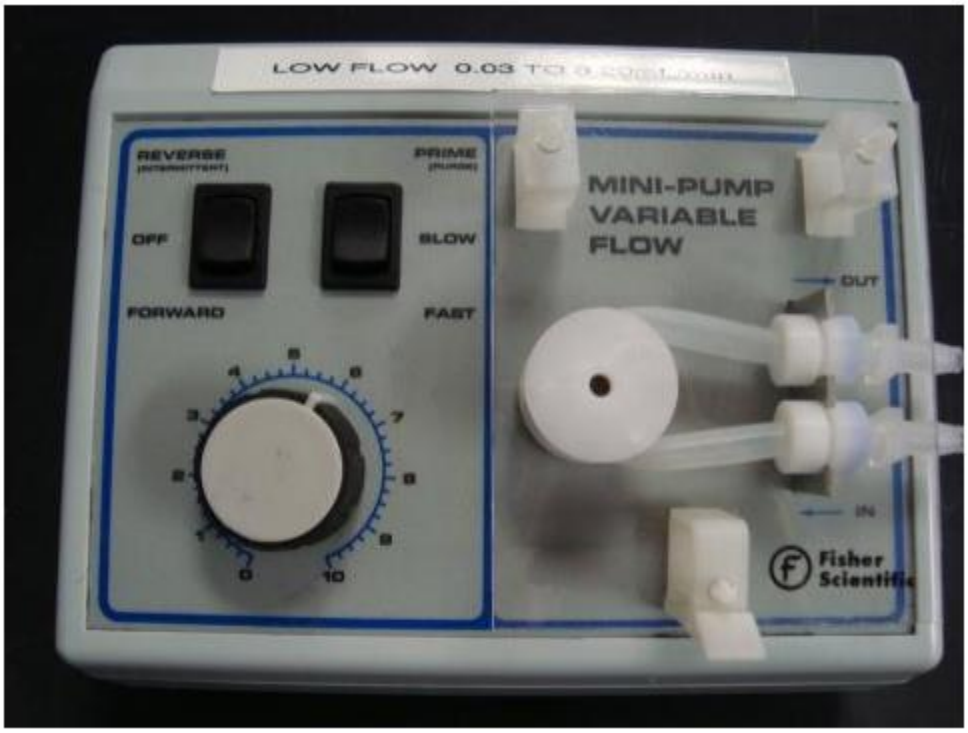

Figure 14: Fischer Scientific Peristaltic Pump

Table 4: Pump flow rate test

Setting	Average Time to Fill 10 mL (s)	Flow Rate (mL/min)
Slow 0	14.5	4.2 ± 0.1
Slow 2	91.0	6.6 ± 0.1
Slow 4	63.5	9.4 ± 0.1
Slow 6	46.5	12.9 ± 0.2
Slow 8	38.5	15.6 ± 0.3
Slow 10	35.5	16.9 ± 0.3
Fast 0	58.5	10.3 ± 0.1
Fast 2	45.5	13.2 ± 0.2
Fast 4	39.5	15.2 ± 0.3
Fast 6	34.5	17.4 ± 0.4
Fast 8	29.5	20.3 ± 0.5
Fast 10	28.5	21.1 ± 0.5

Slow 8 provided a close flow rate to 14.7 mL/min, so we tested values between Slow 6 and Slow 8. After testing Slow 7, we determined the setting needed to be between Slow 7 and Slow 8. These values are shown in Table 5. Because the flow rate of Slow 7 ½ was 14.6 mL/min, we determined that was the most accurate setting for the TEVGs.

Table 5: Pump flow rates between Slow 7 and Slow 8

Setting	Average Time to Fill 10 mL (s)	Flow Rate (mL/min)
Slow 7	43.5	13.8 ± 0.2
Slow 7 ¼	42.5	14.1 ± 0.2
Slow 7 ½	41.5	14.5 ± 0.2
Slow 7 ¾	38.5	15.6 ± 0.3

3.6: Revised Client Statement

After we finalized the objectives and constraints, we constructed this final client statement:

The goal of this project is to create a novel bioreactor system, which incorporates laminar luminal flow and a shear stress of 10 dynes/cm^2 to generate appropriate mechanical conditioning for engineered vascular tissue samples. It should remain sterile and be able to culture samples that are 10 mm in length and have an inner diameter of 1.2 mm. In addition, the bioreactor must not leak, must not damage the tissue samples, and should include an efficient method of mounting the tissue samples. Ideally, the device should be easy to assemble and should create as few waste products as possible through its manufacturing and use.

3.7: Project Approach

The most important design criteria were providing laminar flow, preventing leakage, remaining sterile, and limiting mounting time. Using the design criteria and the literature review, we developed a series of design alternatives. We created preliminary prototypes of the alternative that best addressed the criteria. After several iterations of the initial prototype, the device evolved into the final design presented in this report. We developed experiments that addressed each of the design criteria to determine the success of the device. The results of the experimental testing were then used to make final recommendations to the design.

Chapter 4: Alternative Designs

Considering the constraints, objectives, functions, and specifications presented in Chapter 3, we created a morphological chart that provided different means for each function, shown in Table 6. From this exercise, we were able to organize our design ideas and develop four different design alternatives. These four design alternatives were the plunger design, cross-flow design, jar design, and drawer design.

Table 6: Morphological Chart

Functions	**Means**					
Secure the sample	O-rings	Sutures	Inflatable cuff	Glue	Grow tissue on mount that attaches to bioreactor	Clamps
Permit gas exchange	Permeable tubing	Filter	Direct contact	Dissolved gas in media		
Mount the sample	Needle	Micropipette tips	Barbed tip			
Contain sample	Sample in lid	Sample in reservoir	Between plungers			
Supply media to exterior	Static	Dynamic				

4.1: Plunger Design

The plunger design was modeled after a laboratory syringe and a SolidWorks drawing of the device is shown in Figure 15. The design used two hollow plungers, shown in blue. They were designed to slide independently of one another to adjust to the length of the TEVG, which would be mounted in the middle of the device, shown in red. After they were adjusted, the plungers would be fixed in place using support rods, shown in yellow. The length of the support rods would be modified to prevent the plungers from moving independently when inserting the sample. This addition would increase stability and prevent damage to the TEVG. In order to access and mount the sample, both plungers would need to be pushed out of the device through one side. The design used needles, in gray, attached to the ends of the plungers to mount the TEVG. The inlet and outlet ports of both the reservoir and the plungers would be connected to a pump using silicone tubing, which is not shown in the figure.

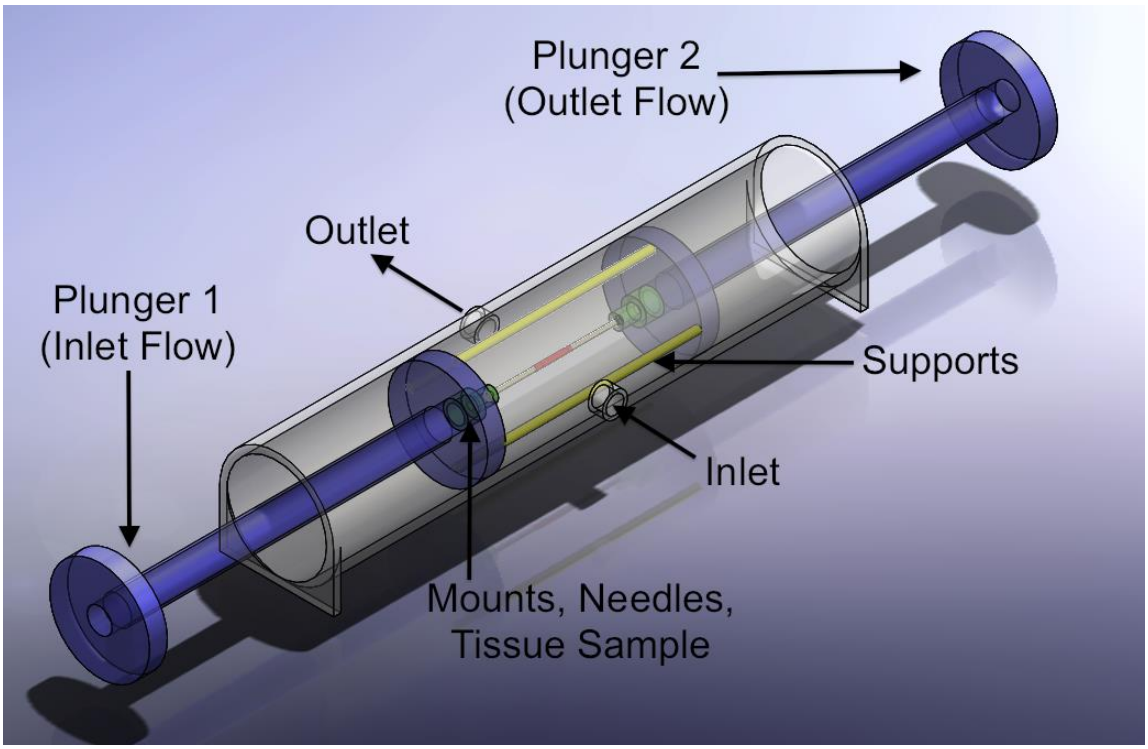

Figure 15: Isometric view of plunger design

Anticipated problems with this design included leakage due to the number of connection points and the gaskets isolating the reservoir. If the gaskets were to fit snugly in the tube to prevent leakage, the seal may have been too tight to put the plungers inside the tube efficiently. For this reason, we foresaw a challenge in putting the plungers back in the tube with a delicate TEVG mounted inside. The distances between the plungers was not fixed, which could cause instability. The reservoir would need a support structure to keep it from rolling in the incubator or in the biosafety cabinet due to its cylindrical geometry. Because the flow in the reservoir is dynamic, we anticipated that the amount of tubing would hinder the assembly of the setup and the transportation from the biosafety cabinet to the incubator.

4.2: Cross-Flow Design

We believed the cross-flow design to be the simplest design alternative, shown in Figure 16. In this design, the TEVG was mounted between two blunted needles inside a rectangular reservoir. In addition to the interior flow loop, an external flow loop was created by the addition of an inlet and outlet port on the reservoir. A simple rectangular lid was also included in this design, but is not shown in the figure. Multiple TEVGs could be cultured in a single experiment by running the reservoir flow loops in parallel

rather than having excessive tubing, and the design could be modified to accommodate multiple TEVGs in one reservoir.

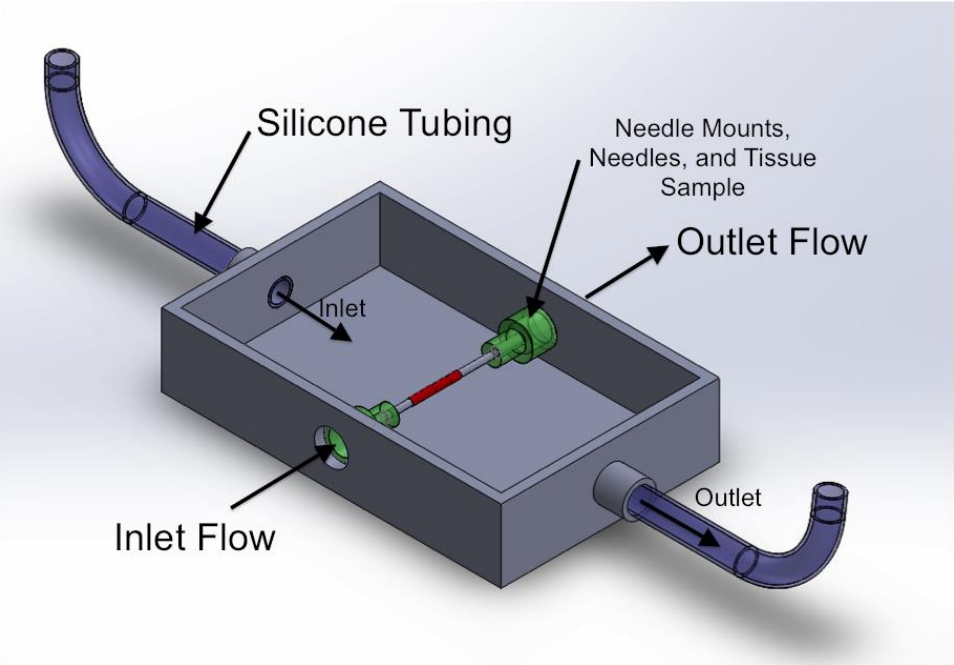

Figure 16: Isometric view of cross-flow design

We expected this design may have problems with leakage at the inlet and outlet ports of the external flow loop because they are below the media level. In addition, this design did not allow for the accommodation of different TEVG lengths; adjustment would require an entirely new device to be manufactured. The reservoir would require milling, a process used in the Cerulli *et al.* bioreactor that we deemed undesirable because more material is needed per bioreactor. Like the plunger design, the reservoir is dynamic. Consequently, we anticipated that the amount of tubing would hinder the assembly of the setup and the transportation from the biosafety cabinet to the incubator.

4.3: Jar Design

The jar design was the only design in which the TEVG was attached to the cap of the reservoir, shown in Figure 17. The TEVG would be then lowered into the media reservoir, which had its own separate flow loop. The cap also had space for an air filter, which allowed for gas exchange in the bioreactor reservoir. This design had the luminal inlet and outlet flow loops above the media level, which

we believed would reduce the chances of leakage at those points. By making use of the vertical space in the incubator, we expected that we could fit more bioreactors in the incubator at one time.

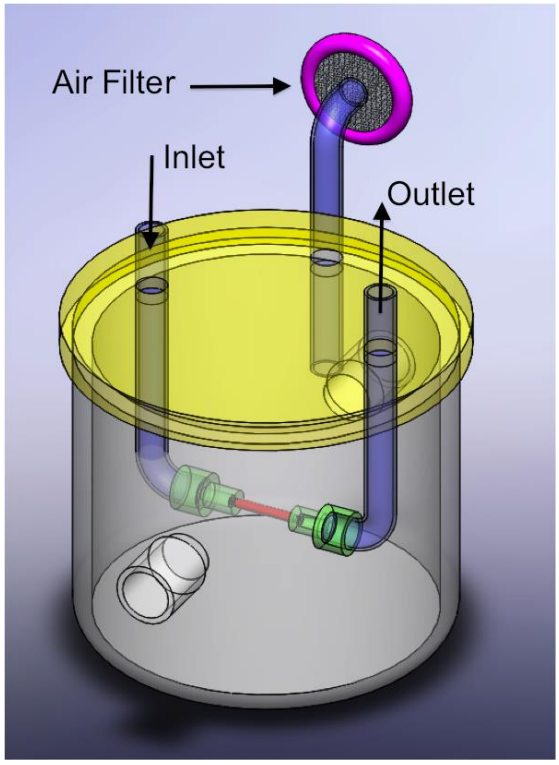

Figure 17: Isometric view of jar design

Similarly, this design had predicted limitations. Movement of the flexible tubing could impart undesired stresses on the TEVG and cause difficulty when mounting. These stresses in turn could cause inconsistent wall thicknesses or damage to the TEVG. Because the exterior flow loop connections are below the media level, the chances of leakage could be increased at those points. Like the previous two designs, we expected that the amount of tubing would interfere with the efficient assembly of the setup and transportation from the biosafety cabinet to the incubator because the exterior reservoir is dynamic. We expected that the TEVG would be difficult to mount in this bioreactor because there is no way to set the cap down steadily.

4.4: Drawer Design

The drawer design included a cartridge that can be removed from the device to aid in efficiently mounting the sample, as shown in Figure 18. It included isolated flow loops for luminal flow and exterior flow. The housing is shown in green in the figure and included four holes: two for interior flow and two

for exterior flow. The interior flow inlet and outlet are the closest to the top left, and the exterior inlet and outlet are labeled in the figure. The drawer in blue had inlets and outlets that corresponded to the green housing part. The TEVG would be mounted in the bioreactor on blunted needles attached to the drawer. A benefit of this design was that the bioreactor would be stable and the user could easily access the TEVG.

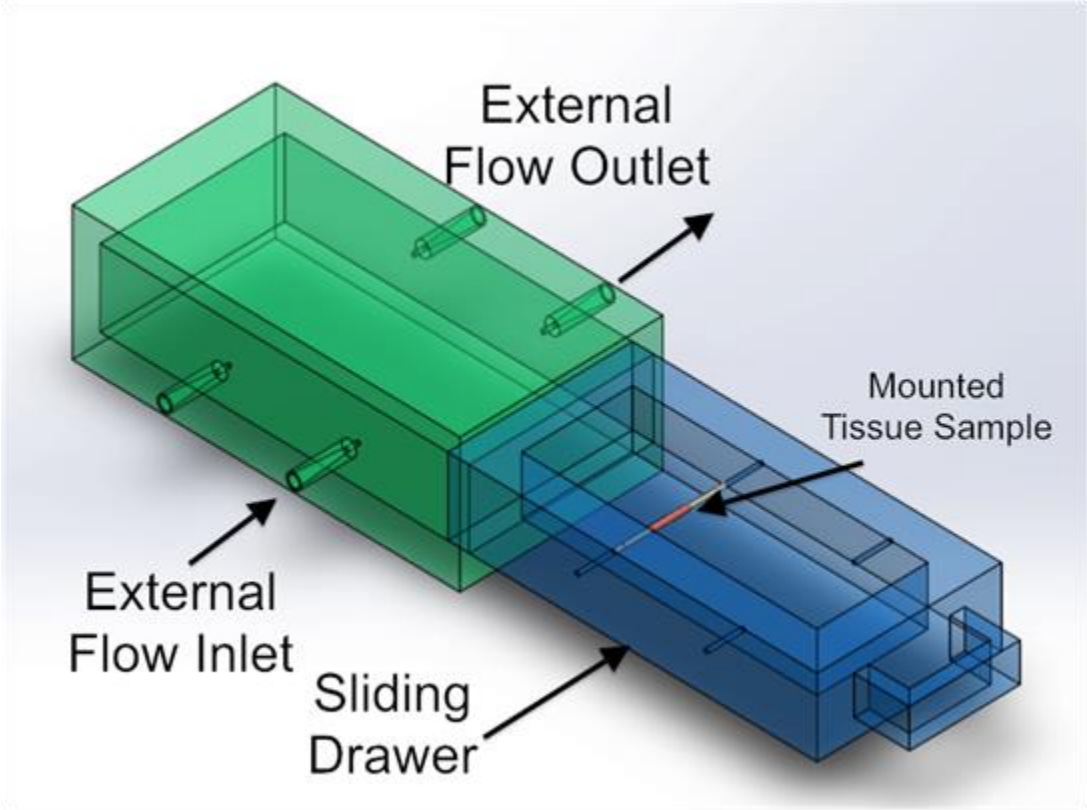

Figure 18: Isometric view of drawer design

This design alternative would require precise manufacturing to align the holes correctly. Because of the small diameter of the inlet and outlet ports, even a slight variation during manufacturing would cause misalignment. Manufacturing errors could cause the drawer to be too large for the housing or if the drawer were slightly too small, media would leak between the two parts. This design would require entirely new drawers to be designed and manufactured in order to accommodate different sized samples. Like the cross-flow design, manufacturing of this bioreactor would require milling, which we deemed undesirable as discussed previously.

4.5: Design Selection and Conceptual Final Design

The conceptual final design was chosen based on our four main objectives, as discussed in section 3.3. We used the sub-objectives as tools to evaluate the corresponding main objective during the design selection phase. However, some sub-objectives were omitted since the same method can be used in any design. For example, we ignored securing the sample because both O-rings and sutures could be used in any design. Further testing was required to determine which method would be most effective in our final design. Using the ranking of objectives and sub-objectives evaluated using the pairwise comparison charts in Appendix A, we rated each design conceptually in a selection-matrix, located in Appendix B. The jar design ranked the highest because of its ease of manufacturing. It could be made from commercial parts and has a simple geometry. We created and tested preliminary prototypes for multiple iterations of the jar design.

4.6: Final Design Prototype

After a discussion with our client, we confirmed that the jar design could meet the design criteria if we redesigned it to address its expected limitations. We decided to prototype a simplified jar design using Tupperware, straws, and a pasta tube as a TEVG model. This prototype can be seen in Figure 19.

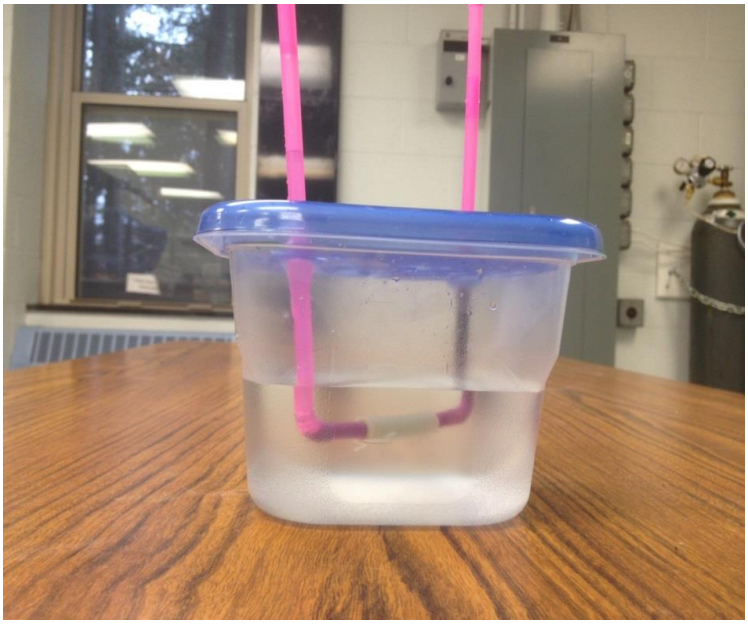

Figure 19: The prototype of the simplified jar design

After creating the first prototype, we decided to mount the TEVG vertically in the device to decrease its footprint in the incubator and the required volume of media. We also hypothesized that the 90º bends in the interior flow loop in the horizontal prototype could disturb the laminar flow of the media through the lumen of the TEVG. The vertical mount model was created out of a plastic container using straws to simulate tubing and pink cuffs to hold the straws in place. The first iteration of the vertical mount design is shown in Figure 20.

Figure 20: An open view of the preliminary vertical mount design

Because silicone tubing is flexible, the pink cuffs in the prototype evolved into a larger support piece. The piece was designed so that the tubing could be fed through the support piece as shown in Figure 21. We expected this would reduce leakage by providing a continuous flow loop. This vertical mount design was modeled in SolidWorks, shown in Figure 21. The original design used a BD Falcon Tube as a reservoir. We chose a BD Falcon Tube because it is a commercially available part that is readily obtainable in a laboratory setting. They are available in polypropylene, which can be sterilized in an autoclave. Test tube holders manufactured to the dimensions of a BD Falcon Tube can be purchased commercially. The material we selected for the support piece, depicted in purple in Figure 21, was polycarbonate because it does not deform in the autoclave, it is inexpensive for the size part we designed, and it can be manufactured in the WPI Machine Shop.

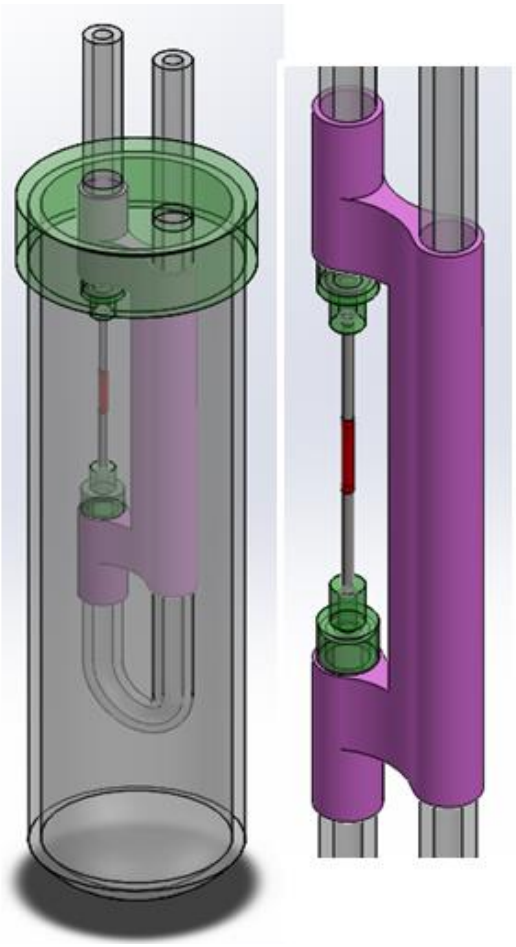

Figure 21: An isometric view of the first model of the vertical mount design

After a conversation with the machinist at the WPI Machine Shop, we decided that several changes to the design could improve the manufacturability and functionality of the support piece. Luer connectors were added at either end of the polycarbonate support piece to decrease leakage and increase reproducibility. The Luer connectors are commercially available, sold by multiple vendors, can be sterilized in an autoclave, and can be replaced if damaged. Female Luer connectors to needle mounts come in a variety of different diameters and length, so they can be changed to house different sized samples. These needle mounts were available in the required entrance length to develop laminar flow. This calculation is discussed in Chapter 5. Additionally, the rounded faces were altered to be rectangular to reduce the time needed to machine the part. However, these changes resulted in a support piece that was too large to fit inside a BD Falcon Tube. To accommodate the new geometry of the design, we chose a glass 38 mm diameter by 200 mm long test tube. We also added a tapped hole where the support piece is attached to a size 8 silicone plug using a number 10-24 3-inch stainless steel screw. We selected a stainless steel screw to prevent rust from affecting the tissue sample. The SolidWorks image of this

design is exhibited in Figure 22. A description of how to assembly the bioreactor system is shown in Appendix D.

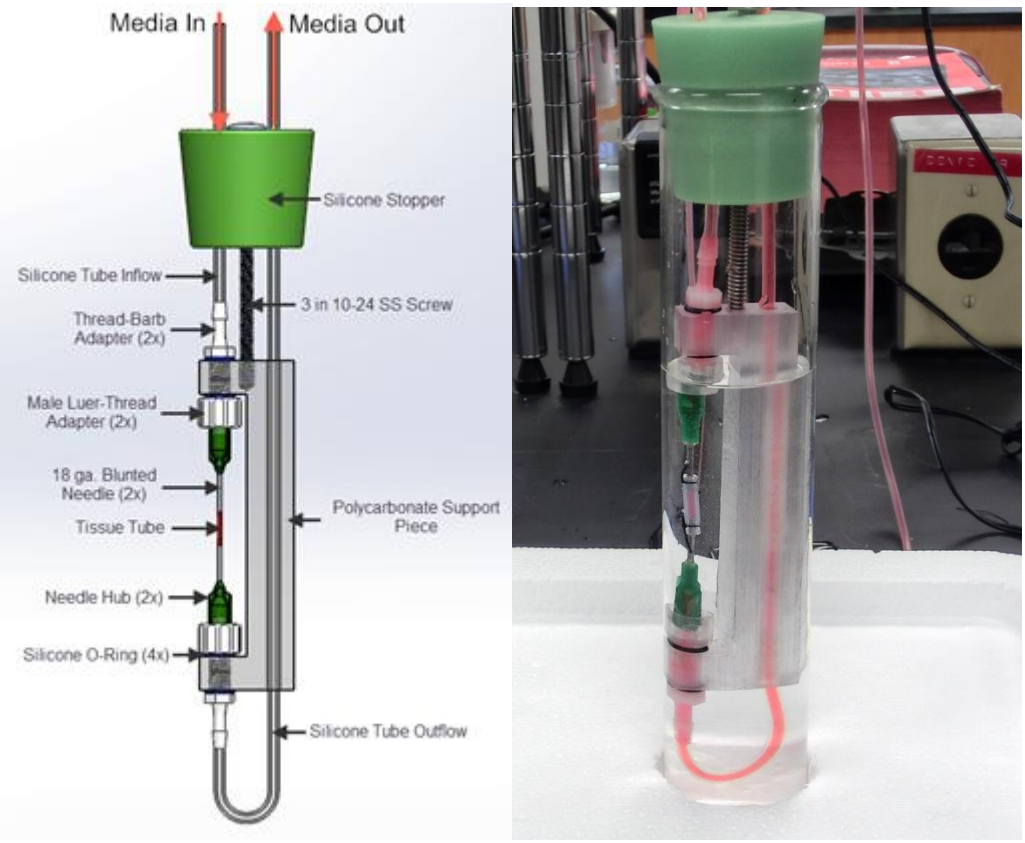

Figure 22: The current iteration of the vertical mount design: SolidWorks design (L) and photograph (R)

We selected parts for the device that would minimize overall cost and difficulty of machining. As seen in Figure 22 and the accompanying picture of the final prototype, all parts of the bioreactor are commercially available, excluding the support piece.

4.6.1: Design Evolution

The first iteration of the design suffered from errors in manufacturing. The drill bit used to create the vertical holes was not long enough to drill through the entirety of the support piece. The machinist needed to drill a hole from either side to pass completely through the support piece, which resulted in misalignment of the holes, and subsequently the needles. This misalignment of the needles was exacerbated by creating the threads for the Luer connectors manually.

During the second iteration of machining, we ordered a six-inch drill bit that could drill the hole in the support piece continuously. The support piece no longer needed to be rotated during manufacturing, and the hole was aligned. However, because the machinist created the threads manually, we still observed misalignment of the needles. When we performed a preliminary isolated luminal flow loop test, we observed leakage at the thread interface and added O-rings at these connection points. We performed preliminary TEVG mounting tests and found the TEVGs are generally 6 mm long after removal from the mandrel. These testing procedures are described in Chapter 5. The results of the preliminary TEVG mounting tests prompted two additional design changes.

The expected length of the TEVGs when being mounted into the bioreactor was 10 mm. However, the TEVGs are approximately 6 mm long after being removed from the silicone mandrel. This discrepancy in the size of the TEVGs caused the gap between the two needle mounts to be too large. Additionally, because both the mounts were stationary it was more difficult to mount the TEVG using the user's preferred method. The mounting procedure is described in Appendix C. In order to make the bioreactor easier to use, we decided to make one of the needle mounts adjustable. We also increased the length of the needle mounts. This was done to reduce the gap to 4 mm to accommodate the shorter TEVGs and to allow for a change in entrance length. Originally, the entrance length was calculated based on the Reynolds number of the TEVG. After personal communication with Prof. Brian Savilonis, we discovered the entrance length should be calculated based on the Reynolds number in the needle (2/15/2013). The new entrance length was 27 mm. The calculation is discussed further in Chapter 5.

The adjustable mount was made by creating a milled pocket in the polycarbonate support piece. The adjustable mount was machined as a separate component that could be inserted into the milled pocket and slid back and forth as needed. A stainless steel pin was used to fix the adjustable mount in place by inserting the pin through a hole in both the polycarbonate support piece and adjustable mount. Figure 23 shows the adjustable design with the adjustable mount locked in place and Figure 24 shows the adjustable mount isolated.

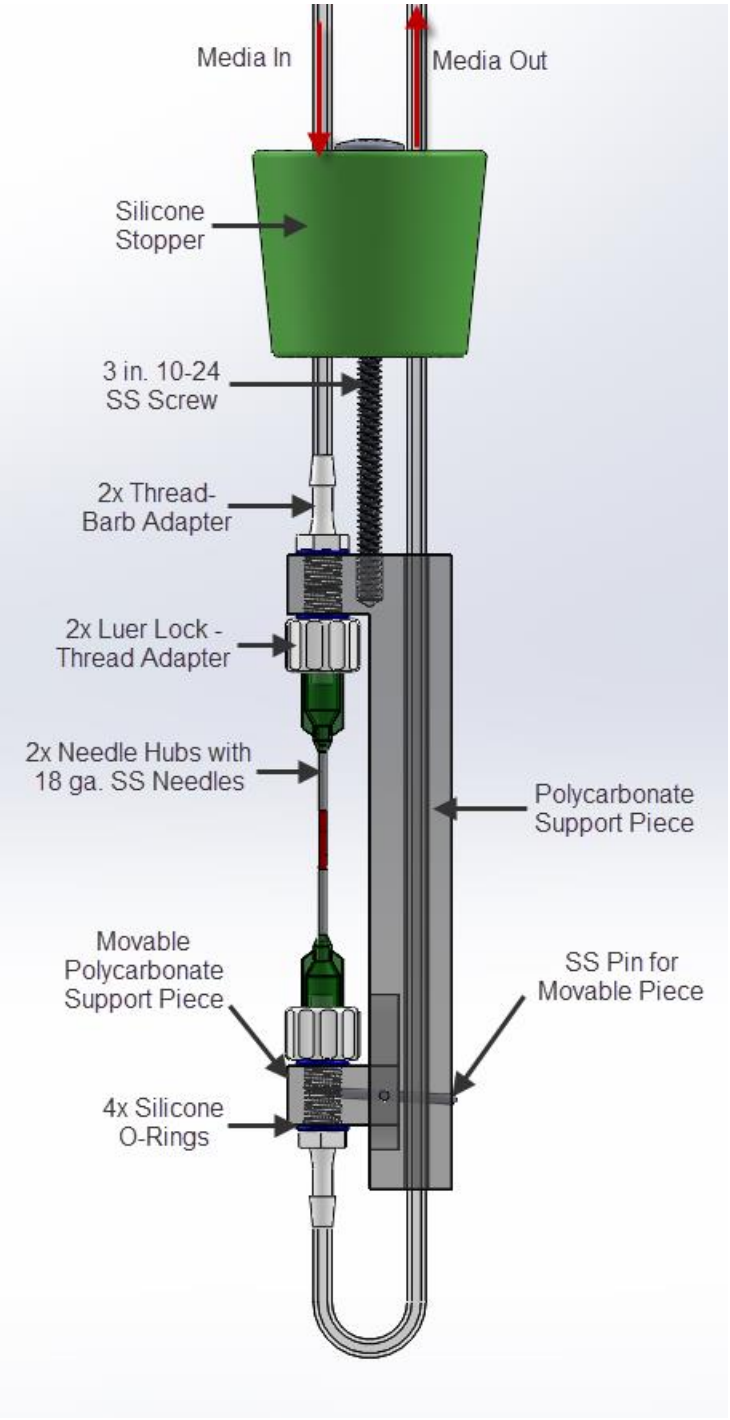

Figure 23: Adjustable bioreactor design

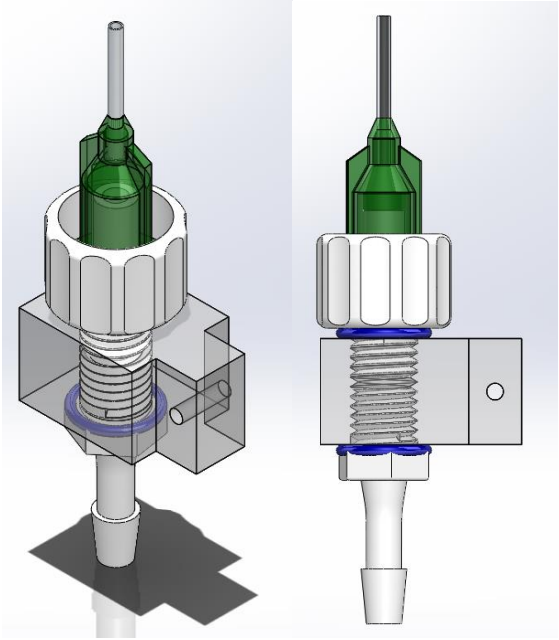

Figure 24: Adjustable mount, isometric view (left) and front view (right)

With these changes, our final design was complete. We began the process of validating the final design through a set of experiments. The experiments evaluated the success of bioreactor based on the project goals and criteria.

Chapter 5: Experimental Testing

In order to test how well our bioreactor addressed the project objectives, constraints, and functions, we experimentally evaluated the bioreactor. The testing methods and materials that were used are discussed in this chapter. These procedures were developed using a thorough literature review and discussions with our client. Of the design criteria discussed in Chapter 4, we tested and, when appropriate, statistically analyzed laminar luminal flow, isolated luminal flow loop, leakage, mounting of the tissue sample, sterility maintenance, gas exchange, and the effect of the bioreactor on the TEVG. We discuss the results of this experimentation in Chapter 6.

Assembling the bioreactor was a prerequisite for many of the experiments described in this section. A brief overview of how to assemble the bioreactor is presented here to assist in understanding the following experiments. To begin the assembly process, all components of the bioreactor need to be sterilized using an autoclave. All components should then be transported to the biosafety cabinet and assembled, after which the TEVG should be mounted into the bioreactor. The luminal flow loop should then be primed inside the biosafety cabinet, as discussed in Appendix E. The bioreactor should then be transported to the incubator with the pump placed on top. A detailed protocol for assembly is provided in Appendix D.

5.1: Laminar Flow

We tested for laminar flow using a dye test. We set up the bioreactor using the directions found in Appendix D, using a 10 mm long and 1.2 mm inner diameter silicone tube as the TEVG. The pump was set to Slow 7.5, correlating to 14.7 mL/min. A syringe filled with food dye was injected upstream of the silicone tube 40 mm before the inlet barb connector. The location was chosen so that the dye bolus would be upstream of the needle to allow laminar flow to develop. The reservoir was not attached in order to see the bolus more clearly. We determined that laminar flow would be present if the flow profile was parabolic with no eddies.

No flow pattern indicative of either laminar or turbulent flow was observed because the dye mixed with the water due to turbulence from passing through the multiple Luer connectors. Mixing also occurred from passing thorough the changing radii from needle hub to needle. This test was inconclusive since it did not show any separation of layers as seen in laminar flow, nor did it show the characteristic eddies seen in turbulent flow.

As a result, we attempted the bolus test again using olive oil instead of water using the same method as the dye test. We hypothesized that the olive oil would not mix with the water since it is more viscous than the dye. The oil was immiscible in the water, which caused the oil to fracture into smaller spheres as the oil entered the silicone tubing. The oil was also difficult to inject using the syringe because of its high viscosity, which resulted in a poor initial bolus. Moreover, the oil is less dense than the water used in the flow loop, consequently the oil rose to the top of the flow loop instead of flowing with the water.

Laminar flow was also evaluated empirically using the Reynolds number calculation. The calculated Reynolds number was 376. This calculation can be found in section 3.4 under specifications and is repeated here in Equation 7.

Equation 7: Reynolds number in TEVG, ρ is the density of the fluid, U is the velocity of the fluid, μ is the dynamic viscosity of the fluid, and D is the inner diameter of the tube.

$$\mathrm{Re} = \frac{\rho U D}{\mu} = \frac{(1000\ \frac{\mathrm{kg}}{\mathrm{m}^3})(0.217\ \frac{\mathrm{m}}{\mathrm{s}})(1.2 \times 10^{-3}\mathrm{m})}{6.92 \times 10^{-4}\mathrm{Pa} \cdot \mathrm{s}} = 376$$

Additionally, we also met with Professor Brian Savilonis, a Mechanical Engineering professor at WPI who specialized in fluid dynamics. Professor Savilonis also agreed that the bioreactor would provide laminar flow to the tissue sample in the bioreactor as long as the needles were long enough for laminar flow to develop. To achieve laminar flow, we needed to calculate the entrance length based on the Reynolds number and the velocity in the needle, which is different from the Reynolds number in the TEVG due to a difference in diameter. This calculation is shown in Equation 8.

Equation 8: Velocity of the fluid in 18 gauge needle, where U_T is the velocity of fluid in the TEVG, A_T is the cross sectional area of the TEVG, U_N is the velocity of fluid in the needle, and A_N is the cross sectional area of the needle

$$U_T A_T = U_N A_N$$

$$U_N = \frac{\left(0.217 \frac{\mathrm{m}}{\mathrm{s}}\right)(1.2 \times 10^{-3}\mathrm{m})^2}{(8.38 \times 10^{-4}\mathrm{m})^2}$$

$$U_N = 0.445 \frac{\mathrm{m}}{\mathrm{s}}$$

Using this velocity, we calculated the Reynolds number in the needle, illustrated in Equation 9, and the entrance length required to develop laminar flow in the needle, shown in Equation 10.

Equation 9: Reynolds number in 18-gauge needle, where Re_N is the Reynolds number in the needle, **ρ** is the density of the fluid, U_N is the velocity of the fluid in the needle, D_N is the inner diameter of the needle, and **μ** is the dynamic viscosity of the fluid

$$Re_N = \frac{\rho U_N D_N}{\mu}$$

$$Re_N = \frac{\left(1000 \frac{kg}{m^3}\right)\left(0.445 \frac{m}{s}\right)(8.38 \times 10^{-4}\ m)}{6.92 \times 10^{-4} Pa \cdot s}$$

$$Re_N = 539$$

Equation 10: Entrance length of needle, where L_e is the entrance length, Re_N is the Reynolds number in the needle, and D_N is the inner diameter of the needle

$$L_e = 0.06 \times Re_N \times D_N$$

$$L_e = 0.06(539)(8.38 \times 10^{-4} m)$$

$$L_e = 27\ mm$$

5.2: Isolated Flow Loop

To test if the luminal flow loop was isolated from the reservoir, we used a dye test. We assembled the bioreactor and filled the reservoir with clear deionized (DI) water. We then filled the internal flow loop with 0.01 mL red food dye per 1 mL of DI water. We ran the bioreactor for one hour, at which point we checked for the presence of red dye in the reservoir. We observed leakage into the reservoir and subsequently placed silicone O-rings at the thread interface, shown in Figure 25. We ran the bioreactor for one hour after implementing the O-rings and observed no visible leakage in the reservoir. To confirm this, we used a spectrophotometer to measure absorbance values.

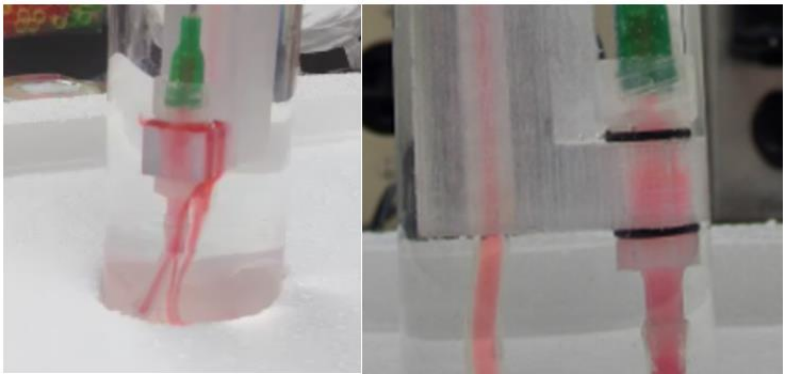

Figure 25: Leakage observed at the thread interface (left) and thread interface with O-rings (right)

Using a Jenway 6305 UV/Vis Spectrophotometer set to 530 nm, a standard curve of absorbance values was created using five samples with increasing concentration of dye. Clear DI water was used as the control. A graph of the standard curve is shown in Figure 26. The standard curve and the absorbance values of the experimental sample were used to determine the presence of food dye in the reservoir. The absorbance value of the water in the reservoir after one hour was 0.06. Using a student's t-test, we statistically compared the absorbance values of the experimental sample and the DI water control and the p-value was 0.08 and n=3. We found that the spectrophotometer used could only detect a difference in the absorbance values between the DI water control and greater than 0.002 mL dye per mL water.

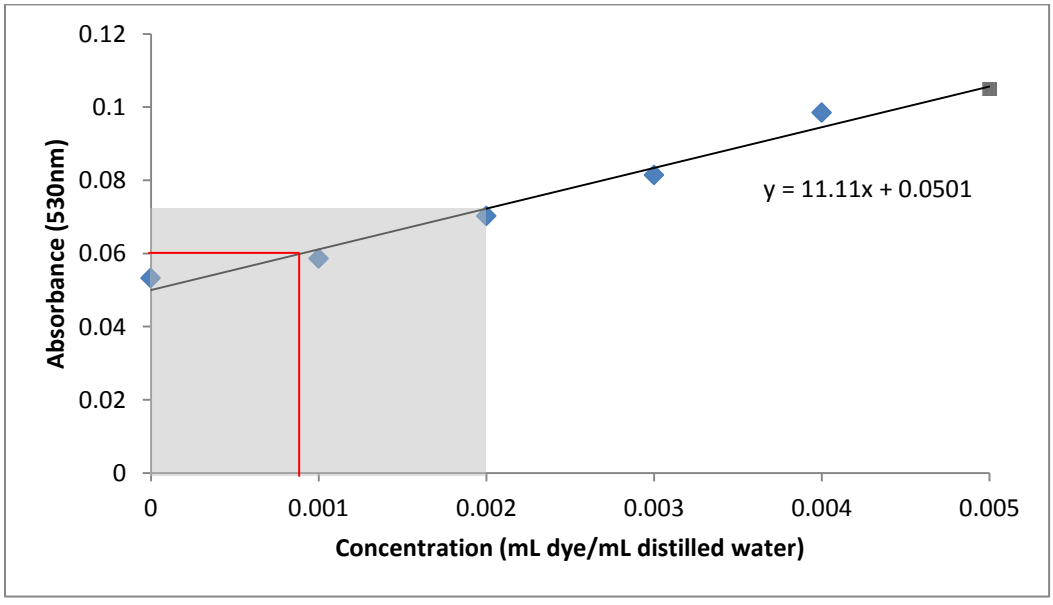

Figure 26: Standard curve of absorbance values: the grey area denotes statistically insignificant readings and the red line shows experimental value

We also used a white cloth to wipe the connectors, tubing, and lid of the bioreactor to test for the presence of red dyed DI water outside the reservoir. Red dyed DI water did not appear on the cloth.

5.3: Mounting Time

In this test, we measured the average amount of time it took to mount a silicone tube onto the needle mounts in our bioreactor. We chose to use a silicone tube instead of tissue in order to conserve tissue samples, as shown in Figure 27. This test was conducted by following the same sterile procedure we would use to mount tissue into the device, described in Appendix C.

Figure 27: A view of the polycarbonate support piece and a mounted and sutured silicone tube, circled in red

Zoe Reidinger performed the mounting procedure because she is the primary user and she used a silicone tube in order to conserve TEVGs. We timed three mounting trials in the biosafety cabinet following the procedure we developed for live TEVGs, detailed in Appendix C, using both the Cerulli *et al.* bioreactor design and our design. The collected data are shown in Table 7 and Figure 28; the Cerulli *et al.* bioreactor design took 158 ± 7 seconds to mount and our device took 133 ± 21 seconds to mount.

Table 7: Mounting time data

Trial	Cerulli *et al.* Bioreactor	Our Bioreactor
1	162	122
2	150	157
3	162	119
Average	158	133
Standard Deviation (±)	7	21

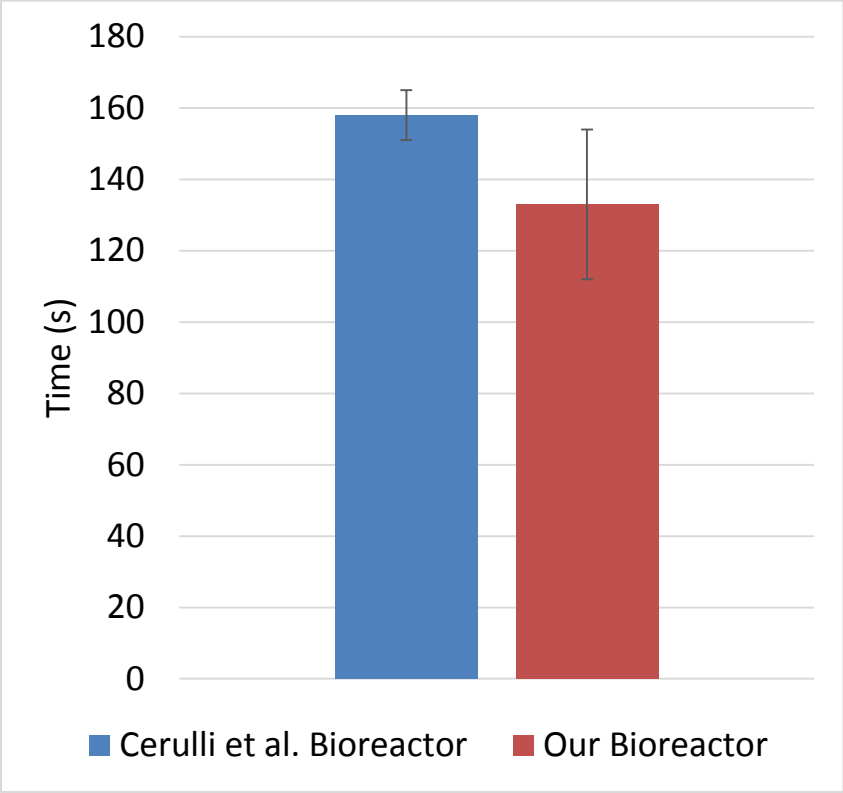

Figure 28: Mounting time

5.4: Sterility During Operation

In order to ensure that the bioreactor remains completely sterile during operation, we developed an experimental plan using microscope observations and clarity of media to evaluate microbial growth in the bioreactor. The bioreactor and other essential components were first sterilized using an autoclave for 15 minutes at 121^0C and at 2 atm, while inside an autoclave bag. Afterward, the bioreactor was placed on a piece of sterilized aluminum foil within the biosafety cabinet to maintain a sterile working space. All the components used in the experiment can be found in Table 8. The necessary tubing connections were assembled and cell culture media was added to the reservoir. Then the tubing was primed using the protocol in Appendix E. The bioreactor was then placed into the incubator. A tissue culture polystyrene petri dish was filled with media and placed in the incubator to serve as a control. The incubator setup can be seen in Figure 29.

Table 8: List of bioreactor components used in the sterility experiment

Silicone Tubing:	Connectors:	Silicone Stopper:	Miscellaneous:
Inlet section	2x Thread to barb	1x Silicone stopper	1x sheet of tinfoil
Outlet section	2x Female Luer to thread	1x 3in Stainless steel screw	1x 38 by 200mm test tube
Priming section	2x blunt ended needle		4x silicone O-rings

Figure 29: Sterility test setup

The sterility experiment was terminated prematurely when leakage was observed at the interface between the pump and silicone tubing causing the luminal flow loop to fill with air. The plastic bin containing the pump, which was located on top of the incubator, was filled with several milliliters of media, shown in Figure 30. Dried media was also present on the pump itself. Upon visual inspection, media in the bioreactor reservoir did not appear turbid indicating no contamination.

Figure 30: Leakage from sterility test

This test was repeated and we followed the same procedure that was discussed previously. In this test, all connection points remained tight and no leakage was observed. The media remained clear and not turbid throughout the four days of running the bioreactor in the incubator. We also concluded that there was no contamination in the system by observing media samples of the control petri dish as well as the bioreactor's reservoir under the microscope. No organisms were observed in the media and the media was not turbid in either sample, shown in Figure 31.

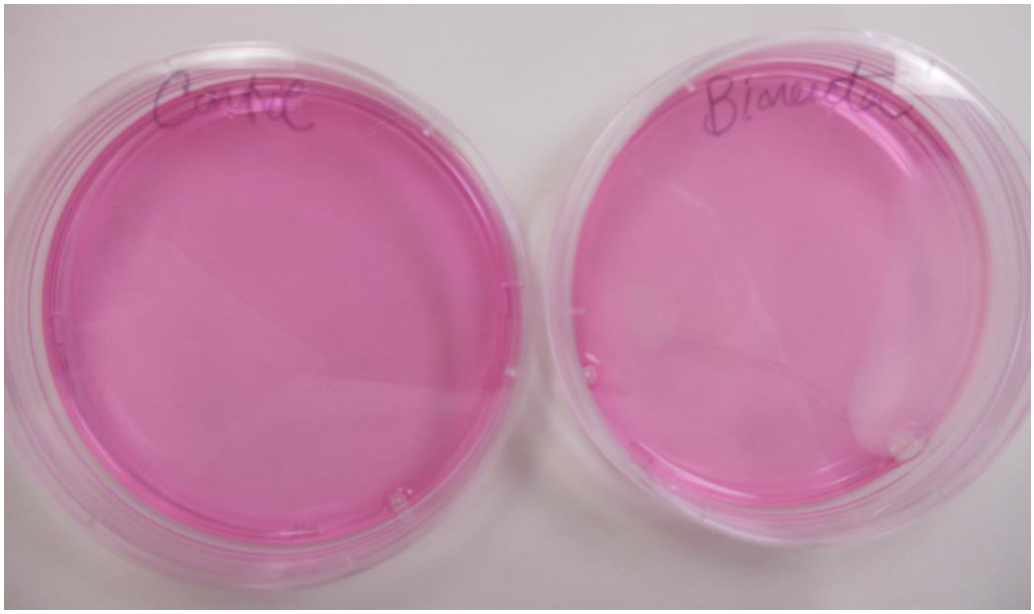

Figure 31: Media after four days in incubator, control (left) and bioreactor sample (right)

5.5: Tissue Culture

We attempted to mount TEVGs into the bioreactor on multiple occasions. The first time we attempted this test, the gap between the needles in the bioreactor was too large, and the device did not allow any adjustability. The TEVGs that were cultured for the second and third trials could not be mounted in the bioreactor because they were contaminated during static culture. The final time we attempted to mount TEVGs into the bioreactor, only two could be successfully mounted and neither stayed securely mounted throughout the experiment.

Chapter 6: Discussion and Conclusions

After performing the experiments discussed previously, we adjusted our final bioreactor design to remedy any limitations discovered. Our final design is composed of several major components; a polycarbonate support piece, a fixed mounting arm, an adjustable mounting arm, a glass test tube that serves as a reservoir, and a silicone stopper. The adjustable mounting arm can be slid along a milled pocket of the polycarbonate support piece and then fixed in place using a stainless steel pin. This arm reduces the mounting time because the needle mounts can be moved together while they are being sutured, providing support to the TEVG. Once mounted in the bioreactor, the TEVG is oriented vertically within the test tube.

The polycarbonate support piece is attached to the silicone stopper using a stainless steel screw to ensure that the system remains stationary and self-contained during operation. The seal between the stopper and the test tube prevents leakage and contamination. Both mounting arms are composed of an 18 gauge blunt needle, a female Luer-to-thread connector, and a thread-to-barb connector. This allows platinum-cured silicone tubing to be connected to each mounting arm and the TEVG to be mounted between them, forming an isolated luminal flow loop. This silicone tubing is threaded through two holes in the silicone stopper and then attached to a peristaltic pump. All materials chosen could be sterilized in an autoclave, which is the preferred method of sterilization in the laboratory. We also chose to incorporate commercial parts to increase reproducibility and allow the bioreactor to accommodate TEVGs of different sizes. The rectangular geometry of the polycarbonate support piece allowed the bioreactor to be easily machined.

Based on the testing results presented in Chapter 5, we were able to evaluate the bioreactor's performance of the objectives introduced in Chapter 3. The following sections discuss the results of the laminar flow testing, the isolated flow loop and leakage testing, the mounting efficiency of the device, and the sterility experiment. We also discuss the qualitative assessment of the bioreactor's tissue culture trials, ease of manufacturing, ability to accommodate different sized samples, ability to permit gas exchange, and material cost. We discuss the limitations of our testing and the relationship between the device and the following topics: economics, environmental impact, societal influence, political ramifications, ethical concern, health and safety issues, manufacturability, and sustainability.

6.1: Laminar Flow Testing

Due to the lack of results in the bolus test with both the dye and oil, we deemed both of these tests inconclusive. However, laminar flow through the tissue tube is still strongly supported by calculations, as discussed in section 3.4. The Reynolds number was 376 in the TEVG and 539 in the needle, indicating laminar flow. The inlet needle length allowed for fully developed laminar flow. The limitations of this analysis were that the Reynolds number calculation is for fluid moving through a rigid tube and the TEVG is compliant. However, published literature regarding biofluid mechanics uses rigid tubes as an approximation for vascular tissue (Waite & Fine, 2007). Another limitation of the laminar flow testing was that the graft was slightly larger in diameter than the needle mount. This would require laminar flow to fully develop again after passing through the needle into the TEVG. However, the discrepancy in the diameters was necessary because the needle had to fit inside the TEVG to be mounted into the bioreactor.

6.2: Isolated Flow Loop and Leakage Testing

The average absorbance value of the water in reservoir was 0.06±0.01. Since this is lower than the resolution of the spectrophotometer, 0.07±0.003, we concluded that there was no statistically significant leakage into the reservoir ($p=0.083$, $n=5$). Because of this analysis and the absence of red dyed water on the cloth, we concluded that our bioreactor did not leak. This test is limited because we only ran the bioreactor system for one hour and therefore the data do not account for leakage over a longer period.

6.3: Mounting Efficiency

Because there was no statistically significant difference between the Cerulli *et al*. bioreactor and our device, we concluded that our design did not increase the mounting time. Because the time needed to mount a TEVG on the Cerulli *et al.* bioreactor did not dehydrate the TEVG and lead to necrosis, we determined that TEVGs would not dehydrate during the mounting process into our bioreactor. A limitation of this test was that Zoe had over six months of mounting experience on the Cerulli *et al.* bioreactor. Additionally, the needles did not align perfectly in our design. This could cause an increase in mounting time. The needle misalignment and its effect on the functionality of our design are addressed further in Chapter 7.

6.4: Maintaining Sterility

We determined the device could maintain sterile over a four-day period because the media samples did not become turbid and we observed no evidence of microbial growth using the microscope.

6.5: Tissue Culture

During the tissue culture trials, we reiterated our original prototype into the final design presented in Chapter 4. The first tissue culture trial resulted in the development of the mobile arm. Because the original design was stationary, it was very difficult for the user to mount the TEVG into the bioreactor. We were also able to finalize the protocols for mounting, assembly, and priming, which can be found in Appendices C, D, and E, respectively. The next two attempts of tissue culture trials were prevented because of contamination of the grafts while they were being cultured statically. The final tissue culture period was successful, and the TEVGs were mounted in the bioreactor. Of the seven TEVGs that were cultured, only two were mounted successfully in the bioreactor. This was due to the delicate nature of the grafts and the fact they were harvested at day fourteen, the earliest they could be harvested. The first graft mounted on the bioreactor fell off the inlet needle when the pump was turned on. Upon investigating an image of the TEVG on the bioreactor before media was flowed through it, we found that it was not properly sutured on this end. The second TEVG mounted in the bioreactor suffered the same fate: it was dislodged from the needle mounts when the pump was turned on. We attributed this failure to the challenges of mounting a delicate graft. Unfortunately, we ran out of time to culture the TEVGs and could not attempt any further tissue trials.

We believe that with more TEVG samples, we could successfully mount a sample into the bioreactor. This is because the Cerulli *et al.* bioreactor was able to accommodate TEVGs for the entire duration of an experiment and it incorporated identical needles and a similar suturing process. The lack of histological tissue culture data is the greatest limitation of this project: we were unable to determine definitively the effect of our bioreactor on the TEVGs cultured in the Rolle laboratory. However, we have developed an in depth experimental plan for the tissue culture process, which is discussed further in Chapter 7 and Appendix F.

6.6: Ease of Manufacturing

Our device was easy to manufacture because there are only two parts that need to be machined at the WPI Machine Shop. Both of the parts, the main polycarbonate support piece as well as the movable mount, have a simple geometry that takes less than three hours to machine. All other parts of the bioreactor are commercially available. Using commercial parts allows damaged parts to be replaced easily, increases the reproducibility of the bioreactor, and decreases the time and costs associated with custom made parts.

6.7: Various Sized Sample Accommodation

The bioreactor was able to accommodate different sized samples by incorporating interchangeable needle hubs and the ability to machine different sized polycarbonate support pieces. The outlet needle can be cut to varying lengths to accommodate variations in tissue length. However, the inlet needle cannot be cut shorter than 27 mm to allow laminar flow to fully develop.

6.8: Gas Exchange

Past research shows that the surface area of 3 meters of gas-permeable silicone tubing in the isolated flow loop will allow sufficient gas exchange (Radisic *et al.*, 2006). Our bioreactor design incorporates this necessary length.

6.9: Cost Analysis

Using one stopper, one test tube, two thread-barb Luer connectors, two thread-male Luer connectors, two needle hub-needle connectors, four O-rings and the necessary polycarbonate, a single bioreactor assembly costs $18.18. This tabulated cost can be seen in Appendix. This is less than the estimated cost of the previous bioreactor of $23.56. Our bioreactor also creates much less polycarbonate waste as it requires a very small amount of milling as compared to the large milled reservoir and lid of the previous bioreactor. This cost can be further reduced by buying parts in bulk, or by selecting a different stopper. This cost does not reflect the tubing costs of either bioreactor as both bioreactor assemblies should use roughly the same amount of tubing.

6.10: Impact of Device

The overall purpose of the following sections is to discuss the implications of manufacturing this bioreactor design to the following areas: economics, the environment, society, politics, ethics, health and safety, manufacturing, and sustainable production.

6.10.1: Economics

Our bioreactor for TEVGs is the next step towards having an off-the-shelf vascular graft for use in medical procedures, which may reduce the time associated with finding a donor graft for use in surgeries. Compared to autologous grafts, the patient would only need to undergo one surgery; therefore, a TEVG would save him or her the cost of the secondary surgery. Our bioreactor is easy to manufacture, with few waste products and minimal labor. The use of this device will decrease the culturing time because grafts will achieve necessary mechanical strength in a shorter period. This will save these patients time and money. The device is also inexpensive to machine and produce, having a final build cost of $18.18 per bioreactor. The main cost of the device derives from the cell culture media required by the bioreactor to properly culture the TEVG. The bioreactor is a feasible method for growing three-dimensional constructs in the future.

6.10.2: Environmental Impact

Few waste products are generated in the manufacturing of our bioreactor because commercial parts are used and the device can be re-used multiple times. The only electricity required to run the bioreactor is for the pump, which could easily be solar powered in the future to decrease its environmental impact. This device does not produce any toxic chemicals that can negatively affect the environment. Polycarbonate is thermoplastic and can be recycled indefinitely (Müller & Rises, 1992).

6.10.3: Societal Influence

Our bioreactor, as well as tissue engineering in general, may one day play a role in improving the quality of life and extending the lifespan of countless people around the world. As tissue engineering becomes more advanced and standardized, tissue engineered organs and organ systems may one day become available to the general populace. Although the bioreactor is scaled for use in simulating the *in*

vivo environment of a rat carotid artery, it could be scaled up for use in human-sized veins and arteries in the future.

6.10.4: Political Ramifications

Because of controversy regarding the ethical use of cells and cell-derived product, the government regulates the use of these products. As a result, this could limit the progression of tissue engineering and eliminate the need for a bioreactor.

6.10.5: Ethical Concern

The use of cells and all cell-derived products used in our device may anger groups such as People for the Ethical Treatment of Animals (PETA) because animals can be harmed in the creation of the tissue. In the future, our bioreactor may aid in increasing the quality of life and lifespan of patients in need or a surgery involving a vascular graft. In addition, the patient will not need a second surgery to retrieve this type of graft or need a donor, and the needed graft can be pulled off the shelf or cultured using the patient's own tissue.

6.10.6: Health and Safety Issues

This bioreactor is safe for the users of the device. No harmful chemical byproducts are produced from the device. Standard procedures for working with blood-borne pathogens should be followed when using any biological tissue in the device. Currently, the TEVGs produced using our bioreactor are not suitable for implantation and therefore are not a major health or safety concern at this time. If the TEVGs were to be implanted then it would need to follow all the regulations set by the FDA so that it does not harm the patient. Because the bioreactor is designed to prevent leakage, it is safe for the user.

6.10.7: Manufacturability

Our bioreactor can be reproduced easily. The only part that needs to be machined is the support piece that holds the tubes in place. Due to its simple geometry, it is easy to manufacture. The machining shop at WPI completed this in less than a week. The other parts of our design are commercially available and just need to be adjusted slightly to fit together.

6.10.8: Sustainability

Our device is made of polycarbonate and polypropylene, which can be autoclaved. Since these materials can be autoclaved, the device can be used many times. It requires very little electricity to run as only the pump uses energy, and there are few non-biodegradable waste products.

Chapter 7: Conclusions and Future Recommendations

To create a luminal laminar bioreactor that aided in maturing TEVGs, we developed design criteria with information gathered through a literature review and client meetings. We organized our criteria into constraints and objectives. The constraints narrowed our design space and stated what our device *must* accomplish. The constraints of the design were that is provide luminal flow, securely mount the tissue sample, permit gas exchange, fit inside incubator, be sterilizable, be within a $496 budget, and be completed within the 28-week time frame. The objectives are defined has characteristics our clients *want* our device to accomplish. The objectives include providing laminar flow, preventing leakage, an isolated flow loop, remaining sterile, limiting mounting time, easy to machine, accommodating different samples, and cost effective. The device that we created effectively met the design criteria that we established at the start of the project, and we believe that this Major Qualifying Project was a success. In this chapter, we discuss the major successes and shortcomings of this bioreactor and suggest future redesigns.

7.1: Comparison to Cerulli *et al.* Bioreactor and Recommendations for Future Designs

We produced a bioreactor that is functional and significant to the academic research that is conducted at WPI. Experiments with the TEVGs cultured in Professor Marsha Rolle's laboratory can be performed in a controllable *in vitro* environment. Currently, the Rolle laboratory uses the Cerulli *et al.* bioreactor, described in Chapter 2. Although this bioreactor provides luminal flow, it cannot ensure laminar flow.

The Cerulli *et al.* bioreactor may not have been exposing the tissue samples to the correct amount of shear stress. The needle lengths were too short to account for the entrance length needed to develop laminar flow, and in turn, a constant shear stress on the wall of the tissue engineered vascular graft. Our bioreactor accounts for this entrance length, ensuring that the desired shear stress is applied to the lumen of the TEVG. Although these needles are now the correct length for the development of laminar flow, any error in manufacturing of the threads designed to hold the Luer lock pipe adapters creates a noticeable error at the meeting point between the two needles due to the small scale of the bioreactor system. To remedy this, we suggest that the next iteration of the device is created using an automated system to prevent misalignment due to human error.

Our bioreactor will not allow leakage at any point outside of the test tube reservoir as our design contains all connection points within the reservoir. The Cerulli *et al.* bioreactor design allows for leakage out of the reservoir, while our design eliminates all possible leakage into an incubator environment.

The Cerulli *et al.* bioreactor can accommodate TEVGs of varying lengths by sliding the adjustable arm back and forth along the magnetic track. This allows many different length samples to be mounted in the bioreactor. Although our design does involve an adjustable mount, it is only designed to accommodate one length sample. This can be somewhat overcome by using different length needle; however it does not allow the same degree of adjustability as the Cerulli *et al*. bioreactor. We propose a reiteration of our final design involving multiple holes for adjustment. This design is shown in Figure 32. This would allow a larger variety of TEVGs to be used in our bioreactor, but would not allow for as much instability as the magnetic mobile arm used in the Cerulli *et al.* design.

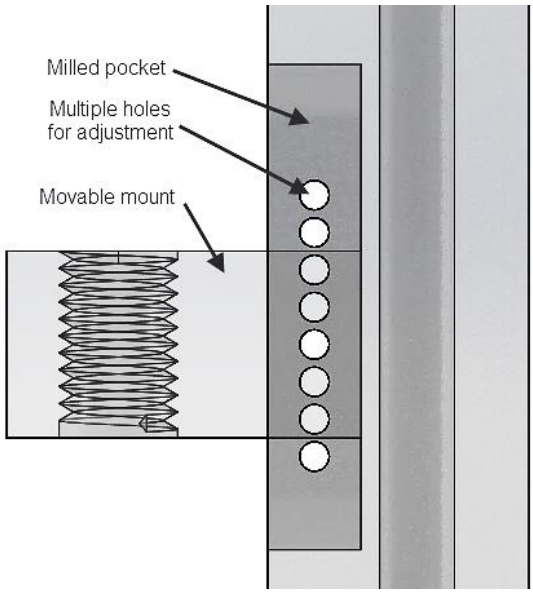

Figure 32: A close-up view of the adjustable design with additional holes to allow for more adjustment, created in SolidWorks.

In the Cerulli *et al.* device, the movable arm could be moved side to side, which could result in damage to the tissue sample or an interruption of laminar flow. Our device manages to be adjusted along only one axis with no possibility of rotation. Because the movable arm in our design must slide inside a milled pocket, the connection between this arm and the rest of the polycarbonate is loose. This could cause damage to the sample. Further testing is needed, but the team has redesigned the movable mount in order to make it more stable. Figure 33 illustrates this new design. The square shape of the main polycarbonate support piece fits within the square hole of the movable mount. This will allow the mount to move in only one direction with no possibility of tipping or rotating. This will create a more stable environment for the TEVG and remove the risk of breaking the TEVG while adjusting the movable mount.

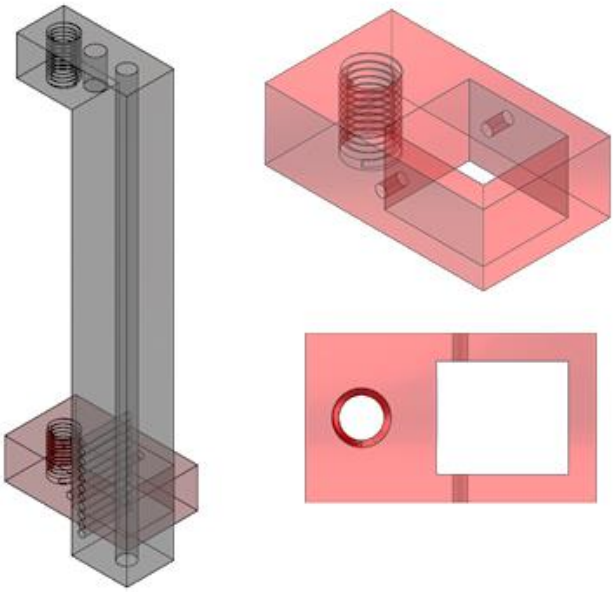

Figure 33 A SolidWorks model of the stable adjustable design: assembly (L) isometric view of movable mount (TR) top view of movable mount (BR)

A major drawback of our device is the silicone stopper. We ordered a four-gauge piercing needle to create holes in the stopper for the inlet and outlet tubing as well as the stainless steel screw. Not only is this process not reproducible, but it is time consuming and difficult. In the future, a polycarbonate cap that rests on the mouth of the test tube reservoir would be an improvement over our silicone stopper solution. A possible design for this cap is shown in Figure 34.

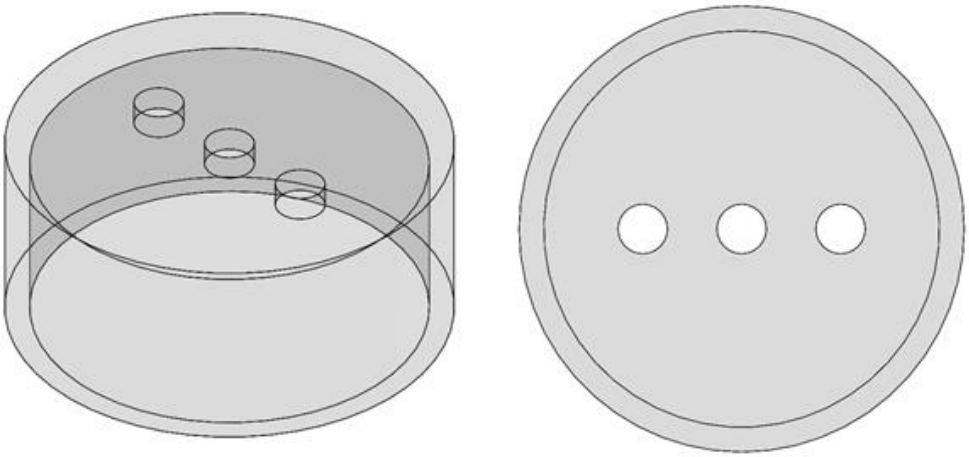

Figure 34: A possible design of the polycarbonate cap

Currently, our bioreactor requires nearly 100 mL of complete media for one tissue sample. We suggest that the device be scaled down in order to fit in a smaller container and still provide luminal, laminar flow to a TEVG. This would decrease the running cost of the bioreactor and concurrently allow improved transportation from the biosafety cabinet to the incubator.

We recommend that the Rolle laboratory use our bioreactor instead of the Cerulli *et al.* bioreactor for their continuing research involving TEVGs. The bioreactor should be tested further using recommendations presented in this chapter. After making the proposed changes to the design, we believe it would completely fulfill the requirements of the Rolle laboratory.

7.2: Recommendations for Future Testing

We recommend the following tissue culture experiments to determine the effect of the bioreactor on TEVGs. The TEVGs should be cultured statically using the direct cell seeding method and harvested at 14-18 days. Two samples should be fixed immediately as the control, two samples should be mounted in the bioreactor without flow, and two samples should be mounted in the bioreactors with flow. The protocols for mounting the samples in the bioreactor and priming the pump are found in Protocol and Appendix E, respectively. The samples should be removed and fixed after 48 hours. One sample from each test group should be embedded in paraffin vertically in order to obtain luminal cross-sections, and one sample from each test group should be embedded in paraffin in such a way to observe the luminal cell alignment. H&E staining can be used to observe changes in the luminal diameter and cell alignment in the direction of media flow. Smooth muscle α-actin staining can be used to assess expression of actin in the lumen of the TEVG. Smooth muscle α-actin is an indicator of SMC behavior. We hypothesize we would see no change in the luminal diameter between the control sample and the sample cultured in the bioreactor under dynamic flow conditions (Fitzgerald *et al.*, 2009). We expect to see increased cell alignment to the direction of flow on the lumen of the TEVGs cultured in the bioreactor when compared to the control and statically cultured samples (Fitzgerald *et al.*, 2009). Finally, we anticipate a decrease in smooth muscle α-actin in the cells that were exposed to flow as they take on a more EC-like phenotype than SMC-like phenotype (Wang *et al.*, 2006). The procedures for all tissue culture experimentation and preparation are found in Appendix F.

7.3: Conclusion

A luminal laminar flow bioreactor is an important aspect of the tissue engineering process of vascular grafts. Because TEVGs have the potential to be vasoactive, non-immunogenic, customizable, and have an off-the-shelf availability, they could be a better option for many patients undergoing CABG surgery and dialysis. However, because the current technology is limited by the culturing period and inconsistency with native tissue, a bioreactor is necessary. In this report, we determined our design criteria, developed design alternatives, then manufactured and validated our final design. Our bioreactor design successfully incorporated an isolated luminal flow loop, remained sterile, provided laminar flow, was easy to machine, and could incorporate different sized samples. In the future, we recommend the mobile arm to be redesigned in order to increase needle mount stability and decrease mounting time. Moreover, the silicone cap should be replaced with a more reproducible and cost effective polycarbonate cap. To determine the effect of our bioreactor on TEVGs, we developed a tissue culture protocol to assist in future testing. After making the proposed changes to the design and conducting further experiments, we believe it would completely fulfill the needs of the TEVGs cultured in the Rolle laboratory.

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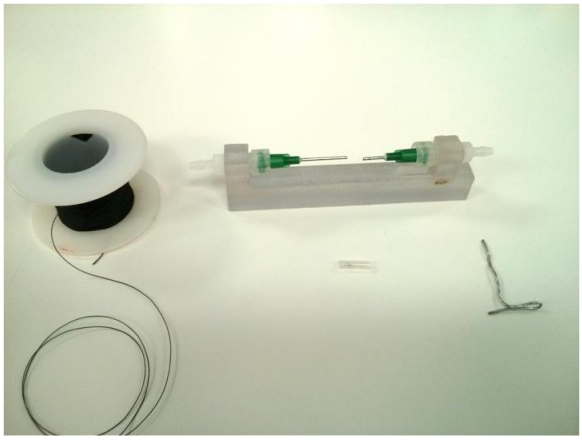
Appendix A

	Isolated flow loop	Remain sterile	Limit mounting time	Easy to machine	Laminar flow	Accommodate different sized samples	Cost Efficient	Prevent Leakage
Isolated flow loop	X	1	1	0	1	0	0	1
Remain sterile	0	X	0	0	1	0	0	1
Limit mounting	0	1	X	0	1	0	0	1
Easy to machine	1	1	1	X	1	0	0	1
Laminar flow	0	0	0	0	X	0	0	0
Accommodate different sized samples	1	1	1	1	1	X	0	1
Cost efficient	1	1	1	1	1	1	X	1
Prevent leakage	0	0	0	0	1	0	0	X
Total	**3**	**5**	**4**	**2**	**7**	**1**	**0**	**6**

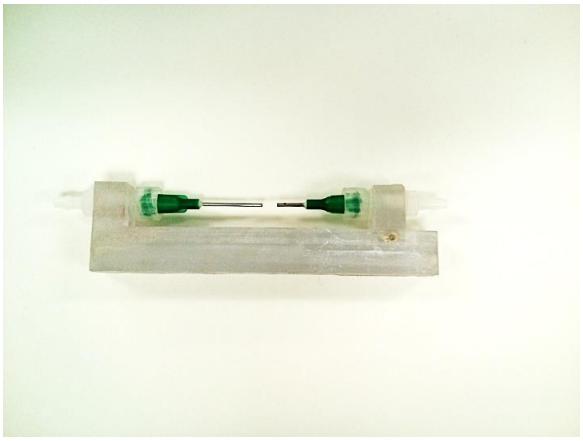
Appendix B

Design Constraints			Plunger Design	Cross-flow Design	Jar Design	Drawer Design
Cannot cost more than $496			Y	Y	Y	Y
Must be able to fit in incubator			Y	Y	Y	Y
Provide laminar flow through lumen			Y	Y	Y	Y
Permit gas exchange			Y	Y	Y	Y
Sterilizable			Y	Y	Y	Y
Not leak			N/A	N/A	N/A	N/A
Design Objectives	**Weight (%)**		**Weighted Score**	**Weighted Score**	**Weighted Score**	**Weighted Score**
Able to flow media	40	Have laminar flow	38.4	38.4	32	38.4
		Have pulsatile flow				
		Able to flow cell media through lumen				
		Able to include isolated flow loops for samples				
Able to house tissue sample(s)	30	Able to securely mount samples	15	15	13	9.6
		able to accommodate different sample sizes				
		able to accommodate multiple samples in a single experiment				
Manufacturable	10	Cost effective	6	6.9	6.5	6.9
		Have a simple design				
		Able to use commercial parts as alternatives				
		Have materials that will maintain its properties after repeated sterilization				
Easy to use	20	Can be operated by an inexperienced user	16	17	19	17
		Take five minutes or less to mount samples				
Total:	100		75.4	77.3	70.5	71.9

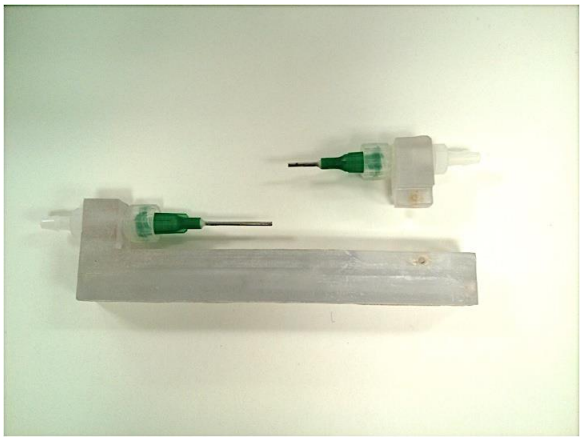
Appendix C



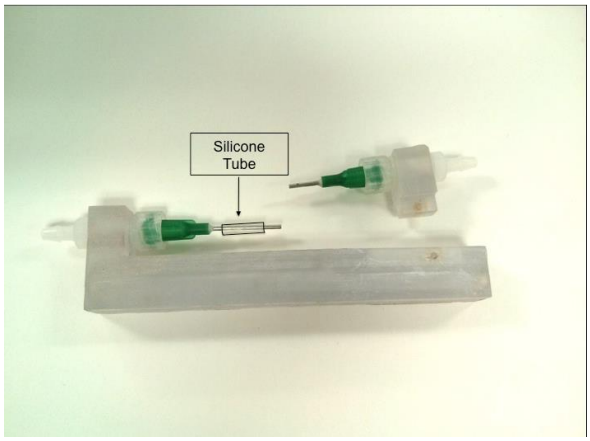
1. Gather the sterile sutures, sterile tissue tube (silicone tube for clarity), stainless steel pin, and the polycarbonate support piece assembly



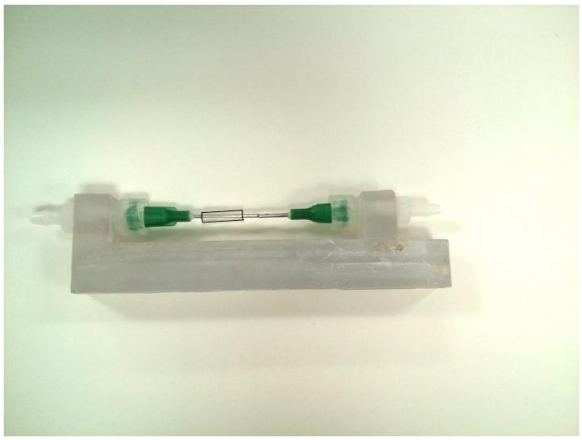
2. The adjustable polycarbonate arm is easily removed from the main support piece



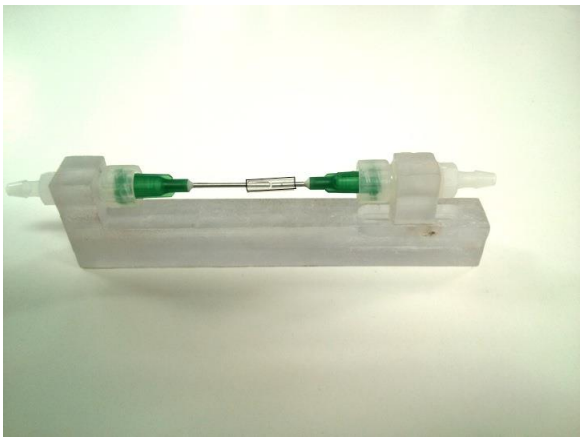
3. The adjustable arm is removed in order to facilitate mounting



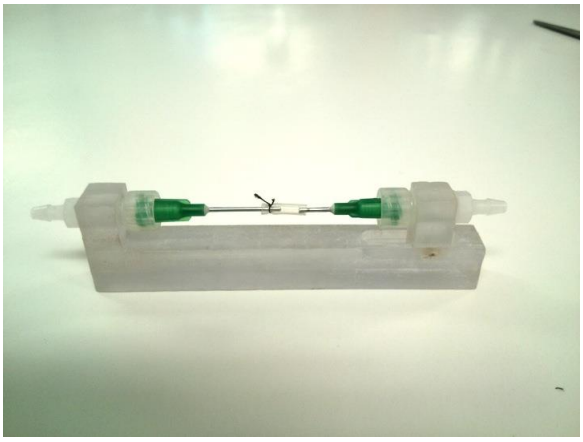
4. The tube is slid onto the longer, static needle mount and the adjustable arm is reinserted into the milled pocket



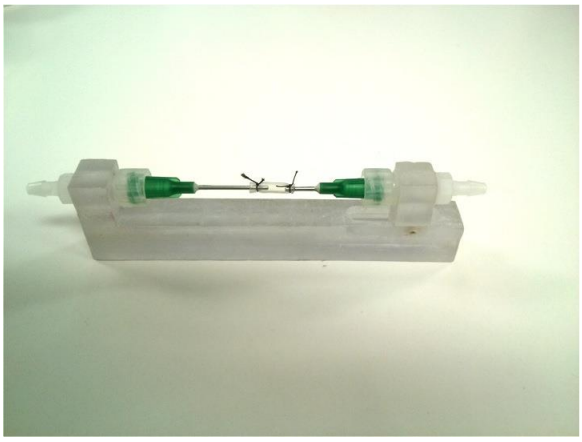
5. The adjustable arm is slid in the milled pocket to aid the needles in meeting



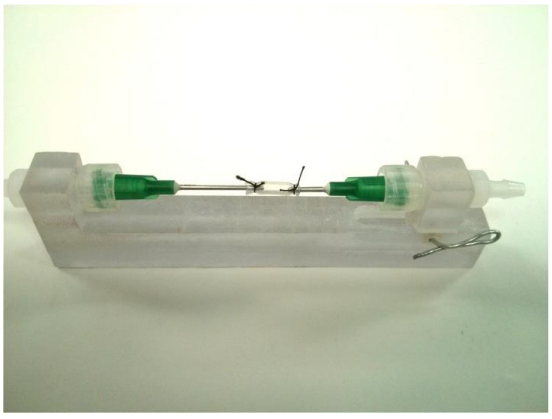
6. The tube is then slid so it rests on both needles



7. The tube can be sutured to one needle



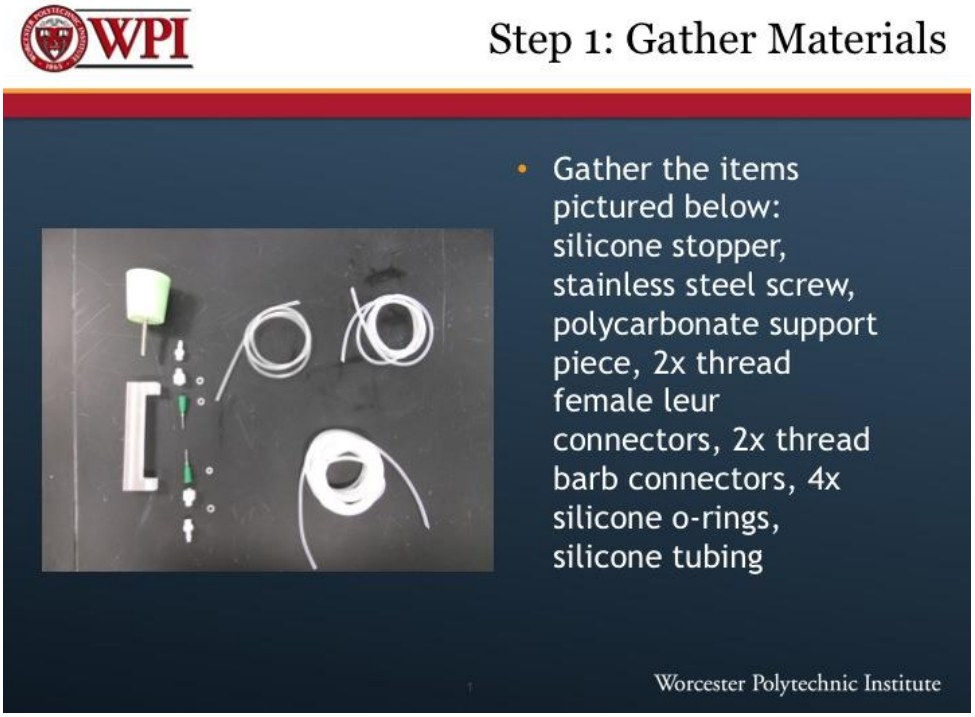
8. The tube can then be sutured completely

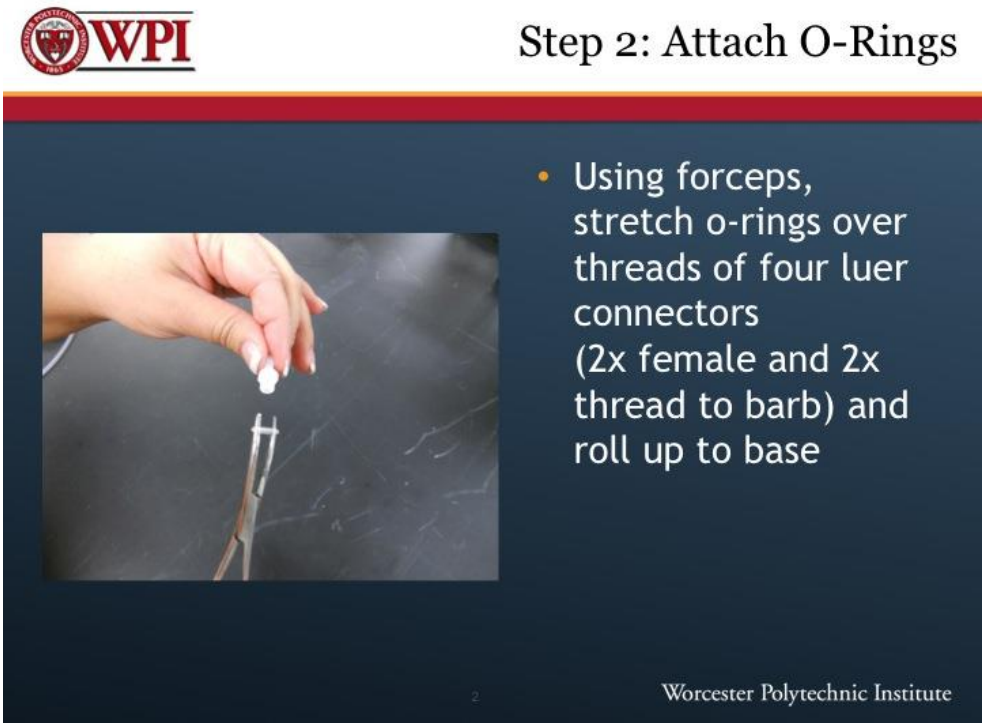


9. The stainless steel pin is reinserted for stability

Appendix D

Note: This protocol was created with the non-adjustable iteration. However, when using the adjustable iteration, insert the pin into support piece before beginning in order to use this protocol.





Step 3: Screw in Pipe Adapters

- Screw luer connectors into polycarbonate support piece:
- Screw 2 x female luer – thread on inner sides of piece
- Screw 2 x thread – barb on outer sides of piece

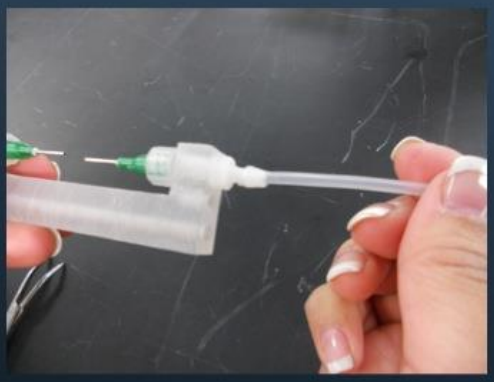
3 Worcester Polytechnic Institute

Step 4: Attach Needle Hubs

- Add needles and hubs to female luer connectors

4 Worcester Polytechnic Institute

Step 5: Attach First Tubing Segment



- Attach silicone tubing to top barb of support piece

5

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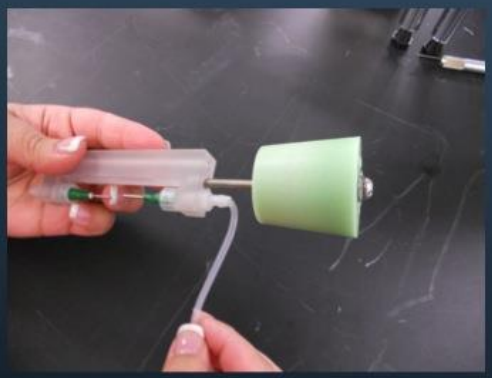
Step 6: Attach Stopper and Screw



- Push screw through middle hole of stopper and screw into middle hole on top of support piece

6

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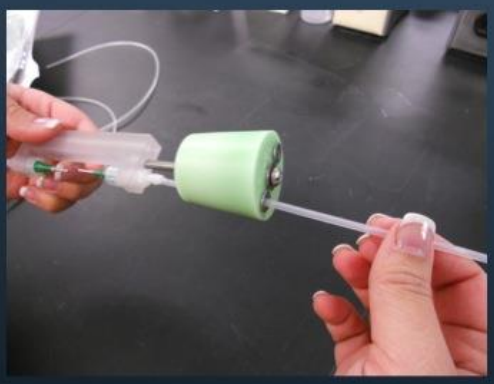
Step 7: Thread Tube Through Stopper



- Thread top silicone tubing through stopper

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Step 8: Thread Second Tube Segment

- Feed second piece of silicone tubing through other hole in silicone stopper and feed tubing through hole in support piece

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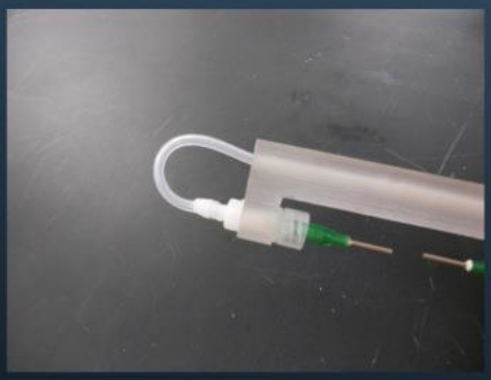
Step 9: Attach Second Tube Segment



- Loop silicone tube making sure there are no occlusions and attach to bottom barb of support piece

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Appendix E

Isolated Flow Loop Priming Protocol

Materials

- Assembled pump with TEVG mounted (Steps 1 and 2)
- 20mL of larger sterile container for priming media
- Rubber stopper with two holes for tubing
- 8 to 10 inches of tubing for the priming section
- 2 sterile clamps for the silicone tubing
- Pump
- Pump tube segments with two barb to barb connectors attached

Procedure

This procedure must be performed after the reservoir has been filled with 95 mL of media and the TEVG has been mounted.

1. Assemble bioreactor as shown in Appendix D- Bioreactor assembly.
2. Mount TEVG.
3. Insert assembled bioreactor with TEVG into glass test tube with media. Set aside.
4. Dispense 15mL of premade media into a sterile container.
5. Feed inlet and outlet tubes attached to the bioreactor through a two holed rubber stopper.
6. Insert the inlet port to the bioreactor into the sterile container with media.
7. Attach the outlet port of the bioreactor to the inlet port of the pump
8. Attach a priming segment to the outlet of the pump with the other end free on top of a sterile surface.
9. Set the pump to “Forward” and the speed setting to “Prime/Purge”
10. Allow media to fill all sections of tubing until half of the priming section if full.
11. Turn of pump.
12. Place clamps near the ends of the inlet and outlet tubes of the bioreactor.
13. Detach the priming segment.
14. Attach the bioreactor inlet segment to the pump outlet and remove clamps. You should now have a closed loop system with no air bubbles.
15. Remove the pump segment with two barb-to-barb connectors from the pump keeping all connection points intact.

16. Place the pump on top of the incubator
17. Feed the tubing and stopper through the hole at the back of the incubator
18. Place pump segment back peristaltic pump.

Appendix F

Tissue Culture Experimental Protocol

- Gas exchange
    - What testing results and statistical analyses mean to the bioreactor
    - Which design criteria it addresses
    - How well this aspect of the device meets the criteria

Before using the bioreactor for any tissue culture, it needs to be sterilized through autoclave the day before the experiment. Steps on how to sterilize the bioreactor are in the section below.

Part 1. Sterilization

Materials: For one bioreactor and one static control

- Autoclave bag at least 7" by 12", or enough aluminum foil to completely wrap all the materials
- Every piece of the bioreactor
    - Support piece
    - 2 thread to barb connectors
    - 2 male Luer to thread connectors
    - 4 female Luer to needle mounts
- 2 for bioreactor
- 2 for static control
    - 4 silicone O-rings
    - Silicone stopper
    - Stainless steel screw
    - 2 test tubes
    - Silicone tubing- 10 ft.
    - Silicone tubing- length needed to reach pump through bioreactor
    - Silicone tubing that goes in pump
    - PDMS support piece for static control
- Aluminum foil 6" x 4"(To prevent contamination of the bioreactor when mounting sample)
- Two forceps
- Time needed: at least 12 hours

Put together the bioreactor if necessary. Place everything inside the autoclave bag or completely cover with foil, and seal it. Make sure that the autoclave has enough water to run the cycle. Place the sealed autoclave bag in to the autoclave and run one cycle. Remove the bag from the autoclave and let it cool for at least 12 hours. Only open the bag inside a well-ventilated biosafety cabinet.

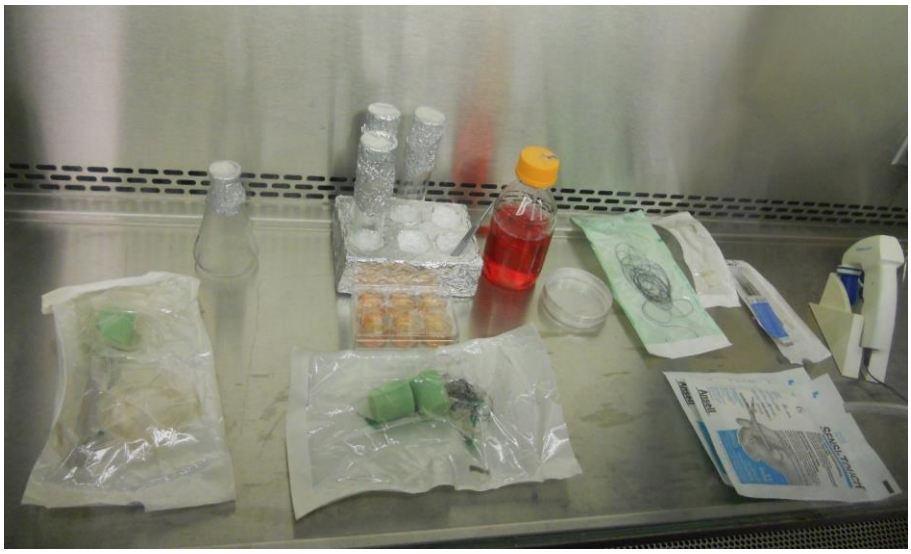
Part 2. Biosafety cabinet setup

In addition to sterilization, the biosafety cabinet needs to be setup before performing the experiment.

1. Gather all materials needed for the experiment:
    a. Latex gloves
    b. Kim wipes
    c. 70% ethanol
    d. Pipette aid
    e. Pipettes
    f. Sterile Sutures
    g. Forceps
    h. Media, warmed to 37˚C in bath
        - Media required for the culturing of TEVGs as of 2/13/13 is high glucose, DMEM with 10% fetal bovine serum, 1% Pen-Strep, 1% glutamax, 1% nonessential amino acids, 1% sodium pyruvate
    i. Styrofoam tube rack for bioreactor glass tube
    j. Pump
    k. Cooled Autoclave bag with bioreactor, forceps, aluminum foil and silicone tubing
    l. Sterile latex gloves
    m. Tissue tubes
        - Only removed from incubator when needed for mounting
2. Turn on hood and make sure that the glass window is raised to the height marked.
3. Put on non-sterile gloves
4. Clean the hood walls, surface, and vacuum tube with 70% ethanol.
5. Spray everything with 70% ethanol before placing in the hood including your gloved hands and excluding the tissue tubes.
6. Open autoclave bag inside hood

7. The placement of each object in the hood will aid in making everything easier. Figure below depicts the orientation recommended.

   a. Pipette aid, pipettes, forceps, and sutures in front to the right
   b. Media in the back right
   c. Tube rack with in the rear middle with glass tube
   d. Bioreactor and tissue tubes in the center. **Do not place bioreactor directly on hood surface, place bioreactor on sterile aluminum and glass tube in the tube rack.



8. Replace non-sterile gloves with sterile gloves
9. Slide tissue sample off the silicone mandrel onto needle mount of mobile arm. Suture tube onto needle using forceps and silk sutures, as shown in Appendix C.
10. Place glass tube into stand and fill with 95 mL of cell media
11. Put support piece connected to stopper into glass tube
12. Prime the interior flow loop with the pump, and clamp each end with ratchet clamps as described in Appendix E.
13. Place bioreactor in incubator (37C, 5% CO_2) with silicone tubing running out the back to the pump, which is placed on top of the incubator.
14. Close incubator door and run pump forward on speed Slow 7.5.
15. Place two other tissue samples in separate petri dishes filled with media to serve as controls.
16. Place these petri dishes in the incubator.
17. Fix one tissue sample at this point with 10% neutral buffered formalin for 30 minutes.
18. Vertically embed the sample in paraffin.

19. Run bioreactor for 48 hours.
20. Perform staining.

Appendix G

Estimated Cost of Bioreactor	
Item	**Cost (USD)**
1 Stopper	6.38
2 male Luer to thread adapters	1.15
1 glass tube	4.00
2 barbed fittings	3.45
2 Luer to needle mounts	0.48
1 x .5 x 4 in. polycarbonate	1.28
3 in. stainless steel screw	1.18
4 O-rings	0.26
Total:	**18.18**

Estimated Cost of Cerulli *et al.* Bioreactor	
Item	**Cost (USD)**
Polycarbonate*	20.12
2 male Luer to thread adapters	1.15
1 thread to barb connector	1.15
Female Luer to thread barb	0.40
2 female Luer to needle mounts	0.48
Neodymium magnet	0.27
Steel bar	Unknown
Total:	**~23.56**

*Previous Bioreactor Dimensions + .25 in per Dimension for Manufacturing			
Part	**Dimensions (mm)**	**mm^3**	**in^3**
Reservoir	133.35	206128.76	12.58
	46.35		
	33.35		
Top	147.35	292441.49	17.84
	61.35		
	32.35		
Mobile arm	27.35	16216.43	0.99
	24.35		
	24.35		
		Total Size:	**31.41**
		Cost:	**20.12**