Design of a perfusable vascularized leaf scaffold housing device

A Major Qualifying Project Report Submitted to the Faculty of Worcester Polytechnic Institute

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Abstract

There are many challenges with the use of engineered skin substitutes including their high cost, fragility, and long culture time. However, the one feature that is missing from many of these substitutes is that there is limited vasculature. George Pins' lab is currently researching the use of decellularized spinach leaves to serve as skin substitutes with its already built-in vascular network. The device currently used for culturing skin cells on the leaves, although effective, has flaws such as its ability to perfuse the scaffold vasculature and to be assembled quickly. The goal of this project is to design and develop a sterile housing device which allows for imaging, user friendliness, and perfusion. Testing will show proof of concept. Results suggest our device to be a viable tool for user friendliness and bilayered cell seeding during perfusion.

1. Introduction

Skin substitutes have been used since the 1880's, starting with cotton wool over gauze (Alrubaiy et. al 2009). Over time, skin substitutes have evolved into cellular or acellular substitutes. Despite the different compositions, most substitutes lack a built-in vascular network. The Pins lab is developing a substitute made out of a decellularized spinach leaf. The decellularized spinach leaves are cultured on both sides with keratinocytes and fibroblasts, eventually leading to skin regeneration (English et. al 2019).

There are various cell culture devices for these substitutes. Many substitutes are grown in tissue culture dishes but in Pins lab, a polycarbonate housing device was utilized. The device features an air-liquid interface along with access to both sides of the leaf. While this device has been used since 2010, it still has limitations (Bush and Pins, et. al 2010). For example, the device was complicated to assemble because there were many parts. As a result, it had a long assembly time. One additional feature that would increase the functionality of the device is the ability to perfuse a liquid into the leaf stem while in the device. This would mimic blood going through microvasculature.

The device would need to be user friendly (shorter assembly time) and have perfusion capabilities. This would give access to the stem and enable liquid to be perfused throughout the leaf. Additionally, the device needs to have autoclaving and imaging capabilities to allow for sterility, clear imaging and enable real-time viewing of samples on fluorescent microscopes. The objective for this device is to meet the aforementioned criteria. The device can save time, allowing for mass production of spinach leaves supporting the growth of human engineered skin.

2. Literature Review

2.1 Clinical Need

It was reported that nearly 500,000 people in the United States sought medical attention for their burn wounds in 2015. Of those 500,000, 40,000 people were hospitalized due to severe burn injuries. It was estimated that the USA spent approximately $7.9 billion on hospital burn care treatments and emergency room visits (Browning et. al 2019). In 2017, 50,000 people were hospitalized for their burn wounds. Subsequently, the United States lost nearly $1.2 billion because of the workforce loss in the country (due to burn injuries) (Carter et. al 2022). As demonstrated, this is a prevalent issue that continues to grow every year.

2.1.1 Wound Healing Process of Burn Wounds

There are three phases for burn wound healing: inflammation, proliferation and maturation. Inflammation begins immediately after the burn wound. Histamine is released which increases blood pressure and capillary permeability. The kinin system is also activated, this increases the arachidonic acid cascade (Abazari et. al 2022). Molecules such as thromboxane A2, prostaglandins and prostacyclins proliferate within the system. This causes fluid formation, a low blood supply and vasoconstriction in the burned tissue. Mast cells secrete chemoattractants which activate the migration of leukocytes to the burn wound. All these factors help remove debris and toxins from the injury (Abazari et. al 2022).

Proliferation is the second phase of burn wound healing and occurs four days after the injury. Once the inflammation decreases, reepithelialization of the burn wound begins with the differentiation/migration of keratinocytes. Keratinocytes will proliferate to form a granulation tissue barrier between the environment and the injury (Abazari et. al 2022). Fibroblasts also proliferate deeper in the wound and create collagen. The collagen is a scaffold that aids in the production and migration of extracellular matrices and fibroblasts. New blood vessels form due to growth factors (ex: fibroblast growth factor) that activate endothelial cells. After formation, the maturation phase commences (Abazari et. al 2022).

The maturation phase is the last phase of burn wound healing. Depending on the severity of the wound, it can last between a few months and a few years. Additionally, the extent at which the wound can heal also depends on the severity of the burn (Abazari et. al 2022). First, fibroblasts will leave the injured area and the collagen will become an organized matrix. The collagen fibrils will cross-link which increases the tensile strength of the skin. Subsequently, myofibroblasts accumulate in the extracellular matrix and secrete extracellular matrix (ECM). This improves the remodeling of the ECM (Abazari et. al 2022). Collagen III is replaced by collagen I in the ECM, this increases the tensile strength in the wounded area. After the wound heals, the apoptosis of myofibroblasts occurs. Unfortunately, some skin components such as sweat glands and hair follicles may not recover depending on the degree of the burn injury (Abazari et. al 2022).

2.1.2 Physiology of Burn Wounds

There are four degrees of burn wounds. All four degrees of injury have three zones: the zone of coagulation, the zone of stasis, and the zone of hyperemia (Teot et. al 2012). The coagulation region is the center of the wound where there is permanent tissue damage. The proteins are denatured and there is a loss of the plasma membrane. The zone of stasis has damaged tissue perfusion with noticeable capillary vasoconstriction. Additionally, cell death in organs and tissues (necrosis) occurs due to the decrease of perfusion. The zone of hyperemia is the outer layer of the burn wound. Vasodilation from the inflammatory mediators helps with tissue recovery (Teot et. al 2012). In general, all four types of injuries have the same wound-healing pathway. The first phase is called systemic inflammatory response syndrome. Macrophages and biochemical cytokines are key factors in this stage. The macrophages secrete proinflammatory mediators such as prostaglandin E2, IL-6, and TNF-α (Nielson et. al 2017). Subsequently, induced hypermetabolism causes an increase in proinflammatory cytokines. TNF-a (biochemical cytokine) is responsible for the apoptosis of different cell elements. It also contributes to the activation of antimicrobial defenses such as monocytes and neutrophils. Lastly, TNF-a can induce the production of other proinflammatory mediators, examples include IL-1 and IL-6 (Nielson et. al 2017).

The second phase of a burn injury is called the counter anti-inflammatory response syndrome. It is primarily controlled by anti-inflammatory mediators. Vasodilation causes an

increase in vascular hydrostatic pressure, this is a major contributor to leakage in the wound. The endothelial lining in the tissue widens which diminishes all barrier functions. Moreover, bradykinin is secreted at the injury site and is a useful vasoactive mediator (Nielson et. al 2017). It will cause pain, venular dilation, increased permeability, and smooth muscle contraction. Following a burn injury, adenosine triphosphate decreases, and adenosine monophosphate increases. Adenosine monophosphate will convert to hypoxanthine and will provide a substrate for xanthine oxidase (Nielson et. al 2017). These reactions lead to free radicals that have beneficial effects on wound healing.

For extreme cases of burn injuries, there are two phases: a resuscitation phase and a hypermetabolic flow phase. The resuscitation phase happens first and lasts for about 24 to 72 hours (Nielson et. al 2017). There are fluid shifts, increased permeability in the tissues, and fluid collecting in the cavities and tissue (edema formation). The goal is to maintain tissue perfusion and avoid shocking the cells. The hypermetabolic flow phase happens 24 to 72 hours after the injury. During this phase, the person's heart rate increases, vascular permeability decreases and vascular resistance decreases. All of these factors cause an increased cardiac output (Nielson et. al 2017).

As demonstrated, all burn wounds have the same general pathology; however, their appearances and treatments are different. First-degree burns (or superficial burns) have redness and swelling but will heal within 5-10 days. Typically the pain will subside between 48 to 72 hours. Second-degree burns (or partial-thickness superficial burns) damage the dermis and epidermis. There are two mosaic subcategories: II A and II B. Type II A is the degradation of the "epidermis and the superficial layers of the dermis." (Markiewicz-Gospodarek et. al 2022) The victim will experience pain accompanied by blisters, which normally take up to 21 days to completely heal. Type II B is caused when the dermis layers are degraded at various depths and the skin becomes moist, red, and filled with blisters. However, this type normally heals between 21 and 35 days if the patient doesn't already need a skin transplantation. Third-degree burns (or full-thickness deep burns) are when the full thickness of the skin is degraded. The skin becomes brown and dry and requires surgery for optimal healing. Lastly, fourth-degree burns are a combination of second and third-degree burns where the wound has penetrated the "subcutaneous tissue layer." Similarly to third-degree burns, fourth-degree burns also require

surgery because muscles and tendons have been severely damaged (Markiewicz-Gospodarek et. al 2022).

2.1.3 Issues with the Golden Standard

The golden standard for burn wound treatments is autografts and allografts. The goal of skin grafts is to replace the lost skin or improve the appearance of the damaged skin. Autografts/Split thickness skin grafting (STSG) is the “golden standard” for treating large skin burn wounds (Tam et. al 2013). The procedure involves removing the epidermis and the upper dermis from various donor sites and transplanting them onto the injured areas. Dermal components such as hair follicles and sweat glands are harvested during the process (Valencia et. al 2000). Scarring at the donor or graft site is a serious issue that results from autografting. Usually, scar tissue is painful and will eventually contract over time, this leads to deformities (Tam et. al 2013). When autografts aren’t available, the next viable option is allografts (ex: cadaver skin).

Allografts/cadaver skin are preserved and maintained in skin banks until a donation is needed. They contain the dermis and are grafted onto the wound which minimizes the loss of proteins, electrolytes, and water (Popa et. al 2021). Their main issue is limited availability. There is currently a worldwide shortage of cadaver skin. Countries such as Australia cannot meet the national demand for cadaver skins due to insufficient resources (Schlottmann et. al 2023). Another potential issue with allografts is immunological rejection. Immunological rejection is directly correlated to the viability of the allograft. According to a study, allografts preserved in 85% glycerol elicit a milder immunological response. Macrophage cells will produce most of the response to the allograft. Allografts that are not preserved correctly can get rejected by CD8 positive T cells (Hermans et. al 2011). Issues with the current golden standard have created a need for bioengineered skin substitutes. However, bioengineered skin still has problems and is not the most viable solution yet.

2.1.4 Issues with Current Bioengineered Skin Substitutes

Skin substitutes are created from polymers and non-biological molecules not found in native skin. They are typically biodegradable and aid in the regeneration of tissue. Its goal is to facilitate the growth of epithelial cells, blood vessels and fibroblasts (Halim et. al 2010). Skin

substitutes are becoming more promising than the “golden standard”, however there are still disadvantages. Skin substitutes insufficiently adhere to the body and can form blisters due to mechanical loading. The acceptance rates (whether or not the body will reject the scaffold) can also be very low which negatively impacts the patient (Horch et. al 2005). Additionally, bioengineered skin contains two cell types which are keratinocytes and fibroblasts. Since there are other cellular components in the skin, these substitutes can not replace the function of the human epidermis and dermis (Supp et. al 2005). There is an absence of a “vascular plexus” in bioengineered skin (Supp et. al 2005). Essentially, it has a slower vascularization process than autografts after being grafted onto the patient. This can cause graft failure due to the increased amount of time the cells are deprived of nutrients and exposed to bacterial contamination. Another limitation is the inconsistent pigmentation of the skin. For regular skin, the epidermal melanocytes are properly distributed and function normally. Melanocytes are vital for protecting the body from UV rays. This helps prevent cancer and other lethal skin conditions (Supp et. al 2005).

2.2 Skin Substitutes

Skin substitutes were used as far back as the 1880s when an absorbent dressing made of cotton wool was sandwiched between layers of gauze. Since then skin substitutes have evolved into epithelial cell seeding on burn wounds, and the growth of human keratinocytes onto lethally irradiated murine fibroblasts. Eventually, a living alternative has been created which was a dermal substitute based on collagen 1 gel containing fibroblasts. Some skin substitutes on the market are composed of type 1 collagen, however, there are others with a different composition. skin substitutes can be broken down into acellular skin substitutes, cellular allogeneic skin substitutes, cellular autologous skin substitutes (Alrubaiy et. al 2009).

2.2.1 Acellular Skin Substitutes

Based on the compositions of these skin substitutes, there are no cellular components and it is mainly synthetic material. Products overall provide adequate wound coverage and promote neo-dermis generation. The main products on the market for acellular skin substitutes are Biobrane®, Integra ® and Alloderm ™ .

2.2.1.1 Biobrane ®

Biobrane ® is a bilayered membrane made of a porcine dermal collagen banded nylon membrane attached to a thin layer of silicone (Feng et. al 2018) (Kim et. al 2006). For placement onto a wound, Biobrane ® initially adheres to fibrin and a clean wound surface. The silicone allows for some but not excessive water loss as it promotes moist wound healing (Kim et al. 2006). There are peptides within the graph that foster wound bed healing and fibrovascular ingrowth (Feng et. al 2018). Once the neo-dermis layer is formed, it separates and can easily be removed from the wound bed (Kim et. al 2006). Advantages of Biobrane ® include a long shelf life, immediate availability, decreased wound contracture, immediate availability and epithelialization within 1 to 2 weeks (Feng et. al 2018) (Kim et. al 2006). Biobrane ® is unique because it is pliable and stretchable, meaning it can be put on complex body parts such as the ear and buttocks (Feng et. al 2018). The downsides are that it is expensive, has a high risk of infection and requires the use of anastasia for its application (Fan et. al 2018).

2.2.1.2 Integra ®

Integra is a bilayer made of type 1 tendon collagen and chondroitin-6-sulfate with an epidermal layer of silicone. The silicone barrier is temporary as within the collagen, the neodermis is remodeled. After 2-3 weeks, the silicone barrier is removed and is eventually replaced with an autograft (Kim et. al 2006) (Nyguen et. al 2010). In terms of vascular formation, Integra obtains its vasculature during matrix formation over the wound bed and lays a new layer of dermis. Once the dermis is formed and receives the necessary blood supply, it can be covered with a skin thickness graft (Hughes et. al 2021). Advantages of Integra include a long shelf life and low chances of host immunologic reaction. However, it has a high cost, a second operation is required, and there is a risk of bacterial infection. There is also the possibility of incorrect application and fluid entrapment (Kim et. al 2006) (Hughes et. al 2021).

2.2.1.3 Alloderm ™

Alloderm ™ is a dermal allograft made of collagen and elastin matrices. It has an absence of undesirable dead cells with associated class I and II HLD antigens (Agarwal et. al 2015). The substitute is typically freeze dried and used in acute and chronic wounds. Additionally, it is often utilized with split thickness grafts (Kim et. al 2006). In terms of aesthetics, it blends well with adjacent tissue with no reactivity (Argawal et. al 2015) (Terino et.

al 2001). Advantages include great elasticity and pigmentation, easy placement (less time consuming), long shelf life and it is ready to use immediately (Kim et. al 2006). The disadvantages is that there is a possibility of resorption and it is expensive (Taban et. al 2005).

2.2.2 Cellular Allogeneic Substitutes

Cellular allogeneic skin substitutes are similar to the base composition of acellular skin substitutes, however they contain neonatal skin cells. These cells are cultured over a period of a few weeks so that a cellular matrix can be formed. Some of these products contain growth factors to promote accelerated wound healing. Example products include Transcyte ™ , Apligraf ™ and Dermagraft ™ .

2.2.2.1 TransCyte ™

TransCyte is composed of a nylon mesh fabric of Biobrane ® that is seeded with allogeneic human dermal fibroblasts. The mesh contains high levels of proteins and growth factors with no viable cells (Kim et. al 2006). This substitute has been used mainly in mid-dermal to intermediate depth partial thickness burns (Kumar et. al 2004). The growth factors in TransCyte ™ accelerate the rate of epithelialization, this decreases the amount of dressing changes needed during treatment. (Kim et. al 2006). However it requires frozen storage and is very expensive. The nylon component of the substitute is also not biodegradable so it only provides temporary coverage (Kim et. al 2006).

2.2.2.2 Apligraf ™

Apligraf ™ is an FDA approved substitute composed of a bovine collagen matrix that contains neonatal fibroblasts with an epithelium of keratinocytes. The substitute provides growth factors to the wound and the dermal matrix produces cytokines, both of which contribute to accelerated wound healing (Hu et. al 2006). Apligraf ™ overall has a low immunogenic response and a low risk of disease. However, it has limited shelf life and can be expensive (Hannen et. al 2023). Another issue with Apligraf is that the cells composed in the graft have unknown origin. More specifically, whether or not the healed tissues contain neonatal fibroblasts/keratinocytes or the patient's cells. (Kim et. al 2006) (Hannen et. al 2023).

2.2.2.3 Dermagraft ™

Dermagraft is a dermal substitute containing allogeneic human fibroblasts harvested from neonatal foreskin. The fibroblasts are cultured in vitro on a bioabsorbable polyglactin scaffold and proliferate to fill interstices of the scaffold. Fibroblasts also secrete human collagen, cytokines, matrix proteins and growth factors. These all contribute to the formation of a new dermis layer. The graft comes frozen in a clear bag for single use application and degrades through hydrolysis in 20-30 days after being applied. Unfortunately, Dermagraft ™ does not contain macrophages or blood vessels and has safety issues owing to allogeneic cells (Tavakoli and Klar, et. al 2021). Examples include slower immune reconstitution and an increased chance of an infection (Champlin et. al 2003). It is also very expensive (Kim et. al 2006).

2.2.2.4 Orcel ™

Orcel ™ is an FDA approved composite allograft that is synthesized by culturing allogeneic neonatal keratinocytes and fibroblasts. They are cultured on opposite sides of the matrix of crosslinked bovine linked collagen (Kim et. al 2006) (Tavakoli and Klar, et. al 2021). It treats split thickness donor sites in severely burned patients with reduced scarring compared to Biobrane ® (Tavakoli and Klar, et. al 2021). The graft contains viable cells that secrete growth factors and cytokines to promote migration and wound healing. One of the major advantages of Orcel ™ is that it has reduced scarring which allows for better aesthetics (Kim et. al 2006).

2.2.3 Cellular autologous skin substitutes

Many of the previously discussed substitutes only provide for temporary coverage and are used with another graft. Since these solutions are not made of autologous keratinocytes, they don't provide sufficient coverage. Solutions include, spray on cells (ReCell ™ by Avita), Cultured Epidermal Autografts such as Epicel and Cultured Skin Substitutes.

2.2.3.1 Epicel ®

Epicel ® is currently one of the only autologous skin products on the market and it is made of the patient's own keratinocytes. The graft is used for superficial wounds and can cover more than 30% of the body's surface area (Supp and Boyce, et. al 2005). The graft can be utilized alone or in conjunction with other split thickness autografts (Nathoo et. al 2014). Despite

its unique properties, it has more cons than pros. Firstly, it takes 3 weeks for the cells to be cultured to produce the graft (Kim et. al 2006). Since it is extremely fragile, friction on the wound leads to blisters (Kim et. al 2006) (Supp and Boyce, et. al 2005). The blisters on the wound are also susceptible to infection (Kim et. al 2006). In terms of costs, the graft is expensive and also requires intensive labor (Supp and Boyce, et. al 2005).

2.2.3.2 Cultured Skin Substitutes (CSS)

Cultured skin substitutes are a class of materials that are composed of both the epidermal and dermal components of the skin. The base is typically made with a biopolymer sponge and has been used to treat full thickness burns. The sponge is typically electrospun and provides for increased mechanical strength and stability. There is also a reduction in wound contraction and promotion of engraftment of epidermal tissue substitutes. Yet, it is expensive and does take an extensive period of time for preparation (Powell and Boyce, et. al 2009).

2.2.3.3 Recell ™

Recel ™ is a spray on skin composed of the patient's own cells and is used for full thickness burns. Spray on skin is versatile as the wound can either be sprayed or dipped (in the spray). It can also be spread on wounds with large surface areas. Along with its unique application and composition, the main company ReCell ™ by Avita is FDA approved as it provided the first spray on treatment for burns in 2018. However, spray on skin is very expensive (Beltran et. al 2022).

2.3 Engineering Need

Despite the advantages and disadvantages of these skin substitute products, all have unique compositions and provide adequate coverage for burn wounds. Skin grafting has progressed to the point where live cells can be used to treat burn wounds, whether or not it's aesthetically pleasing. They also provide for skin regeneration and vasculature which can form within the substitute as a part of the wound healing process. However, when vasculature is developing, it may not completely form. This can affect the overall structure of the skin and potentially leave scarring. A graft with a built in vascular system can eliminate all of those issues and provide a faster healing process. More importantly, the graft can provide for an additional layer of security. Currently the Pins' lab wants to use decellularized spinach leaves to treat burn

wounds on different types of patients. The stem and the microvessels provide for a vascular system similar to the human body which shows more promise. Additionally, it provides a bilayer template for seeding of keratinocytes and fibroblasts.

2.4 Leaf Decellularization Process

Before the leaves can be used as skin scaffolds, they need to undergo decellularization. A specific process, constructed in Gaudette lab, has been put into place so that this is possible. It first consists of agitating the leaves vigorously in hexane rinse for two minutes. This removes the cuticle, or outermost layer, of the plant. It is followed by a two minute rinse in deionized (DI) water. This cycle of hexane rinse and DI water is repeated 3 times to completely pull off the cuticle layer of the spinach leaf. After this, the leaves are submerged and continuously agitated (gently) in 1% SDS in DI water for five days. The leaves are then submerged in 1% Polysorbate-20/3% bleach in DI water for 2 days, followed by DI water for 1 day. It is important to know that the solutions used in this process are exchanged daily. Regarding storage, the samples are stored at 4 degrees Celsius in DI water until used (L. Perreault, personal communication, February 26, 2024). After this process, the samples encounter cell seeding through a process discussed in the next section *2.5 Previous Culturing System*.

2.5 Previous Culturing System

Previous tissue engineered skin created by the Pins lab is known as Microfabricated Dermal Epidermal Regeneration Matrix (μDERM). The μDERM contains a dermal-epidermal junction or DEJ. The DEJ provides “instructive cues critical for modulating keratinocyte function”, and enhances “overall architecture and organization of skin.” (Bush, & Pins, et. al 2012) This is due to the DEJ closely mimicking the basal lamina because of its topographical modifications (using photolithography). μDERMs created by the Pins lab involved the use of collagen–glycosaminoglycan (GAG) coprecipitate crosslinked scaffolds. These GAG sponges contain a covalently conjugated fibronectin surface layer (Bush, & Pins, et. al 2010). The process for making these μDERMs can be seen below in Figure 1.

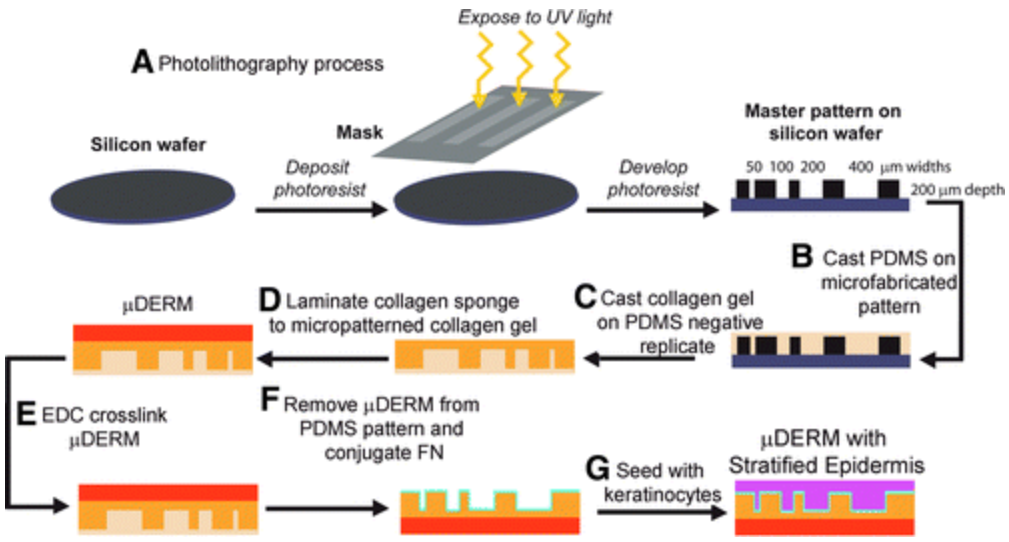

Figure 1: Creation of µDERM

The µDERM is transferred to an air/liquid (A/L) interface culture device. The device was created in 2010 by Katie Bush in collaboration with George Pins. The device features a sandwich design with a rigid top and bottom piece made from polycarbonate, a silicone o-ring, and a surgical grade stainless steel mesh. There is a stainless steel mesh screen sitting on the bottom piece and a silicone gasket on the underside of the top piece. Images and drawings of the device can be seen below (Bush, & Pins, et. al 2010).

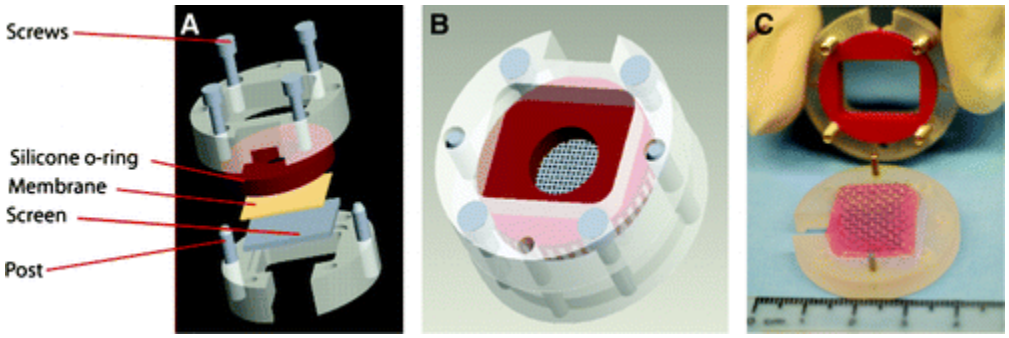

Figure 2: Current air/liquid (A/L) interface culture device

Once the µDERM is transferred in the A/L interface culture device, primary human foreskin fibroblasts (NHFs) were seeded on the dermal side of the scaffold at a concentration of 8×10^4 NHFs cm^{-2}. The seeded cells are submerged and incubated for 2 hours, and then cultured another 48 hours. Neonatal primary human foreskin keratinocytes (NHKs) were seeded on the micropatterned side at a density of 5×10^5 cells cm^{-2}. They were then incubated for 2 hours at the

A/L interface and then submerged for 24 hours. The medium was changed and cultured for a further 48 hours and then cultured for another 3-7 days (Clement et al., 2013).

A similar method was utilized to create the skin grafts using the decellularized leaf. NHFs and NHKs are seeded onto the leaf and submerged into keratinocyte growth medium for 24 hours. After 24 hours, the cell seeded leaf is raised to the air-liquid interface and cultured for a further 14 days. The mesh in the air/liquid (A/L) interface culture device provides structural support for the devices. It also enables passive diffusion of nutrients from the culture media into the scaffold which is utilized by the dermal and epidermal layers. The o-ring provides a seal that allows for the controlled seeding of cells onto the matrix. This seal is only possible due to the rigid nature of the polycarbonate which permits o-ring compression. The aim of this project is to use the existing leaf vasculature present to support vascular ingrowth into the skin graft.

3. Project Strategy

3.1 Initial Client Statement

The challenge presented to the MQP team was to create a device that would allow for a cannula attached to a decellularized scaffold to be present throughout the growth of the skin scaffold. This device would create a watertight seal around the cannula to ensure that any media or other solutions used do not escape. To create a strategy to approach this design challenge, objectives and constraints were identified. The initial client statement provide goes as follows:

"Design a housing device for a scaffold that allows the skin to grow on the back of a decellularized leaf. The skin graft would treat people with burn injuries, open wounds, or other skin-related defects"

Professor Pins' lab has become skilled with growing the skin grafts from the decellularized leaves. With the current housing the scaffold is grown in, the cannula cannot be attached as the graft is growing due to the confined space the current design affords. This means that the graft must be taken out of the media to allow for the insertion of a cannula. This presented a significant risk of rupturing the cannula or destroying it. The next phase of the development of these grafts is to create an easy method of injection for different solutions through this cannula.

3.2 Stakeholders

After learning the initial client statement, the design team determined the primary stakeholders. The stakeholders were the designers, clients, and users. The designer's role is to design a device that benefits other stakeholders. The clients are people who support and sponsor the project, and the users are people who utilize the product. In this project, the designers were the MQP team, Codey Battista, Nishan Grandhi, Aidan Kaufman, and Ariel Shirzadi. The clients include Professor Pins and Bryanna Samolyk. The users are also Bryanna Samolyk and Luke Perrault., as well as researchers in the pharmaceutical industry and academia. After establishing

the stakeholders, interviews were conducted to help create the initial objectives. Constraints were also produced after further analysis of the initial project requirements. All of these components, along with a quantitative comparison of objectives, allowed the team to define the final objectives and project strategy.

3.3 Initial Objectives

After discussing with primary stakeholders, 6 main objectives were determined for the design. These objectives were: *Enable Facile Culture of Bi-layered Skin Equivalent, Cost Effectiveness, Direct Injection System, Reproducibility, User Friendly, Scalable, and Imaging Compatibility.* Table 1 displays each objective with a short description. These objectives are expanded upon in the following sections.

Table 1: Descriptions of Objectives

Objective	**Description**
Enable Facile Culture of Bi-layered Skin Equivalent	Allow for culturing of cells on both sides of the scaffolds within a submerged and air-liquid interface culture environment
User Friendliness	Create a design with no mechanical parts and as few parts as possible to provide easy assembly
Cost Effectiveness	Have a product that is reproducible at a fair market price
Perfusion	Allow the liquid to flow through microvessels throughout the leaf scaffold
Scalability	Ability to grow multiple grafts at multiple sizes
Imaging Compatibility	Design should account for precise and accurate microscope imaging as part of the scaffold growing progress
Reproducibility	Create a design that produces consistent quality skin grafts.

3.4 Constraints

A set of constraints was generated based on the ideation of objectives for this project. The two main categories of constraints were cellular and technical. The constraints along with further breakdown are shown in Table 2 below.

Table 2: Description of Cellular and Technical Constraints

Constraint Category	**Constraint**	**Description**
Cellular	Enable Facile Culture of Bi-layered Skin Equivalent	Allow for culturing of cells on both sides of the scaffolds within a submerged and air-liquid interface culture environment
	Biocompatibility	- Material choice - Does Not interfere with Cell function
	Sterility	Device must be able to be sterilized to limit contamination
	Cell Seeding	Device must allow for cells to be seeded on the decellularized leaf
	Scaffold Access	The scaffold must be accessible during the growing process.
	Downstream Compatibility	Must be able to integrate with all downstream processing of the scaffolds
Technical	Time	The project must be completed by the end of the 23-24’ academic year

	Cost	Material costs must stay within the budget
	Size	Design must fit within a standard well plate
	Manufacturability	- Device must be able to be able to be made with common manufacturing methods - Must be able to be put together in a laboratory hood
	Longevity	Device must be able to be last multiple runs without signs of fatigue or failure
	Reliability	Device must be reliable through multiple runs and produce consistent product
	Bilayer	Device must allow ability to seed keratinocytes and fibroblasts on both sides of leaf scaffold
	Watertight	Device must prevent any leakage of media from exiting the well plate

Cellular constraints are of extreme importance due to the nature of working with cells. Ensuring the success of the product means that it must be *biocompatible, sterile, cell seeding capable, allow access to the scaffold, and be downstream compatible.* The materials and coating must be biocompatible to promote a positive growth environment. Sterilization highly reduces the chance of contamination within the scaffold. The device will be sterilized by using an autoclave. This impacts the size of the device because it must fit inside the autoclave and the materials used have to withstand its high temperatures and pressures. Seeding is the initial step for creating these skin grafts and our design must permit easy cell seeding to increase skin graft production. It’s crucial to the procedural processes that there is access to the scaffold while it's growing. Downstream compatibility ensures that our product will work within the entire process of manufacturing.

Technical constraints include *time, cost, size, manufacturability, and longevity*. This project must be completed within the 2023-2024 academic year which is from August 2023 to May 2024. The budget provided to each student is 250 dollars, meaning the total budget for this project is 1000 dollars. The functionality of the device relies on its size because it must fit within a standard well plate. This ensures that our device is image compatible with a range of commonly used laboratory microscopes. The device must be constructed using common manufacturing methods such as 3D printing and CNC machining. As a part of manufacturability, the device should be assembled in a laboratory hood to ensure sterility. This allows for user-friendliness because no complex tools are required in the assembly process. Longevity ensures that the device will have continued use after creating multiple grafts. This device is not intended to be a single-use product. Therefore, it should be able to withstand any stresses the graft-growing process exerts on it. The bilayer component of the device enables keratinocytes and fibroblast layers to be seeded. On the top side of the leaf, the keratinocytes are seeded. Once the device is flipped, the fibroblast layer is seeded. Watertightness is essential because it facilitates the cell media where the cells proliferate. Any leakage could hinder cell growth along with potential contamination.

3.5 Final Objectives

After discussing specific objective goals with clients, the team created an objective tree which is shown in Figure 3. The main objectives are shown on the left, and secondary and tertiary objectives are shown on the right branching off from the main objectives.

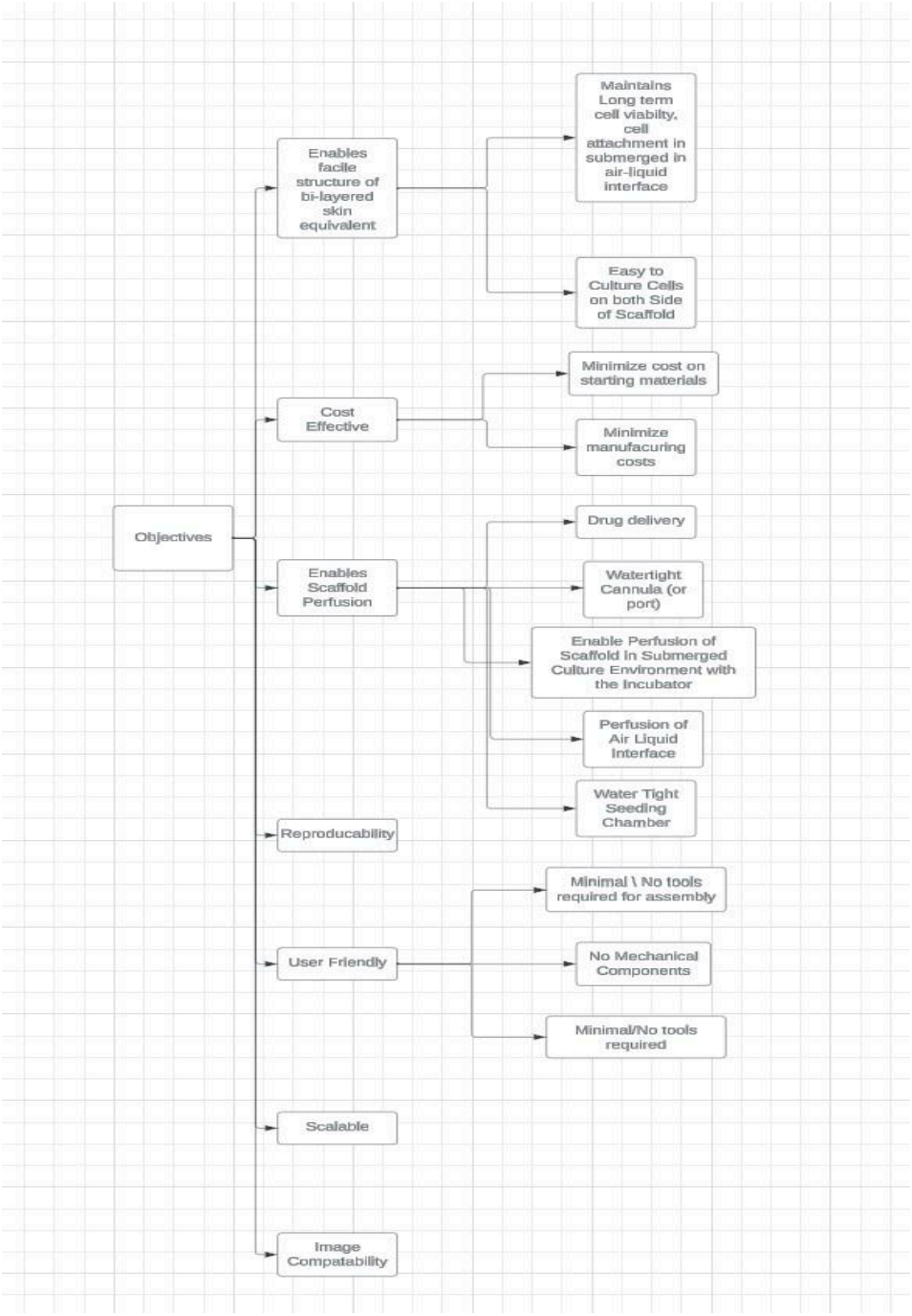

Figure 3: Project Objectives Tree

The primary objectives were rated using a pairwise comparison chart to determine their importance. Each objective received a score of 0, 0.5 or 1. A 0 means that it is less important than the other objective, 0.5 indicates equal importance and 1 indicates more importance. After the resulting score from each objective was totaled, the pairwise comparison chart was

completed by the design team, the client, and the users. Each party completed a Pairwise Comparison Chart without the knowledge of the other groups rankings for each objective. Table 3 below shows the results of the pairwise comparison charts completed by the stakeholders.

Table 3: Primary Pairwise Comparison of Primary Objectives

Primary Objective	**Designers**	**Clients**	**Users**	**Average**
Enable Facile Culture of Bi-layered Skin Equivalent	6	3.5	5.125	4.875
Cost Effective	0.875	1	0.5	0.79
Enables Scaffold Perfusion	3.625	5.5	4.5	4.54
Reproducibility	1.875	4	3	2.96
User Friendly	4.375	2.5	4.125	3.67
Scalable	0.75	1	0.5	0.75
Imaging Compatibility	3.75	3.5	3	3.42

The design team, the clients and the users all agreed that enabling facile culture of a bi-layered skin equivalent was the most important objective. The goal of our project is to create a housing device that can support the growth of a scaffold. If this isn't possible, then there is no use for the device. The second and third important objectives were enabling scaffold perfusion and user-friendliness. The housing device must have a cannula port that allows liquid (ex: hormones, medicine, etc.) to be injected into the scaffold through the leaf stem. User-friendliness is a key design factor. People who utilize the team's product shouldn't have issues with

assembling/disassembling the device and it should be minimal assembly time. In addition, the device should be easy to sterilize (ex: autoclavable). The last important objective was image compatibility. If there are problems imaging the scaffold under a microscope, then data can't be collected. Preferably, the scaffold would remain inside the housing device while being imaged. This reduces the risk of damaging the scaffold. Everyone agreed that cost-effective, reproducibility and scalability were not as crucial.

After the primary objectives were rated, Pairwise Comparison Charts were completed for the Secondary Objectives. These consisted of subcategories for the Cannula, User Friendliness, and Cell Viability. A breakdown of the objectives is shown below in Table 4 gives descriptions for each of the secondary objectives.

Table 4: Description of Secondary Objectives

Primary Objective	**Secondary Objectives**	**Description**
Enable facile culture of bi-layered skin equivalent	Maintain long term cell viability in submerged and air-liquid interface	Ability to ensure cell proliferation while being submerged within culture media.
	Easy to culture cell on both sides of scaffold	Culture and grow Keratinocytes and Fibroblasts on their respective Epidermal/Dermal side of Scaffold
User friendly	Minimal / No tools required	Assembly is mostly done by hand to provide easy assembly and disassembly
	No mechanical components	No screws, bolts, etc for simplicity of assembly
	Minimal assembly time	Doesn't take too much time to assemble/disassemble and preferably no external tools

Enabling of scaffold perfusion	Drug delivery	Provide medicinal injection with a cannula through stem
	Watertight cannula (or port)	Perfusion port will prevent leakage during drug delivery
	Enable perfusion of scaffold in submerged culture environment with the incubator	Allow for flow of medicine within “veins” of leaves
	Perfusion of air / liquid interface	Allow the leaf to be partially submerged in the media
	Watertight seeding chamber	None of the cellular components or media leaks out of the chamber
Cost effective	Minimize cost on starting materials	Want to minimize upfront costs so that our group doesn’t go over budget
	Minimize manufacturing costs	Want our device to be easily mass producible

The results of the pairwise comparisons of the secondary objectives can be found below in Tables 5, 6, 7 and 8. The pairwise comparison can be found in Appendix B.

Table 5: Secondary Objectives for Enable Facile Culture of Bi-layered Skin Equivalent

Secondary objectives for	Designers	Clients	Users	Average

for enabling facile culture of bi-layered skin equivalent				
Maintain long-term cell viability in submerged and air-liquid interface culture environments	.5	1	.75	.75
Easy to culture cells on both sides of scaffold	.5	0	.25	.25

Table 6: Secondary Objectives for User Friendliness

Secondary objectives for the user Friendliness	Designers	Clients	Users	Average
Minimal / no tools required	1.125	1	.5	.875
No mechanical Component	.5	0	.5	.33
Minimal assembly time	1.375	2	2	1.79

Table 7: Secondary Objectives for Enabling of Scaffold Perfusion

Secondary objectives for the enabling of scaffold perfusion	Designers	Clients	Users	Average
Drug delivery	.5	1	.75	.75
Watertight cannula (or port)	2	3	2.75	2.58
Enable perfusion of scaffold in submerged culture environment with the incubator	2.25	1	1.375	1.54
Perfusion of air/liquid interface	2.25	1	1.375	1.54
Watertight seeding chamber	2.625	2.5	3.25	2.79

Table 8: Secondary Objectives for Cost Effective

Secondary objectives for cost effectiveness	Designers	Clients	Users	Average

Minimize cost of starting materials	.5	0	.5	.33
Minimize manufacturing costs	.5	1	.5	.66

Based on the primary objective “Enabling Facile Culture of Bi-layered Skin Equivalent” the designers, clients, and users agreed that maintaining long-term cell viability was more crucial than having it be easy to culture on both sides. While double sided culturing is important for use, the project is a failure if cell proliferation is not successful.

In addition to enabling a facile culture, user friendliness was also rated in our pairwise comparison charts. The most important objective here is to minimize the assembly time. This ensures increased production of skin scaffolds. The second rated objective is no external tools. This permits the user to only utilize their hands during assembly (makes it easier). Lastly, after evaluation, mechanical parts were ranked last since it is not critical but could possibly lead to a simpler assembly.

Regarding scaffold perfusion, a watertight cannula and seeding chamber were the most essential objectives because cell culturing would not be possible if leaks occurred. Enabling the perfusion of a scaffold in a submerged culture environment with the incubator and enabling perfusion of an air/liquid interface were both second in the pairwise comparison charts while drug delivery was rated last. Drug delivery would be a good addition to the project but is still not as important to the clients, designers, and users.

The last secondary objectives that were rated were for cost effectiveness. Ultimately, minimizing the manufacturing costs was more important than minimizing the cost of starting materials. While the grade for both was close, manufacturing costs are more important. If the team’s device can be mass produced, then the startup costs will be more insignificant.

3.6 Revised Client Statement

Professor Pins research on growing skin grafts on decellularized leaves has the potential to make a groundbreaking impact in the healthcare industry as there are no vascularized grafts on the market. His current model only enables him to seed cells because there is no perfusion system. A new housing device that can allow for perfusion throughout the microvessels of the leaf can be a crucial step in terms of vascularized scaffold development. Another issue that the current device has is the assembly and disassembly time. The screws in the device are bolted very tight, requiring a lot of force and time. This prevents the user from working through many different scaffolds. Lastly, to see how the skin cells develop, it is important to see their proliferation on the leaf with precise imaging. From there, the team came up with a revised client statement which is seen as follows along with specifics:

"Design the housing of a leaf scaffold with ports that enable a perfusion rate of between ~ 25 - 300 μL/min for about a week continuously. Additionally, the device must be user friendly allowing assembly time between 0.5-5 minutes. To see how the cells proliferate on the scaffold, the device must be imageable, giving clear and transparent images of keratinocytes and fibroblasts at a scale of between ~3600 x 2700 (4X) and ~240 x 180 (60X) microns. The skin grafts would treat people with 2nd and 3rd degree burn injuries, open wounds or other skin related defects."

The revised clients statement reflects the updated objectives as well as the narrowing of the scope to target the product to a more focused audience. The objectives were also further specified to better represent stakeholder requests.

3.7 Project Approach:

3.7.1 Management Approach:

The team stayed organized by utilizing management plans. Microsoft Planner was used to break down large deliverables into weekly tasks. These tasks were assigned to members of the

team based on their role in the project. Once a task was completed, it would be checked off by the respective team member.

Two weekly meetings were established by the team. The first was a team only meeting to discuss the weekly deliverables and have an effective discussion on the progression of the project. The second weekley meeting was with both the team and the clients. These meetings ensured the team stayed on the correct path to deliver the desired product. Meetings with users occurred on a need basis. These meetings ensured that all the stakeholders' wants and needs were represented in the project design process.

3.7.2 Design Approach:

Throughout the design process, the team used the constraints and objectives determined by all involved parties to brainstorm multiple concepts. The design concepts were discussed between team members during meetings. Additionally pros' vs. cons' were weighed for each design along with how well they achieved the project objectives. The best design concepts were presented to the stakeholders to receive feedback. The designs were altered per the user's feedback and then ranked to determine the final design. After the final design was determined, a 3D model was generated in Solidworks with accurate dimensions as well as material choices. Subsequently, the model was printed and tested by ordering the parts/materials to create prototypes for the final design. Design modifications were made based on prototype testing to further improve the product.

3.7.3 Financial Approach

The team was given $250 per student, or $1000 total by Worcester Polytechnic Institute to complete the project. Due to the limited budget, the team determined which materials were vital to the design before anything was purchased. A list of all materials and costs for our final design was formed to verify the team stayed within the budget.

4. Alternative Designs

After determining the primary and secondary objectives, the design team created a functions means table for the final device. The functions means table, as seen in Table 12 included the potential features that could be incorporated in our final design. The following sections highlight the needs and wants along with the functions and specifications of the final device. This enabled the team to produce a specific final model.

4.1 Needs Analysis

Based on the final objectives and PCCs, the design team developed the wants and needs of the design. From there, a list of requirements to meet the objectives was made. Each requirement was listed as either a need or a want. A need is required in order for the device to be successful. A want is not a requirement for success within the scope of the project. Objectives of higher importance are listed as needs and objectives of lower importance were listed as wants. Table 9 is a list of wants and needs along with a description of each.

Table 9: Design Needs and Wants

Needs	**Definition**
Imageable	Ability to be imaged clearly without losing clarity
Perfusible	Ability to perfuse liquid throughout the leaf
User Friendly	Ability to disassemble/assemble and clean easily
Sterile / Autoclavable	Ability to be placed in autoclave for sterility
Bi-layer seeding	Ability to plants skin cells on both sides of leaf
Wants	**Definition**
House multiple leaves	Ability to house multiple leaf scaffolds
No mechanical components	Ability to assemble/disable device with no

	extra mechanical components
Cost effective	Ability to made made of material for little money
Scalable	Ability to hold scaffolds of multiple sizes

4.1.1 Design Needs

Needs were identified from our design constraints along with the objectives from our primary PCCs that were of greater importance. These were based on the discussion between the client and user. Based on the constraints and the primary PCCs, imageability, perfusion, userfiendliness, sterility/autoclavability, and bi-layer seeding were more important.

Since user friendliness was one of the higher ranked objectives, the device must be easy to assemble and disassemble. The assembly/disassembly time must be quick so that multiple scaffolds can be made in one day. This is crucial for burn wound patients that require immediate treatment.

Perfusibilty is extremely important when treating burn wound victims because the leaf is vascularized. A non perfusable device would restrict fluid movement, hindering oxygenated blood from reaching the site of the graft. This would negatively impact the healing process of the wound. Adding nutrients such as growth factors and hormones into the leaf can lead to the proliferation of skin cells. The perfusion system can be versatile, making it essential to have this feature in the device.

4.1.2 Design Wants

Wants were identified from objectives in the PCCs that were less important and based on discussions between the client and user. Based on the PCCs, the housing of multiple leaves, no mechanical components, and scalability were not as vital. Including no mechanical components in the design is not necessarily needed, however it would add to the user friendliness aspect.

The housing of several leaves would be useful since cells could be seeded on multiple leaves simultaneously. Yet, based on the PCCs, it was scored less compared to other objectives. Based on the tools the team was given in terms of the well plates, it would be difficult to incorporate since the well plates are very small.

Scalability would definitely add to the device because the user could work with multiple leaf sizes. Additionally, there would be more mechanical components to adjust to each leaf size. This was still less important but would be a nice addition to the device design.

4.1.3 Design Decision Matrix

After clearly defining the wants and needs, a design decision matrix was created. The goal of the design matrix was to list the design considerations and identify the important wants and needs. Table 10 shows the complete design matrix. The needs and wants are listed across the top and the design considerations are listed down the leftmost column. A X indicates whether a want or need influenced the design consideration. For example, scalability would affect the size of the device so it would receive an X.

Table 10: Design matrix of Wants and Needs

Needs	Imageable	Perfusible	User Friendly	Sterile/ Autoclavable	Bilayer Seeding	Wants	House Multiple Leaves	No mechanical components	Cost Effective	Scalable
Size of device	X	X	X	X	X		X	X		X
Perfusion system	X	X					X			
Well size		X	X	X			X			X
Device material				X				X	X	
Weight of device		X	X		X		X	X		

4.2 Functions and Specifications

The main functions of the device are having the ability to culture cells on both layers, to be easy to assemble/disassemble, have perfusibility, and be imageable. The bilayer component of

the device is a constraint because it is necessary to plant cells on both sides of the device. There would be culture media in the well plate and the device would contain the leaf. One side would have fibroblasts seeded to form the dermis layer. After a few days of incubation, the device would be flipped and the keratinocytes would be planted.

The user friendliness aspect of the device is crucial to the design, but more specifically the assembly and disassembly time. The total device assembly/disassembly time should be around .5-5 minutes to ensure multiple scaffolds can be seeded in one day. This range of time is ideal because the current device has an assembly/disassembly time of more than 5 minutes. It can also take multiple people to assemble it while under the hood. The device can be assembled by using a mesh, the scaffold, a gasket, and exterior features that clamp the housing together. When the scaffold is done being utilized, the device can be disassembled. Afterwards, the device can be easily cleaned/sterilized/autoclaved and ready for use once again.

For perfusibility, the leaf's vasculature allows liquid to perfuse through its microvessels. The function is the same whether it's a direct injection system or an indirect perfusion system. In a study on the development of microvascular grafts, perfusion rates were approximately 25μL/min (Redd et al., 2019). As a result, it is crucial that perfusion rates in the team's system are similar. Slower rates may hinder the liquid from flowing throughout the leaf and faster rates could cause the vessels to burst or leak. The leaf must also have continuous perfusion for approximately 2 weeks.

When it comes to the device and its imageability, it must be able to sit on an inverted microscope stand which is around 8" by 5". In order to see the proliferation of the cells precisely, scales of 3600 x 2700 um (4X) and ~240 x 180 um (60X) must be utilized. The imaging mesh must also be at least 85% transparent to view the cells clearly. Subsequently, there would need to be an open cavity for the microscope to see the leaf vasculature.

The functions and specifications of the device as mentioned previously are summarized in Table 11.

Table 11: Functions and Specifications Table

Functions	**Specifications**
User Friendly	● Assembly disassembly time between 0.5-5 minutes

Perfusable	● No bursting of leakage of the leaf ● 25-300 µL/min perfusion rate ● Continuous perfusion for a 2 weeks
Imageable	● Must fit on standard 8” x 5” microscope stand ● 3600 x 2700 um (4X) and ~240 x 180 um (60X) scale for precise imaging

4.3 Design Alternatives

Once our group determined the function and specifications of our device, we brainstormed ideas to fit our design criteria. Subsequently, pros and cons were created for each idea. After analyzing all options, the team created a design matrix that ranked each idea. Our group utilized these rankings to form a final design idea.

4.3.1 Brainstormed Design Elements

The first step of creating a conceptual design was for each member of the team to brainstorm concepts on their own. The group then met to discuss each concept. Figure 4 illustrates how the group came up with each design.

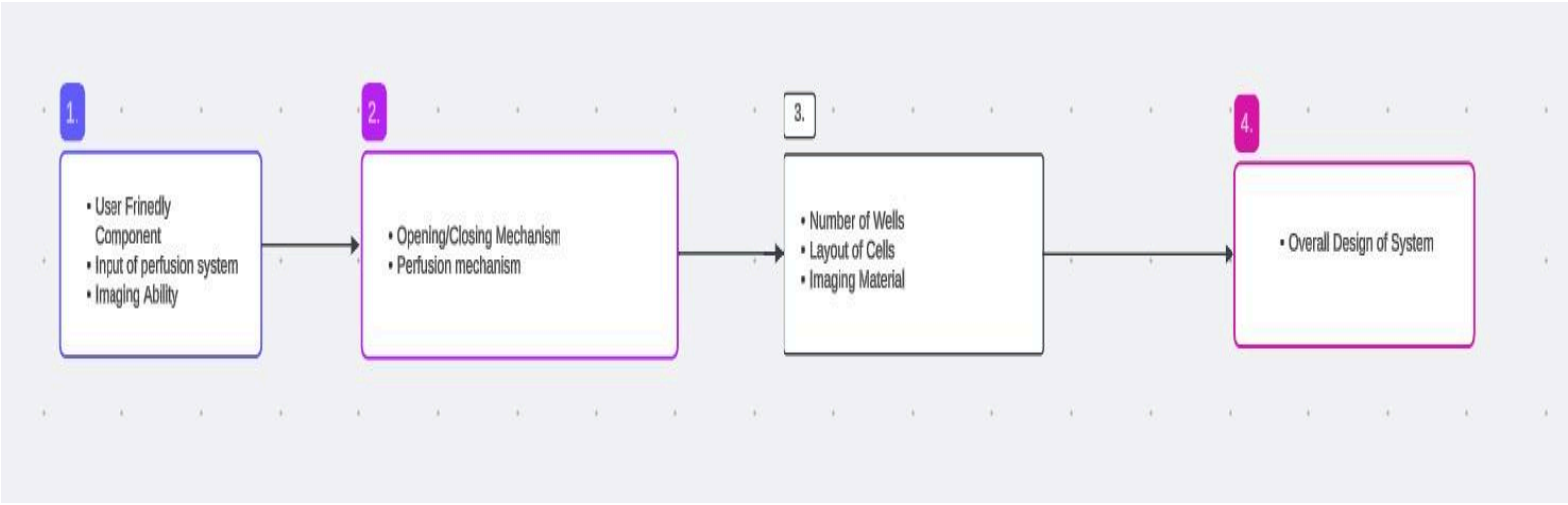

Figure 4: Shows the pathway for creating designs

First, the user-friendly, perfusion, and imaging components were brainstormed. From there, the group discussed the opening/closing components of the device, and how to input perfusion without being restricted to the shape and structure of the device. The last component debated was imaging material of the device. The means the design team came up with collectively can be seen in Table 12.

Table 12: Functions Means Table

Element:	User Friendliness	Perfusion System	Imageable
Means:	- Sliding system - Hinge system - Peg system - Magnetic system - Zip ties - Screws - Metal latch - Plastic clip - Hose clamps - Anchor screws - Alligator clip through pin - Quarter turn	- Direct injection System - Screw in direct Injection system - Continuous gravity flow	- Open cavity - Glass screen - Metal screen - Nylon mesh - Removal from device

Each of these brainstormed ideas were evaluated for their pros and cons in the following section. All ideas were evaluated in a decision matrix based on the top ranked objectives of the PCCs described in the previous section.

The overall ideas took into account the type of well plate, whether it would be a one or six well plate, how the perfusion system will be incorporated into the device and where, the types of material used, and how the device will be assembled making the device more user friendly. Once completed, the design team could determine the type of well plate that would contain the

device. Subsequently, the team can determine the size of the device and its dimensions. Each component in the means table was evaluated into their pros and cons.

4.3.2 Evaluating Design Elements

4.3.2.1 User Friendliness

Table 13: Pros and Cons of Sliding System

Sliding System	
Pros	**Cons**
● Simple ● Easy to clean ● Little time to assemble/disassemble ● Can run through multiple scaffolds during the day	● Large piece ● Material can be worn out from sliding ● Harder to flip ● Sliders may fall out of place

Although broad, assembly and disassembly of the device fall under user friendliness. Sliders would make the device design simpler. The ability to open up the device and place the leaf in within a short timespan enables the user to work on more scaffolds throughout the day. The sliding mechanism makes the device easier to disassemble and clean. However, this would only work in a one well plate because it would be difficult to incorporate in a six well plate. The sliding between parts will eventually cause material deformation, making them non-functional. It would also be harder to flip since the slides could fall out of place.

Table 14: Pros and Cons of Hinge System

Hinge System	
Pros	**Cons**
● Easy to open and close	● Difficult to incorporate perfusion

● Can work in one or six well plate ● Modified into different shapes	system ● Tools may be required

The use of a hinge system is a simple mechanism as it would simply open and close when putting the scaffold into the device. It is also convenient that either a one or six well plate can be utilized. This means the device can be modified into different shapes. One issue with this mechanism is the incorporation of the perfusion system. It may be difficult to fit the device into a six well plate because it is small. When completely disassembled, it may require tools to reassemble due to the hinge. This also makes it harder to clean.

Table 15: Pros and Cons of Peg System

Peg System	
Pros	**Cons**
● Simple ● Easy to assemble ● Extra security of the scaffold in the device	● Extra mechanical components to prevent slippage ● Imaging could be compromised

Pegs would provide extra security especially when the scaffold is in the device. Assembly/disassembly time would be less because it is easier to slide pegs into the device. It is also a very simple component as the top portion of the device slides onto the bottom portion of the piece. In order to prevent any slippage when flipping the device, another mechanical component is required to prevent the device from falling apart. The pegs would also be used as a stand which causes the scaffold to be further away from the microscope. This can negatively impact imaging.

Table 16: Pros and Cons of Magnetic System

Magnetic System	
Pros	**Cons**
● Simple ● Easy to Incorporate ● Easy to assemble disassemble	● May fall apart when flipping ● Opening and closing may wear corrode material

The incorporation of a magnetic system would make the device easy to assemble/disassemble because the pieces would stick together. It would be simple to include in the device since there are different ways it can be installed. However, one issue is the device can fall apart while flipping over due to the strength of the magnet. This would disrupt the cells and negatively impact the experiment. Also, the magnet can wear out because of the constant opening and closing of the device. This could deform the surface of the magnet and lead to corrosion. Lastly, the magnetic pieces could get into the media creating a cytotoxic environment for the cells.

Table 17: Pros and Cons of Zip Ties

Zip Ties	
Pros	**Cons**
● Secures device and scaffold ● Adjustable ● Abundant	● Long assembly/disassembly time ● Requires other tools to get off

Zip ties are widely used to secure objects and they are readily available. They can easily be adjusted depending on the desired tightness of the device. Consequently, zip ties can provide extra security to the device so the leaf does not slip out. However, removing the zip ties requires an extra tool such as scissors. This adds unnecessary steps to disassemble the device.

Table 18: Pros and Cons of Alligator Clips

Alligator Clips	
Pros	**Cons**
● Widely used ● Potential to secure device	● Metal can corrode with opening and closing ● Bulky material ● Wear out housing device

Alligator Clips are another tool example that would provide optimal security to the device. They would make a great addition because they are versatile. Although the constant opening and closing of the clips can cause the metal to deform overtime. The wear debris can enter the media and harm the cells. Additionally, the well plate may not close because of the clamping mechanism. The well plate lid needs to be fully closed when placed into the incubator.

Table 19: Pros and Cons of Metal Latch

Metal Latch	
Pros	**Cons**
● Secures scaffold ● Cheap, durable and autoclavable ● Can be implemented anywhere on device	● Won't fit in six well plate ● Difficult design tolerances

A metal latch (similar to one found on a toolbox) is a viable solution to close the device. Latching the device ensures that the scaffold will be properly sealed and it will not float. Metal latches are also cheap, durable and autoclavable. In addition, they can be implemented anywhere on the device (ex: on the side), this provides flexibility while designing the product. One disadvantage is that the device won't fit in a six well plate due to the latch. Also, the design tolerances for the latch would need to be extremely precise. If the latch doesn't close fully, then liquid can leak which would negatively impact the experimental results.

Table 20: Pros and Cons of Plastic Clips

Plastic Clips	
Pros	**Cons**
● Cost effective and readily available ● Should secure scaffold well ● Allows device to fit in six well plate	● Not as durable as other materials ● Not all types of plastic are autoclavable

Plastic clips are a common tool used to close items. Similar to the metal latch, it will guarantee that the scaffold is secured. Also, they won't exert too much force on the leaf which decreases the chance of the scaffold rupturing. They are readily available, cost effective and come in various sizes. Depending on the size of the clips, the housing device can fit in a six well plate. One disadvantage is that not all plastic is autoclavable, the team would need to buy specific plastic clips for the project. Additionally, plastic isn't the most durable material and constant usage can lead to early deformation.

Table 21: Pros and Cons of Hose Clamps

Hose Clamps	
Pros	**Cons**
● Cost effective, readily available and durable ● Autoclavable ● Available in various sizes	● Causes obstruction ● Extra mechanical component needed

Hose clamps are typically made of metal which means it will be autoclavable. They are cost effective, readily available and durable. Subsequently, they are available in multiple sizes and can be adjusted depending on the user's needs. This provides more design freedom while

choosing the dimensions of the device. One disadvantage is it requires a screwdriver to close the clamp. This adds an extra mechanical component and increases the time it takes to assemble the device. Since hose clamps are circular, they would completely wrap around the entire device. This limits accessibility because the top and bottom portions of the device would be covered (more difficult to seed cells).

Table 22: Pros and Cons of Anchor Screws

Anchor Screws	
Pros	**Cons**
● Cost effective, autoclavable and durable ● Various types ● Will secure the device	● Need a screwdriver ● May clamp down too hard on the leaf

Anchor screws are commonly used for holding heavy objects such as bookshelves, large mirrors, etc. There are various types of anchor screws like hollow wall anchor screws, drive anchor screws, sleeve anchor screws, etc. Anchor screws are cost effective, autoclavable and durable. They are available in different sizes and shapes which gives more design freedom while creating the holes on our device. In addition, they will securely close the device allowing no leakage. One disadvantage is that anchor screws are permanent. Once they are screwed in, they cannot be removed. They also require a screwdriver which is an extra tool. Additionally, you would need to bring the screwdriver into the hood which increases the chances of contamination. Another disadvantage is that anchor screws are used for heavier objects. The screws may exert too much force and rupture the leaf.

Table 23: Pros and Cons of an Alligator Clip through a Pin

Alligator Clip through Pin	
Pros	**Cons**

● Cost effective and durable ● Easy way to close device ● Can be implemented anywhere	● Can fall off the device ● May not be autoclavable

An alligator clip through a pin is a cost effective and easy way to close the device. They are durable and are utilized for multiple applications. Alligator clips can be implemented anywhere on the device since they can clip down on any surface. One disadvantage is it can fall off the device and liquid can leak out. In addition, the outer material of alligator clips (usually rubber insulation) wouldn't be autoclavable. The rubber would have to be taken off which adds unnecessary steps to the assembly process. Lastly, the device wouldn't fit in a six well plate due to the size of an alligator clip.

Table 24: Pros and Cons of a Quarter Turn System

Quarter Turn	
Pros	**Cons**
● Cost effective ● Easy way to close device ● Will secure the scaffold	● Shear force can negatively impact the leaf

Using a quarter turn mechanism would decrease the amount of components needed for the device. The device would click into place after turning each piece. This is the most cost-effective solution since there are no external parts. It would also ensure the leaf would be secure and would not float. One disadvantage is the shear force from turning the pieces can exert too much pressure on the leaf. There is a higher chance of rupturing or crushing the sides of the scaffold.

4.3.2.2 Perfusion System

The perfusion system would allow for medical or other types of injections to be delivered into the scaffold.

Table 25: Pros and Cons of a Direct Injection System

Direct Injection System	
Pros	**Cons**
● Less mechanical components ● Can allow different types of flow	● Port can allow too much pressure to be exerted onto the scaffold

A direct injection piece on the device would allow a cannula injection port to directly perfuse liquid through the leaf. This would enable continuous flow or pulsation flow. Continuous flow indicates the amount of liquid (discharge) perfused through the leaf will be the same at all points. Pulsation flow implies periodic variations in the amount of liquid (discharge) being perfused through the port. An advantage of having a built-in injection port is having less mechanical components and different types of flow can be utilized. However, the leaf can rupture if the flow rate (from the port) exerts too much pressure.

Table 26: Pros and Cons of a Screw In Direct Injection System

Screw In Direct Injection System	
Pros	**Cons**
● Can tailor the system to handle different amounts of pressure ● Can allow different types of flow ● Can be placed anywhere on the housing device, more flexibility with design	● There's an extra mechanical component making device more complicated ● Makes it harder to position housing device on a well plate

A direct injection system that screws into the device can be very useful. Similar to direct injection, it could allow for either continuous flow or pulsatile flow. One advantage is that there can be different types of systems which can each handle a specific amount of pressure. This ensures the scaffold won't rupture during perfusion. It could also be placed anywhere on the device, this allows for more design flexibility. A disadvantage is it is an extra mechanical component which complicates the overall device. Another disadvantage is that it may be harder to position the device on a well plate depending on where the direct injection piece is located.

Table 27: Pros and Cons of a Continuous Gravity Flow

Housing Device Stands Upright (Continuous Gravity Flow)	
Pros	**Cons**
● Not as much pressure exerted on the scaffold ● A direct injection piece is not required ● less components	● There could be leakage from the scaffold ● Inconsistent results during experiments

The last perfusion system idea is the housing device stands upright. Essentially, the liquid would perfuse through the leaf via capillary action. Capillary action occurs when the adhesion of the walls (in a narrow tube) have a stronger force than the bonds between the molecules being attracted. Additionally, capillary flow is completely driven by the force of gravity. One advantage is that a direct injection system isn't required. The device can be placed near liquid and the liquid will perfuse through the leaf by itself. This limits the amount of pressure exerted on the leaf which decreases the chances of the scaffold rupturing. A disadvantage is that there may be liquid leakage from the scaffold. In addition, the absence of external forces can cause inconsistent results during experiments.

4.3.2.3 Imageability

Imageability was the final design need that was included in the design concept for the device. The 4 main ideas were to have an open cavity, or a nylon, glass, or metal mesh screen.

Table 28: Pros and Cons of an Open Cavity

Open Cavity	
Pros	**Cons**
● Doesn't provide any obstacle to imaging	● Would be difficult to engineer as the device still has to support the graft

The open cavity idea provides no obstacles that could hinder imaging. However, the problem is that the de-cellularized leaf needs an obstacle to prevent it from floating and the mesh screen contributes a good structure for the cells to grow on.

Table 29: Pros and Cons of a Glass Screen

Glass Screen	
Pros	**Cons**
● Transparency should not affect imaging ● Could apply surface coating to support cell adhesion	● Doesn't have mesh counts so seeding on both sides may be difficult ● Durability concerns

Glass screens are the next viable option to an open cavity design. They are clear and provide clear imaging through both sides of the graft. Glass can also have a surface coating which can enhance cell attachment. The problem with glass is that it isn't very common. For our purposes, it isn't feasible to engineer a new type of glass screen. In other words, the screen used in this device would have to be re-purposed from its original function.

Table 30: Pros and Cons of a Metal Screen

Metal Screen	
Pros	**Cons**
● Proven in practice ● Widely available in multiple different mesh counts ● Easy to clean	● Specifically need surgery grade stainless steel

A metal screen is a proven method from its past iterations of this device. It has been tested and verified for its application to this project. Metal mesh screens are also widely available, although, for this project, surgical-grade stainless steel would be needed. Metal is able to withstand any type of sterilization that would be required for the device.

Table 31: Pros and Cons of a Nylon Screen

Nylon Screen	
Pros	**Cons**
● Cheap ● Biocompatible ● Widely available ● Lots of research on its use within cell culture	● Not proven for this type of application ● Not Autoclavable

Nylon mesh is the cheapest of all the options. It has been widely researched for its use within cell culture, but this is not applicable to the current project. As mentioned before, metal is widely available in multiple mesh counts. These mesh counts would have to be retested for optimal growing conditions. One concern with this choice is nylon is not autoclave compatible. As a result, another method of disinfection/sterilization would be required.

Table 32: Pros and Cons of Removing Scaffold from Device

Removal from Device	
Pros	**Cons**
● Would provide most adaptable imaging	● Would only allow for fixed imaging ● More work for the user

The last idea is to remove the leaf for imaging. This would yield the most accurate and precise imaging out of all the ideas. Since the leaf would be removed from the device, it could be manipulated so that it could be imaged in various ways. One consequence is that Terminal fixed imaging is the only option. It would be challenging to put the leaf back into the device and continue growing cells on the scaffold. In addition, taking out the leaf adds another step for the user in order to complete imaging.

4.3.3 Quantitative Assessment of Design Elements

To rank the design element ideas, a decision matrix was utilized. The decision matrix was completed to determine which elements would be incorporated in the final design. A rating from 1 to 5 was created for each objective. The rubric for the ratings can be seen in Appendix F.

The decision matrix analyzed each design element that was brainstormed in the following 3 categories: Imageability, Perfusion, and User Friendliness. Both Constraints and Objectives were assessed. First a Yes or No was given to each element if they met a constraint. If a design element did not satisfy a constraint, the design element was not assessed further.

Table 33: The decision matrix used to analyze design elements

	Alligator Clip through pins	
Score	Normalized score	Weighted Sum
Y		
Y		
Y		
Y		
Y		
Y		
Y		
Y		
Y		
Y		
N		

For the elements that met all of the constraints, objectives and sub-objectives were analyzed. Using the rubric from before, a score of 1 to 5 was given to each element. The score was then normalized by dividing the score by the highest score or 5 within the sub-objective. The normalized scores for each sub-objective was then added up to obtain a total normalized score for an objective. The normalized scores were then multiplied by the main objective's weight which was determined based on the scores from the primary PCCs from the previous chapter. The weights were calculated out of 100%. Finally the scores for each objective were added to acquire a final score.

The final scores for each design element are assessed in Tables 34, 35, 36. Completed design matrices resulting in these scores are seen in Appendix F. The highest rated elements are highlighted in yellow.

Table 34: Final scores for user-friendliness

					User Friendliness					
Sliding System	Hingle System	Peg System	Magnetic System	Zip Ties	Screws	Metal Latch	Plastic Clip	Hose Clamps	Anchor Screws	Alligator Screws Through Pin	Quarter Turn
213.36	213.84				207.08	207.56			152		207.72

Table 35: Final scores for the type of perfusion system

	Perfusion System	
Direct Injection System	Screw in Direct injection system	Capillary Action/Continuous gravity flow
221.56	235.4	200.8

Table 36: Final scores for the imageability technique

Imageability				
Open Cavity	Glass	Metal Sceen	Nylon Mesh	Removal from Device
	171.72	235.4		235.4

5. Design Verification

After the quantitative analysis, the team's design elements were approved by the clients and users. Based on this, the team incorporated a hinge or clipping mechanism for the inner housing device. It also included a steel mesh and a silicone gasket. Lastly, the inner device would fit inside a six well plate. The outer housing device would contain the six well plate and facilitate a gravity flow perfusion system. The following sections demonstrate our design ideas and how they encompass all of the chosen elements. The goal of this analysis was to understand how each element can play a role in the entire device design. This ensured that the highest-scored elements would be part of the overall design along with the other elements. Each component was tested to decide on a final design.

5.1 Designs

As a group, the team brainstormed four concept designs. All designs had a hinge system and they would all be placed in the outer casing that contained six cannula ports for perfusion. Since there would be no cannula port attached to the housing device, there would be more space to work within the well plate. Lastly, all the designs can be autoclavable.

5.1.1 Concept Design 1

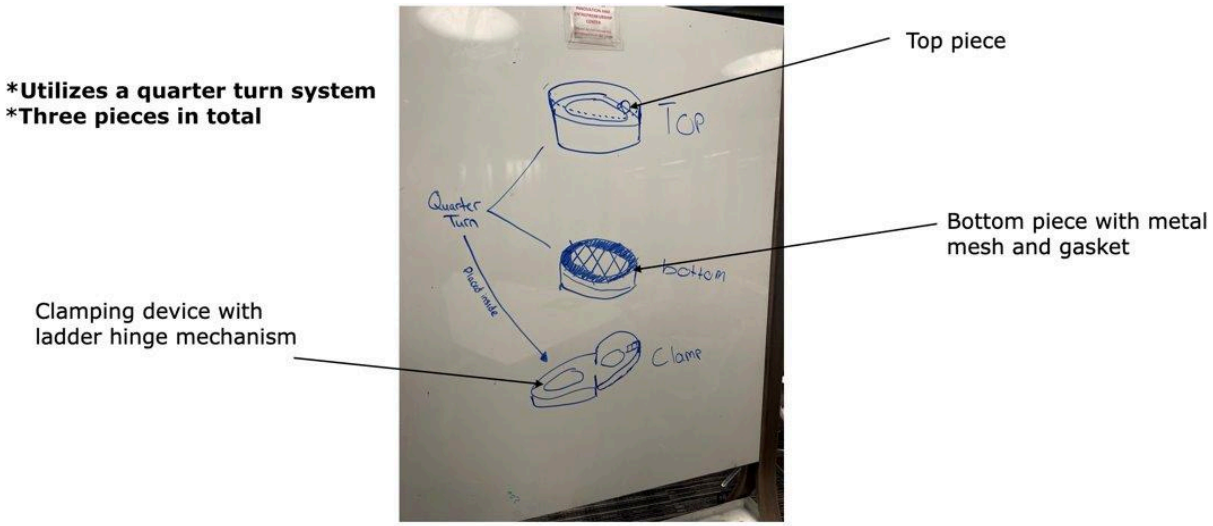

Figure 5: Preliminary drawing of concept design 1

This design utilizes a quarter-turn system and clamping mechanism. The top piece and bottom piece contain the metal mesh, the rubber gasket, and the leaf scaffold. The two pieces can be put together by quarter turning the top and bottom pieces together. The rubber gasket would protect the leaf from any mechanical forces from the turning. These two pieces would then be placed inside a housing device with a rung/clamping mechanism. Essentially, the rung would have different levels (each separated by a millimeter) and it would have a slot on the bottom of the device to click into place. There would also be a rod to secure the rung system. This design can exert different amounts of pressure on the leaf which guarantees the scaffold will not rupture. On the side of the device, there would be a space to feed the tubing into the leaf. This allows the user to perfuse any drugs or medical injections into the leaf scaffold. There would be a hole on the top and bottom of the housing device for cells to be seeded onto the leaf scaffold. Lastly, the entire housing device would be made from plastic (would need to be autoclavable). One disadvantage is that there are three separate pieces in this design, which makes the overall housing device more complicated.

5.1.2 Concept Design 2

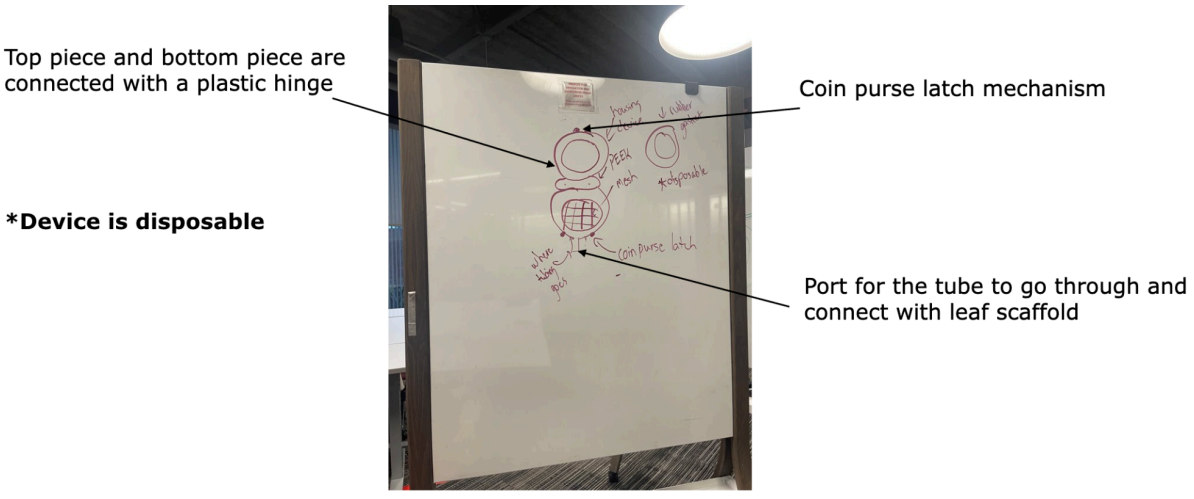

Figure 6: Preliminary drawing of concept design 2

This design is completely made of plastic which makes it disposable (after a few uses). The metal mesh, the rubber gasket, and the leaf would sit on the bottom of the device. There

would be a hole on the top and bottom to seed cells onto the leaf scaffold. On the front of the device, there would be a port to feed the tubing into the leaf. This enables the user to perfuse any drugs or medical injections into the scaffold. There will be a hinge mechanism to close the device. In addition, there is a coin purse latch on the top and bottom of the device. This secures the leaf scaffold in place while in the media. One disadvantage is it has lower durability because it is composed of plastic.

5.1.3 Concept Design 3

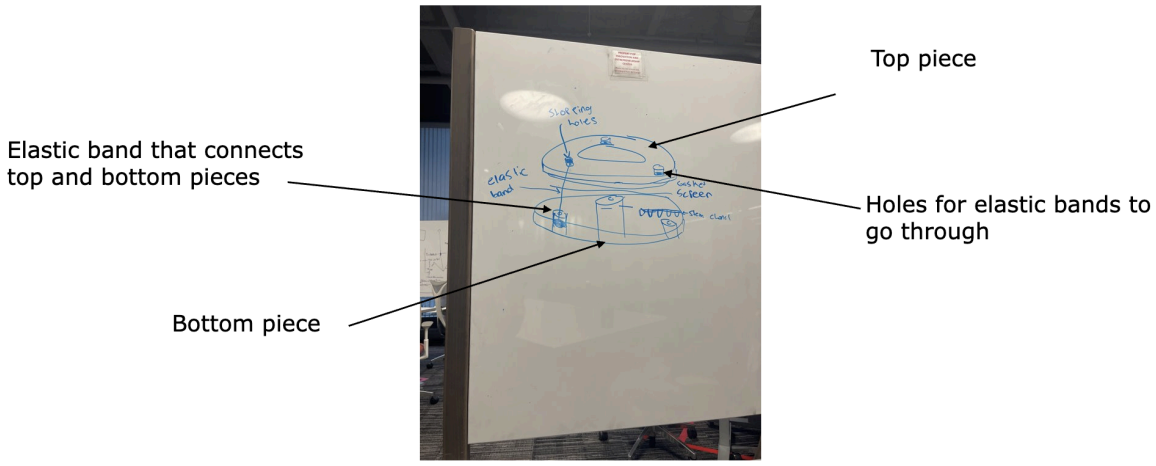

Figure 7: Preliminary drawing of concept design 3

This design uses rubber bands as the clamping mechanism. There will be a top and bottom piece. The bottom piece contains the metal mesh, the rubber gasket, and the leaf scaffold. The three holes on each piece allow the rubber bands to connect the components. There would be a hole on the top and bottom to seed cells onto the leaf scaffold. On the front of the device, there would be a port to feed the tubing into the leaf. This permits the user to perfuse any drugs or medical injections into the leaf scaffold. Despite its simplicity, a major disadvantage is not having the ability to control the amount of pressure exerted on the leaf. Also, the rubber bands will deform over time and the pressure exerted will decrease after each usage.

5.1.4 Concept Design 4

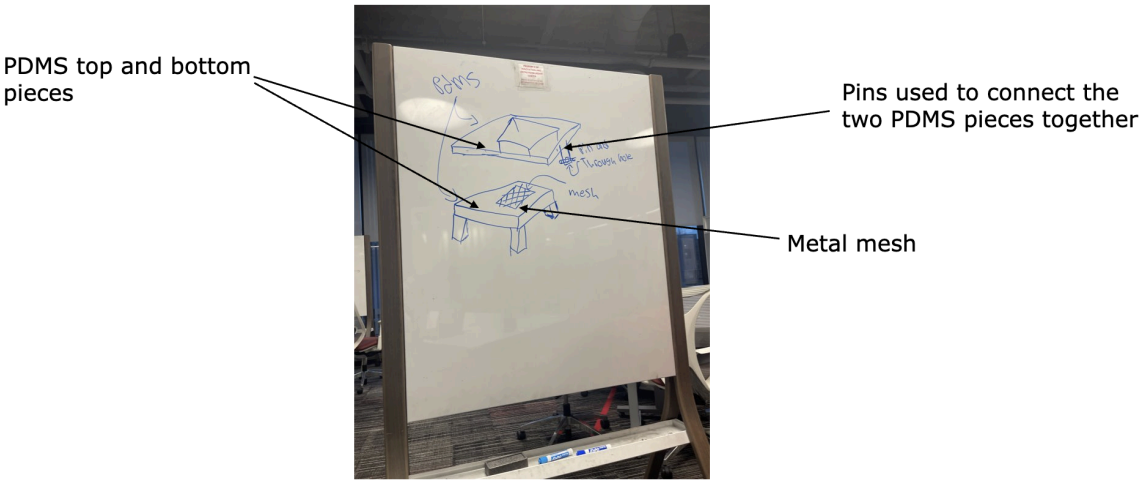

Figure 8: Preliminary drawing of concept design 4

This design is made from polydimethylsiloxane (PDMS) and has two pieces. The bottom contains only the metal mesh and the leaf scaffold. A rubber gasket is not required because the top piece can push the leaf down and secure it. There would be a hole on the top and bottom to seed cells onto the leaf scaffold. This allows the user to perfuse any drugs or medical injections into the leaf scaffold. There are two pins on the side of the device, this will connect the top and bottom pieces. A disadvantage is that a mold is required to create the housing device. An alternative would be 3D bioprinting the PDMS housing, however, those 3D printers are very expensive. Lastly, it would not be durable, and multiple devices would need to be created for one day of experiments. This can be a time-consuming process.

5.1.5 Concept Design 5

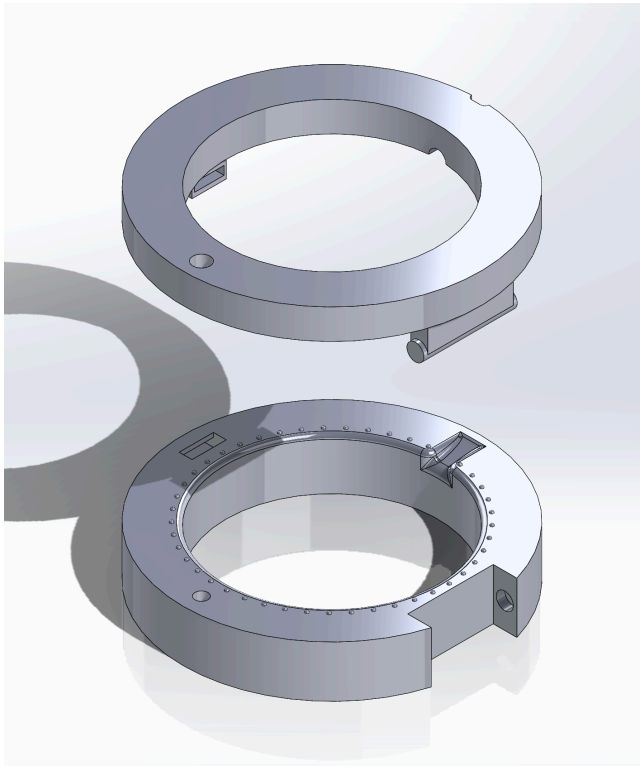

Figure 9: Inner Device Exploded Back View

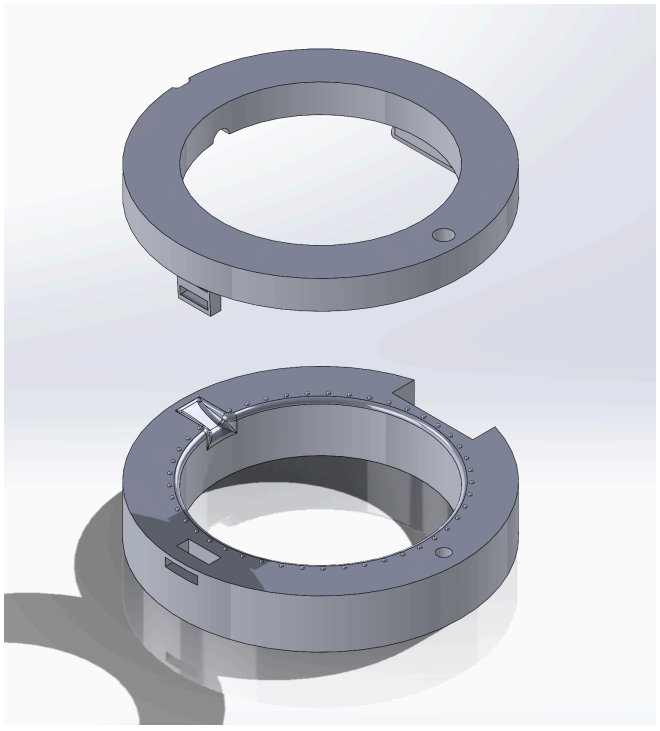

Figure 10: Inner Device Exploded Front View

This concept design includes a hinge system with a ladder adjuster. The ladder component is used to adjust the pressure that the top piece exerts on the leaf. There is a gasket underneath the top portion of the device which holds the leaf down. The stem comes out of a small cylindrical indent that is within the top and bottom portion. This enables the device to connect to the tubing. There is a small hold within the device that allows access for the pipette to change the media. The device would fit in a 35mm cell culture plate which will go into an outside device mentioned in the next section.

A Pugh Analysis was required to compare the different designs of the new device to the design of the current device. Each primary objective was weighted depending on their score from the primary objective rankings. Based on those scores, the weighted scores were rounded to the nearest whole number. If the team thought the requirement performed better in one of the designs than the current device, it received a score of 1. If it was similar, it would score a 0 and if the requirement performed worse, it would receive a score of -1.

Table 37: Pugh Analysis of Concept Designs

Requirement	**Weight**	**Baseline**	**Design 1**	**Design 2**	**Design 3**	**Design 4**	**Design 5**
Imageable	3	0	0	0	0	0	0
Perfusible	5	0	.5	.5	0	0	1
User Friendly	4	0	1	1	.5	0	1
Total (with weight)	N/A	0	6.5	6.5	2	0	9

5.1.6 Outer Device Concept Design

While designing the inner device that holds the decellularized leaf, the team encountered an obstacle with the 35mm cell culture plate. The top plate would have to be modified so that it would be easier to separate it from the perfusion system. This is necessary to obtain accurate imaging of the skin grafts. To engineer around this problem, the team brainstormed an outer housing device. This would make it easier to disconnect the perfusion system from the leaf for imaging. Examples of this design are shown in Figures 9, and 10.

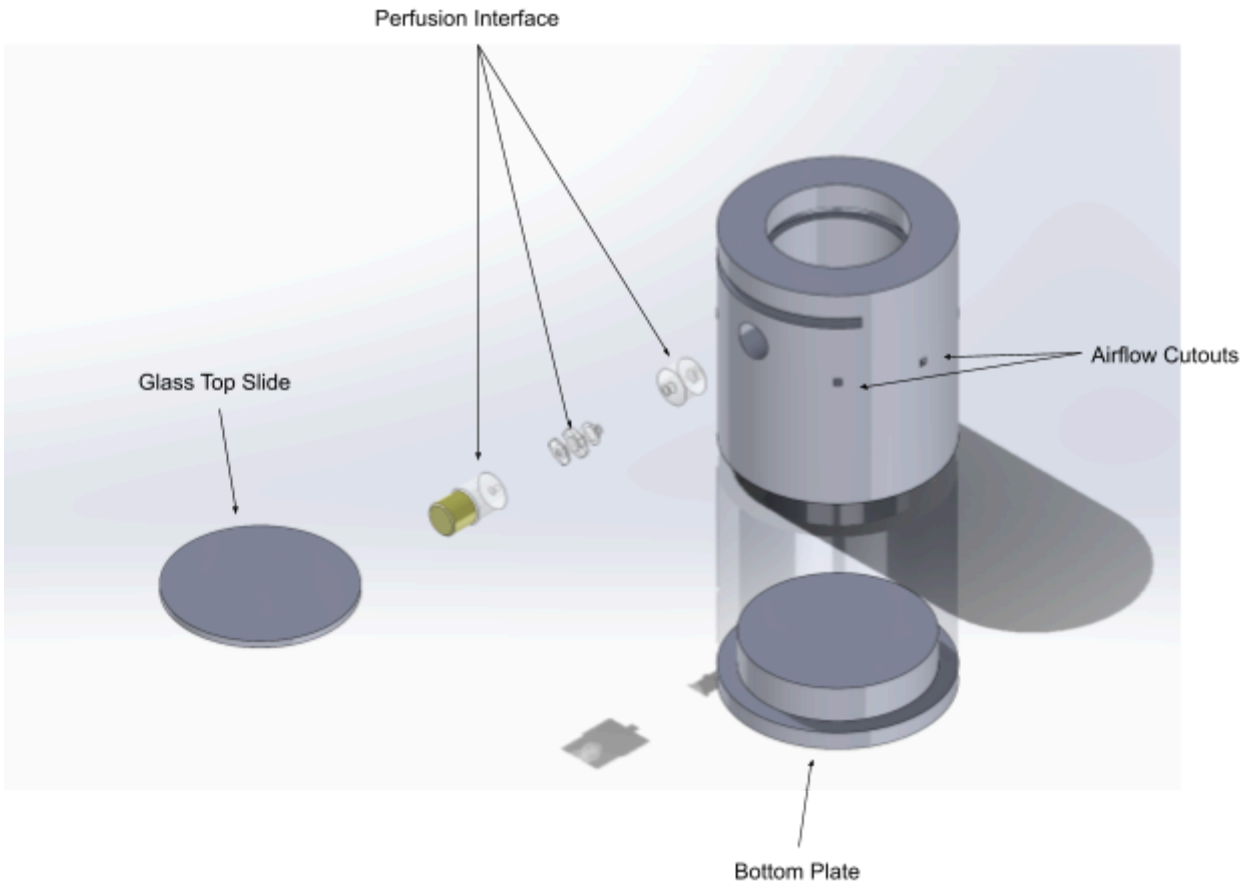

Figure 11: Shows a CAD model in exploded view of the entire design including the housing and outer chamber (contains perfusion system)

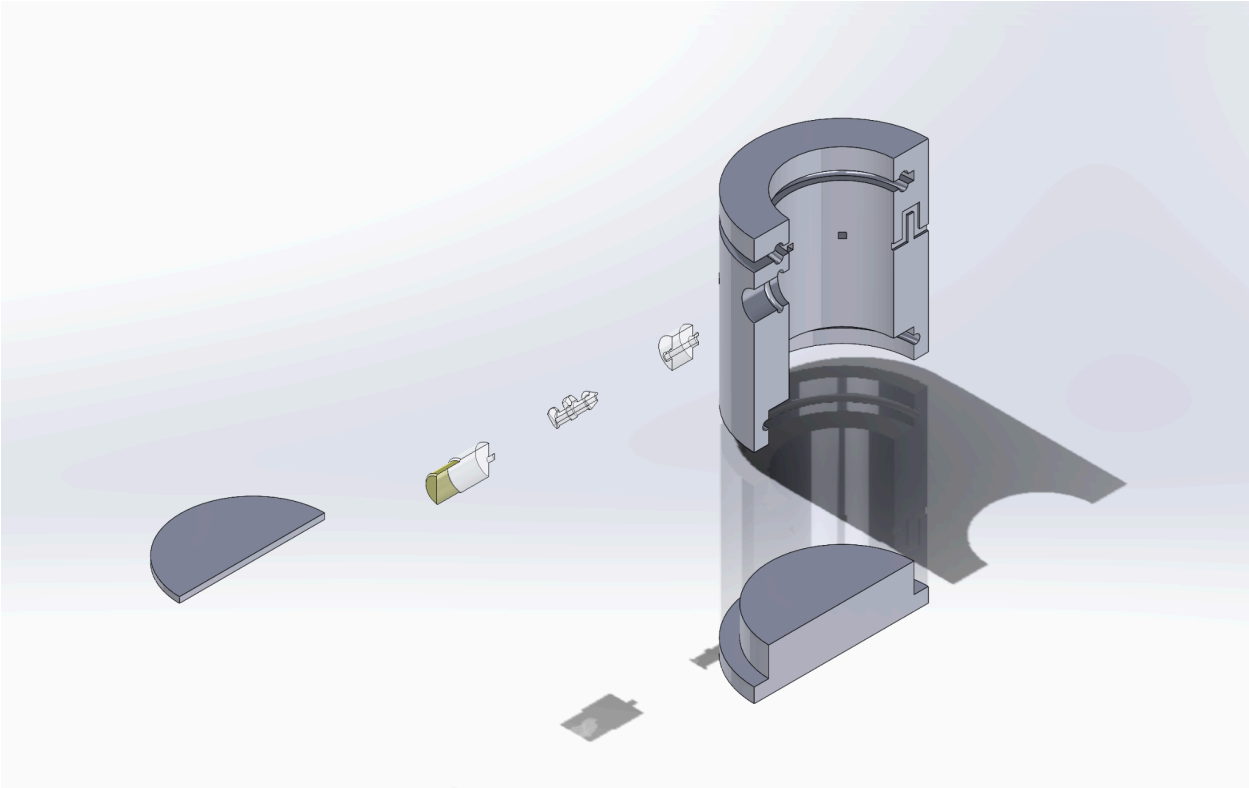

Figure 12: Section view of the exploded CAD model.

Similar to the inner device, the outer device needed to be user friendly. The team accomplished this by removing mechanical components and relying on pressure fittings (using silicone gaskets). The main shell has openings for the bottom piece that contains the 35mm plate, the luer lock perfusion system, and a glass disk which acts as the top plate. The main shell also includes an inverted "U" shape extruded cut to mimic the airflow in a traditional 35mm dish. This airflow is essential because it decreases the risk of contamination. The technical bulletin *Understanding and Managing Cell Culture Contamination* from Corning states "It is very easy for the space between the top and bottom sidewalls of a dish, or a flask and its cap to become wet by capillary action with medium or condensation. This thin film of liquid then provides a liquid bridge or highway for microorganisms to either swim or grow into the culture vessel" ("*A Guide To*", 2017).

The main shell of the outer device incorporates the perfusion system interface as well. This perfusion system was repurposed from the 2016-2017 Major Qualifying Project titled

"*Design of a Universal Microscope Incubator for Drug Screening of 3D Models of Engineered Myocardium*" (Connolly et. al 2017). The perfusion system included a male and female luer lock as well as a connector piece. These pieces were incorporated in the device by creating a circular hole in the outer shell with a slightly bigger diameter than the connector. The connector had the largest diameter of any piece in the perfusion system subassembly. A gasket was cut out on the inside of the hole to hold the connector piece securely and create an airtight seal. The male luer lock is exposed from the outside of the device creating an easy interface with the perfusion pump.

A problem with this design is that it has many intricacies. This could pose a challenge both when 3D printing the device as well as inserting the silicone gaskets. Two of the three gaskets are positioned in spots that may require the use of outside tools to insert them correctly, increasing assembly times.

5.2 Material selection

After initial prototyping, different materials were explored to determine which best fit within the constraints and objectives of this project. The two main parameters that this material needed to meet was being non-cytotoxic and autoclavable. The initial materials investigated were polypropylene (PP), glass filled polypropylene (GFPP), and polyphenylsulfone (PPSU). GFPP and PPSU were both infeasible because they required specific equipment to 3D print which was not available to the group. Polypropylene was selected due to it being autoclavable, non-cytotoxic and not needing specialty parts to be printed. The polypropylene filament selected was manufactured by Fiberology. This was chosen because the filament was tested under ISO standards which ensured its uniformity. Literature also shows minimal deformation in 3D printed polypropylene parts ensuring the longevity of the device (Fischer et. al 2021).

Silicone and surgical grade stainless steel were chosen to support the leaf matrix and to ensure the device had a watertight seeding chamber. The team believed these would be useful because they are utilized in the current device in the Pins Lab. Both of these materials are biocompatible and autoclavable, adhering to both the constraints and objectives of this project. The selection of polypropylene filament from Fiberology was a strategic choice. It aligned with the technical requirements and quality standards of the project. This decision laid a solid foundation for the subsequent stages of development.

5.3 Design Iterations

Initial prototypes were made using 3D printing. This method was chosen because it is easily integrated with 3D modeling CAD softwares. 3D printing is also cheap and timely with parts only taking a few hours to create and costing less than 50 cents per printed piece. 3D prototype printing was done using an Ender 3 3D printer with PLA as the material choice. An image of the Ender 3 can be seen below (Figure 13).

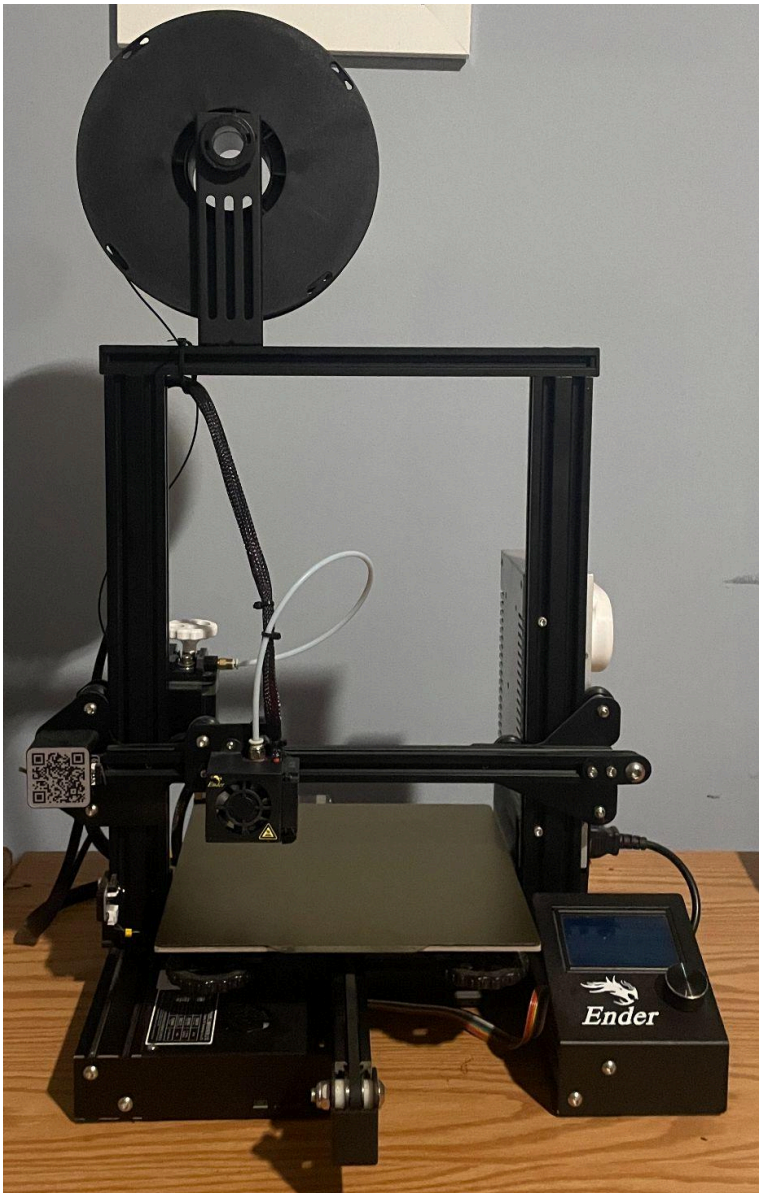

Figure 13: Creality Ender 3 3D printer

While prototyping, multiple errors were found with the selected inner and outer device design. Many of these errors were foreseen as stated in section 5.1.6 due to the intricacies and small dimension in the chosen concept design. In total, 5 iterations of the final design were completed before the final design was achieved.

5.3.1 Inner Device Design Iterations

The inner device had many issues when first being printed. The latch mechanism of the design did not work due to the small dimensions of the rectangular cutouts in the top piece. Despite it working in the CAD model, 3D printing could not achieve the correct definition in reality. The second problem with the latch was that the pin used to lock the device into the plate was too small and delicate. There was a large risk of the pin breaking when trying to remove it from the device. This would cause the device to be permanently closed. The iterations before the final design were achieved by involving the holes for the pin to run perpendicular to the origin pin placement. This iteration had larger holes for the pin and the pin ran through a longer section of the device. However, this was infeasible because the tolerance requirements were not compatible with 3D printing.

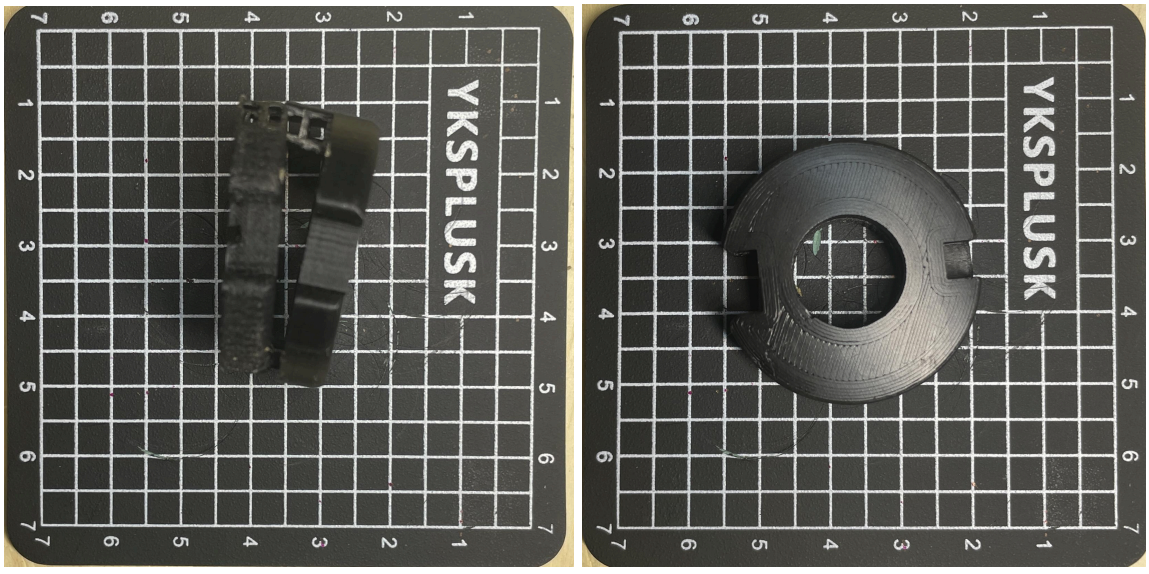

Figure 14: Latch Mechanism Design Iteration. As seen in the image the rectangular cuts are not consistent due to 3D printings limited definition.

The micro-indentation used to hold the mesh in place did not work either due to the same 3D printing issue. Consequently, the team reverted back to the current device design for the mesh mechanism. It would involve an extruded cut in the top side of the bottom piece.

The hinge also had issues because of the rigid structure of the bottom piece. Since it was too rigid, the critical extension on the top piece would not fit into the cut out slots when compressed. The circular cutouts on the bottom piece that the hinge slid into were also too small and not printer compatible. There was only one iteration of the hinge and it is shown below in the final device.

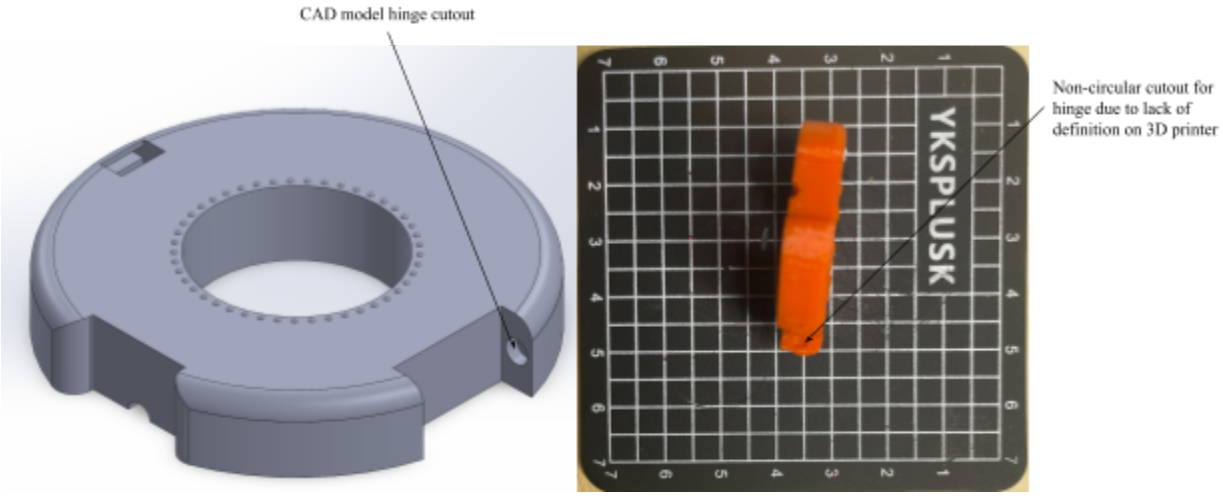

Figure 15: Initial hinge design after printing. As seen in the image the cutouts for the top components of the hinge are not circular nor consistent

The circular cutout for pipetting was modified because the dimensions were too small. While the cutout would have been compatible with small pipette tips, it would not have been compatible with stereological pipette tips or 1000 µL tips. As a result, the design was modified to make the cut out bigger and rectangular. A channel was also added between the cut out and growth chamber due to media flow and aspiration concerns.

There were also issues with the silicone insert because of the specificity of the shape. When searching for compatible o-rings from established suppliers, the correct sizing could not be found. The o-ring insert had to be modified to facilitate easily sourceable o-rings.

The leaf stem cutout was also modified because the initial cutout did not facilitate the flipping of the leaf. If the device was unable to flip properly, keratinocytes could not be seeded.

The design was changed by mirroring the cutout made in the top piece for the leaf stem onto the bottom piece.

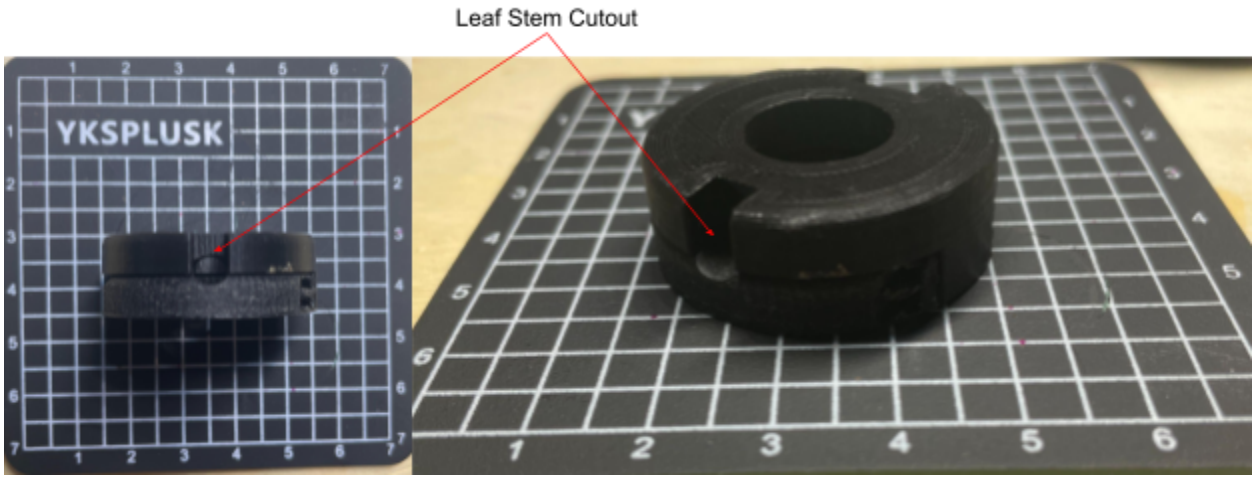

Figure 16: Design Iteration of the leaf stem cutout. This iteration also has the problem of not allowing for the stem to exit either side of the device

The outer diameter dimension of the inner device had to be changed as well. This was due to two reasons. The first reason was that the device would be compatible with a 6 well plate, this required reducing the outer diameter. The second reason was due to the bulging of the part. This occurred because of the 3D printer method. 3D printers function by heating plastic filament to its glass transition temperature and then laying lines of that plastic filament down in layers. As a result, each layer compresses the layer below which causes bulging at the base of the part.

5.3.2 Outer Device Design Iterations

After initial printing of the outer device, multiple issues were identified due the complexity of the device. The inverted U channels, as well as the glass disk and associated silicone o-ring were unnecessary. These parts could be replaced by re-using the top lid of the existing plate. The cutouts for the perfusion system were also difficult to design because of its intricacies. The support structure required to print the design was not easily removable. The midplane cutout for the o-ring was larger than the circular through cut for the perfusion system. This made it impossible to insert. The design of the through cut and midplane cut also did not allow for different perfusion systems to be utilized. This narrowed the scope of its potential use of the device. The o-ring insert on the bottom of the device was also removed because the seal

created flat bottoms for the main shell and bottom plate which was sufficient. The outer device was altered to facilitate a 6 well plate rather than a 35mm plate. This would be more efficient and practical at growing multiple skin grafts simultaneously.

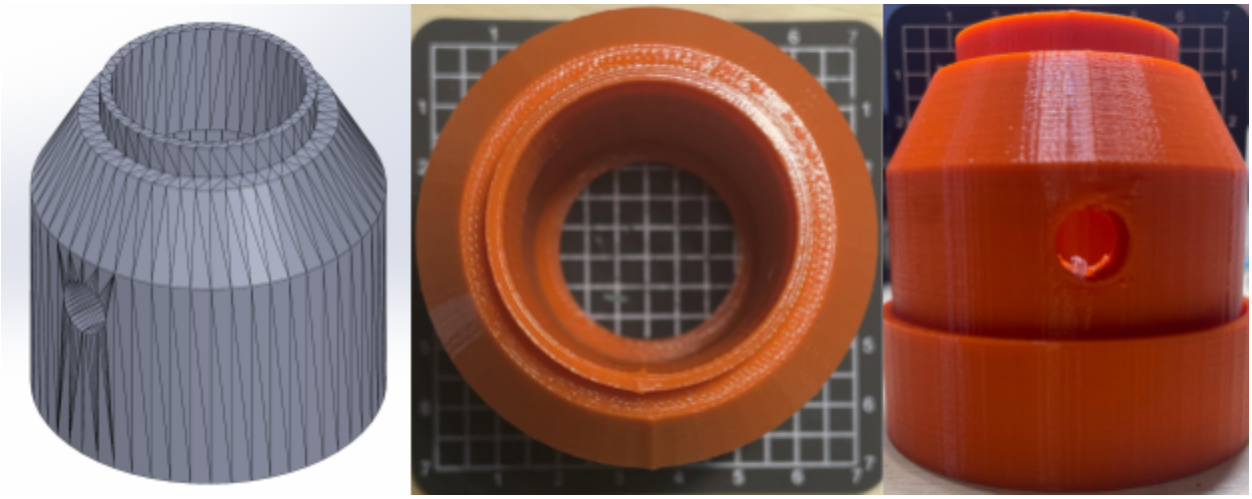

Figure 17: Design iteration of the outer device. This design was for the 35 mm plate rather than the 6 well plate.

5.4 Final Device

A final design was reached after all design interactions. The final device was manufactured using 3D printing. This was done on a combination of 3D printers including the Ender 3, and Ultimaker S3 due to availability. 3D printing was used as the manufacturing method due to its affordability when producing a low quantity of parts, and its wide availability to students across the institution. 3D printing was also appealing due to its low barrier to entry with printers only costing a few hundred dollars, and relatively easy learning curve for its basic functions.

This design featured an inner and outer device that worked together to create a perfuseable system that can be utilized by the Pins lab. CAD assembly models and the exploded view can be seen below Dimensional drawings of components used in the final design can be found in Appendix H.

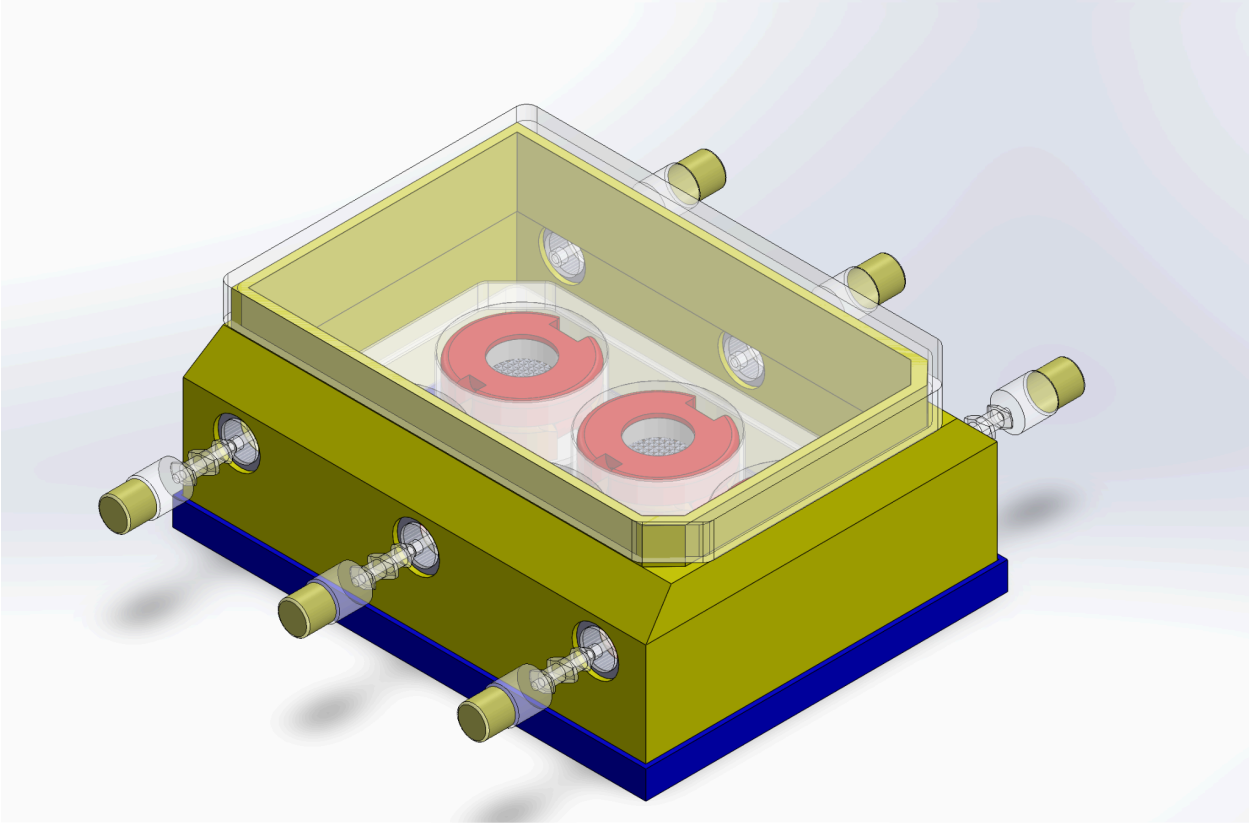

Figure 18: Assembled Final Device CAD Model

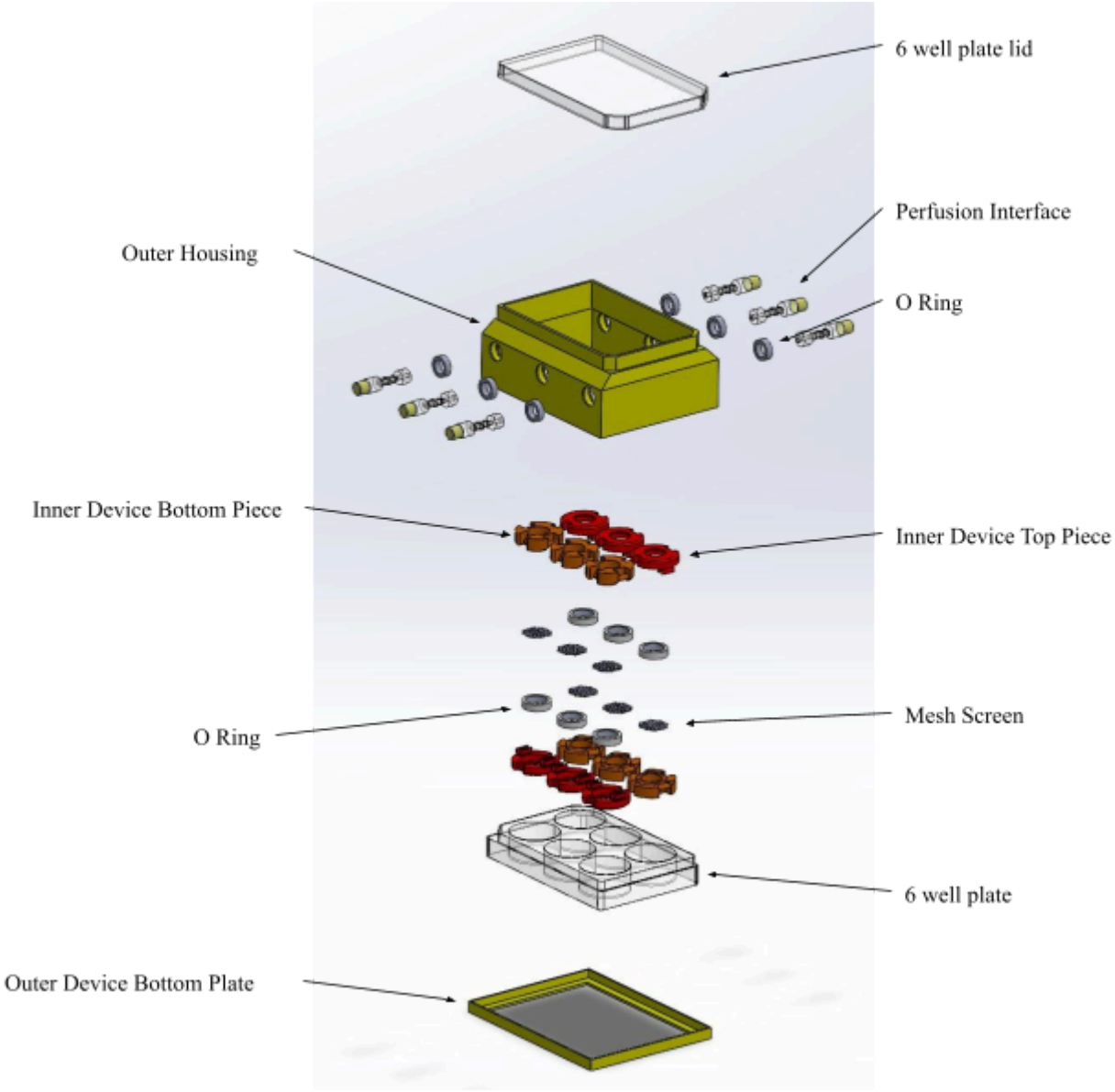

Figure 19: Exploded view of full assembly

5.4.1 Inner Device

After further iterations on the chosen 5th concept design, a final A/L interface culture device was produced. The device followed a similar theme, it had a four piece construction of a rigid top and bottom piece, a silicone o-ring and a stainless steel mesh screen. The device features five distinct cutouts and a growth area of 1.767 cm^2. These cutouts are for the o-ring,

mesh, leaf stem and pipette. An image of the device and an exploded view of the full inner assembly can be seen below.

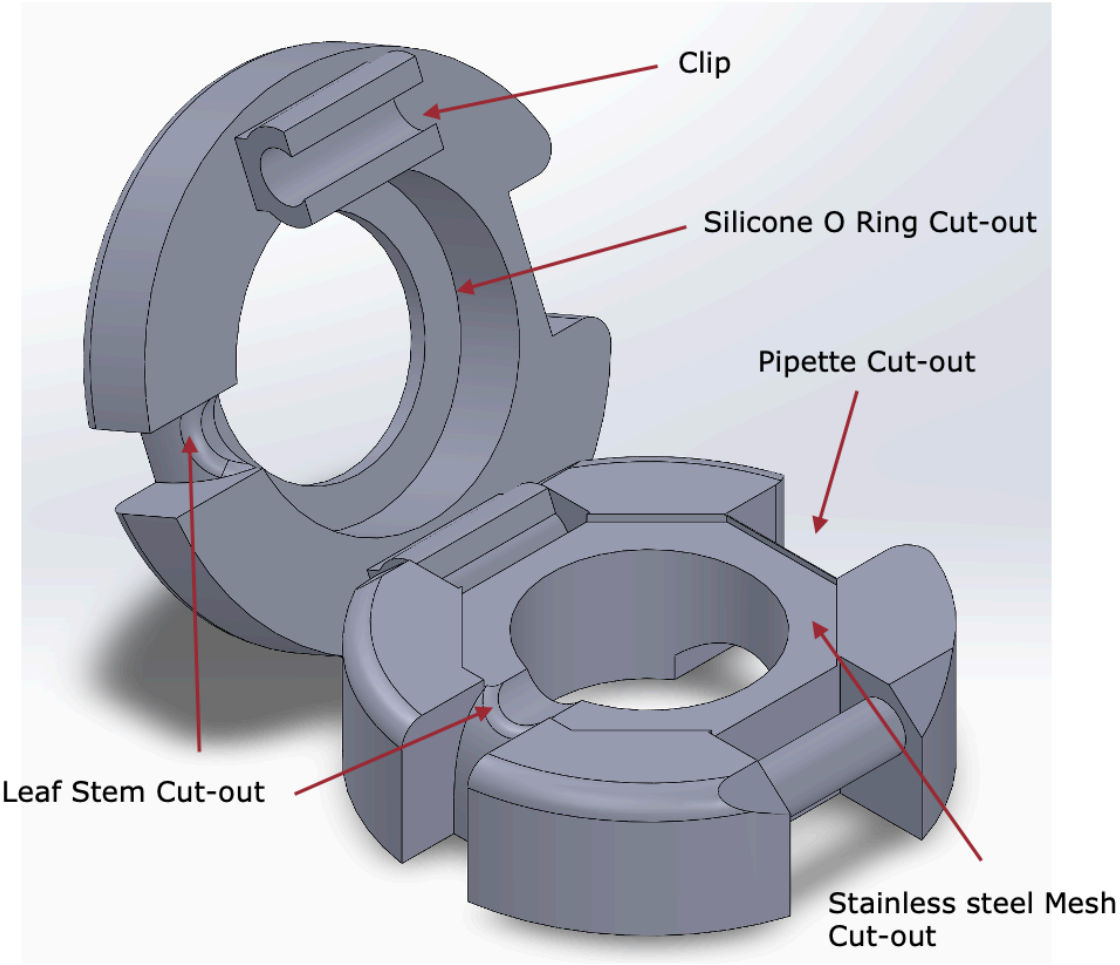

Figure 20: Assembled Inner Device (does not include silicone o-ring or mesh screen)

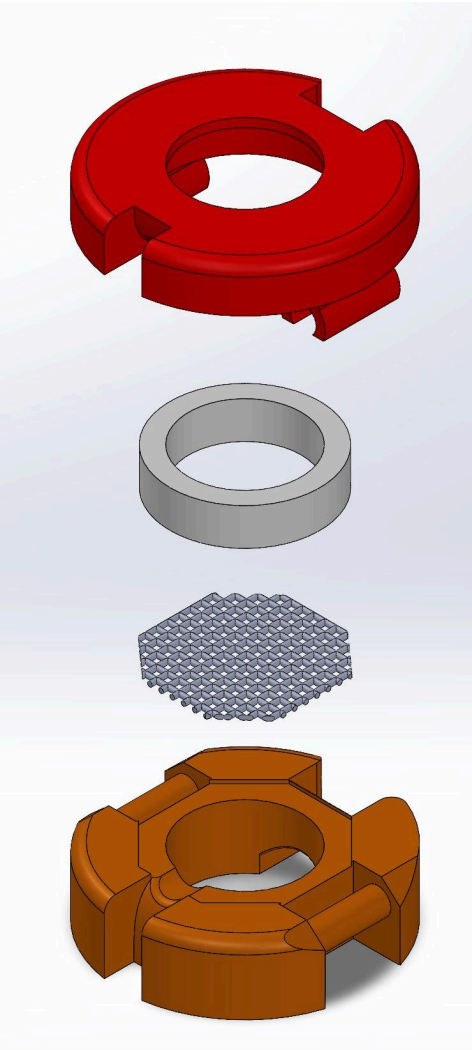

Figure 21: Assembled Inner Device Exploded View

The latch used in the final device was inspired by that used in LEGO. This features a bar latched onto by a semi cylindrical extrusion. The design was selected due to its simplicity and ease of manufacturing. This style of clip also acts as a hinge allowing for easy ambidextrous use of the device. The clips enable easy separation of the device using a simple pry tool.

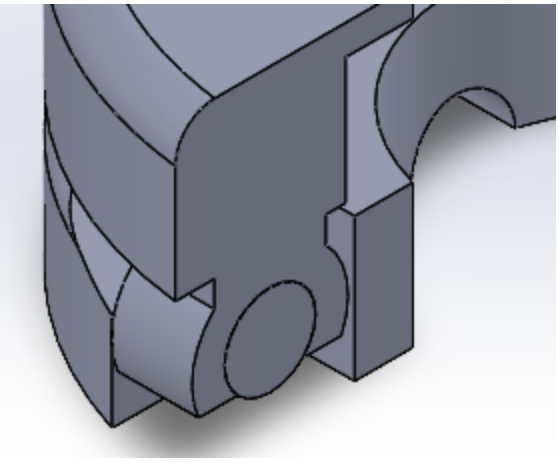

Figure 22: Close up of Latch and hinge mechanism

The cutout for the leaf stem was a crucial design parameter for this device. It is the method by which the leaf is being perfused. Since the leaf stem is delicate, the cutout was smooth and curved. This eliminated concerns of the stem or the attached tube crimping. In both scenarios, there would be a blockage of perfusion or the leaf stem would rupture. Additionally, this could cause pressure spikes which would damage the delicate decellularized vasculature of the leaf. A close up of the leaf stem cutout can be observed below.

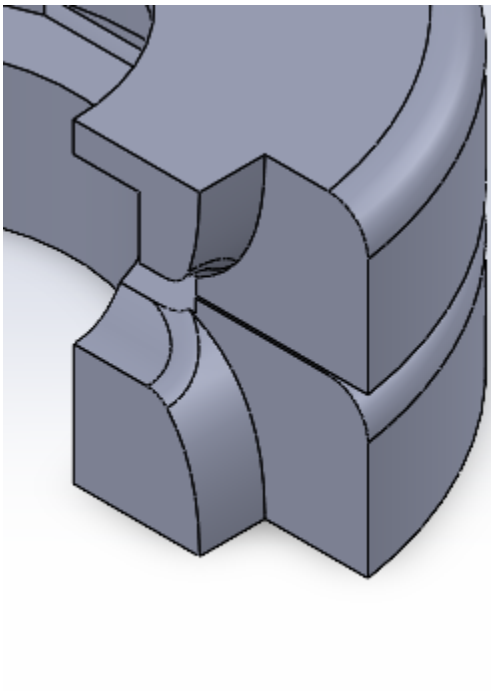

Figure 23: Close up of Leaf Stem Cut Out

The cutout for the pipette features a channel that runs into the growth area. The cutout is designed to fit up to a 1000 µL pipette tip. The channel underneath ensures there is no vacuum created during aspiration, allowing for media flow. A close up image of the pipette channel can be seen below.

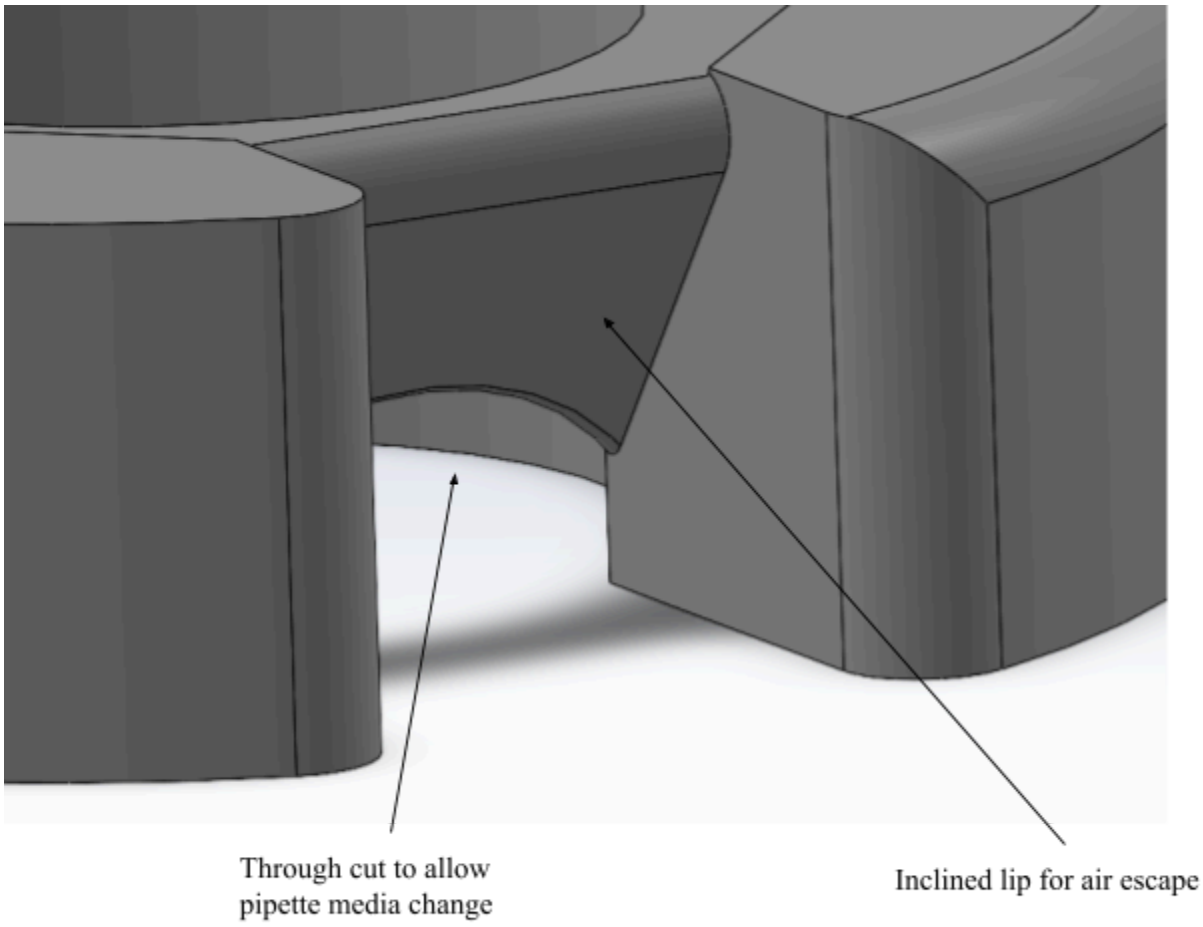

Figure 24: Close up of Pipette Cut Out and Media Channel

5.4.2 Outer Device

The final design of the outer device facilitated perfusion through the leaf, allowing the inserted tube to be connected to a central reservoir for gravitational perfusion. The device has 4 main components. An image of the assembled outer device can be viewed below.

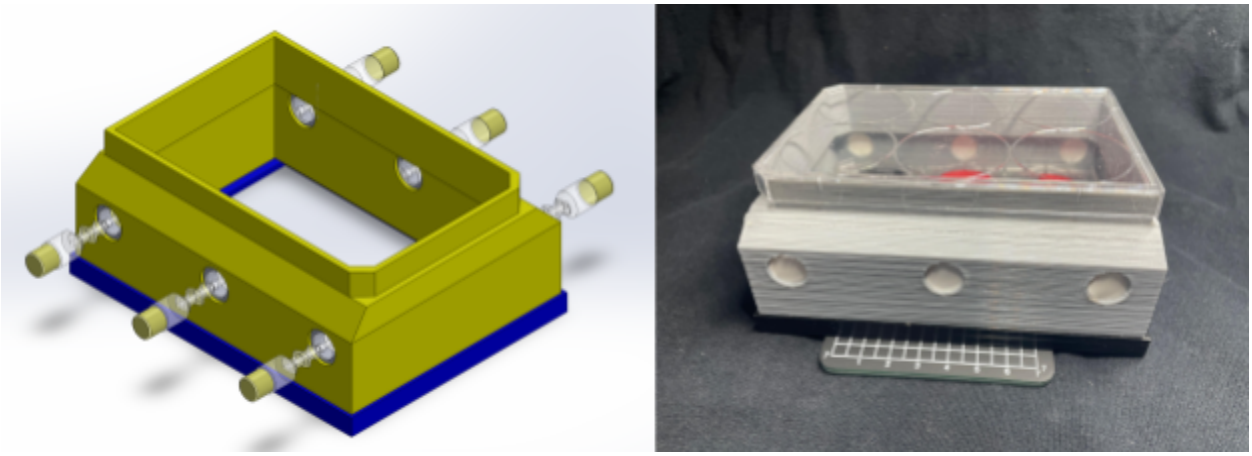

Figure 25: Full Assembly of the Outer Device

The first component is the bottom plate of the device. This piece slides around the outer shell of the device and holds the 6 well plate used to grow the skin grafts. The plate cutout in the middle promotes easy imaging of the wells. It also eliminates the need for the graft to be removed from the inner device during imaging. An image of of the bottom plate can be observed below

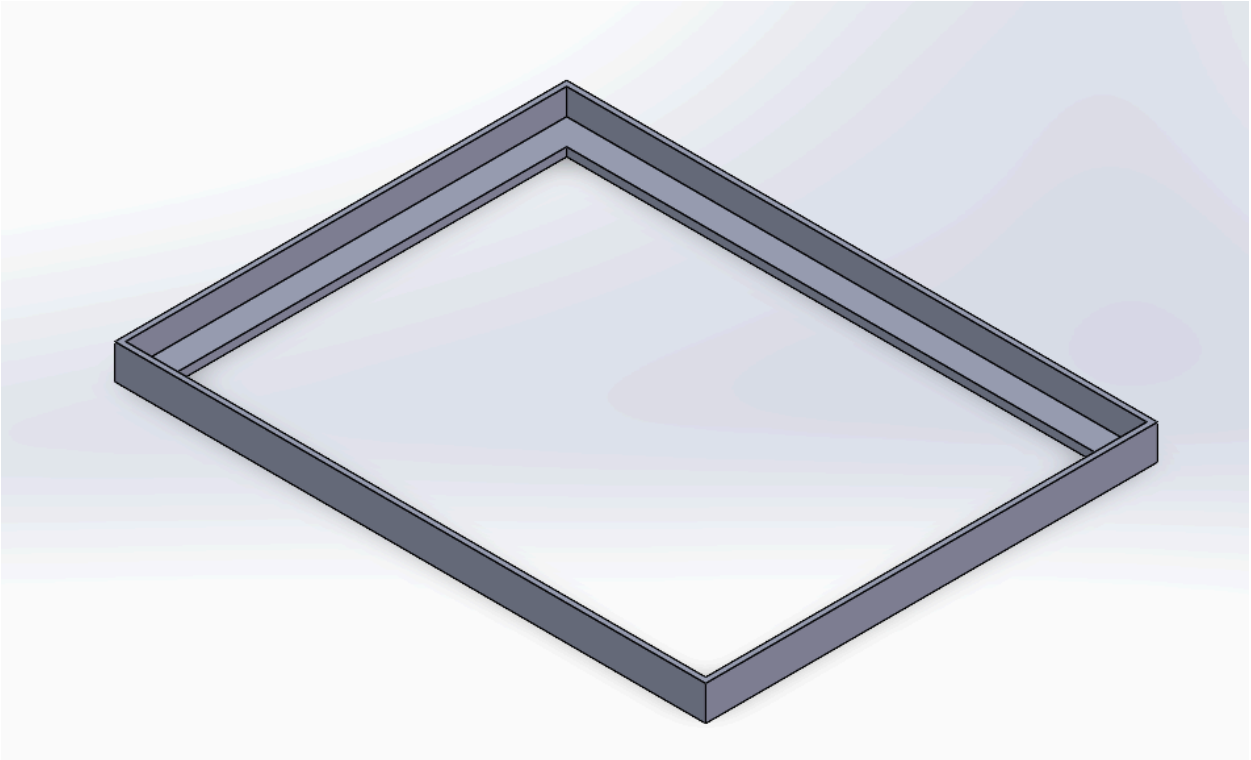

Figure 26: CAD Model of Bottom Plate

The second component is the main shell. The cutout is toleranced to ensure an easy fit with the 6 well plate. It features a slimed top extrusion to enable the lid of the 6 well plate to allow airflow through the device. The main shell also features six circular cutouts for the injection system. These cutouts have a midplane that allows for silicone disks to be inserted. This design promotes customization because it can fit different amounts of skin grafts. There is another cutout in the silicone disks that accommodate the perfusion system. Images of the exterior shell and midplane cut are shown below.

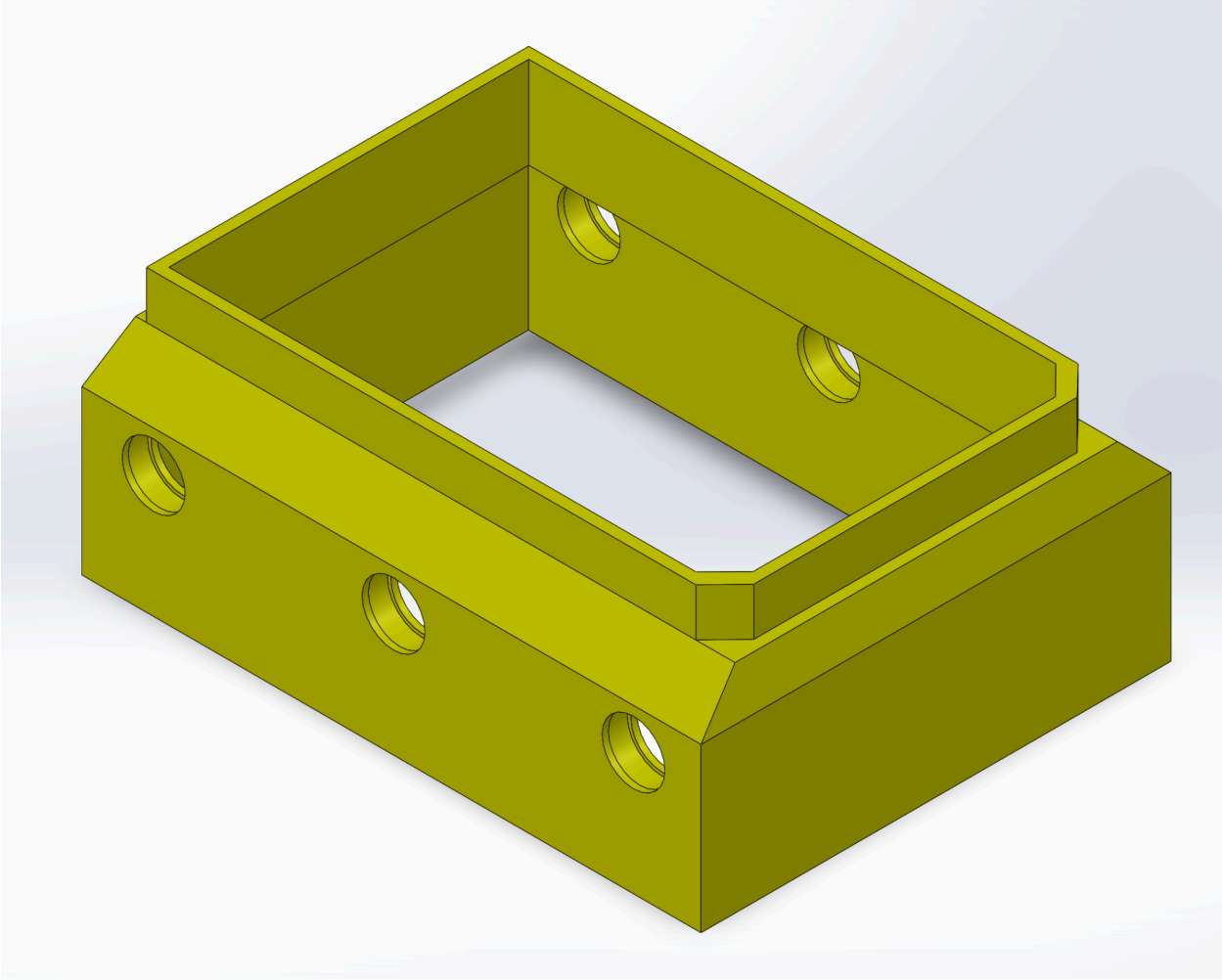

Figure 27: CAD Model of Outer Shell

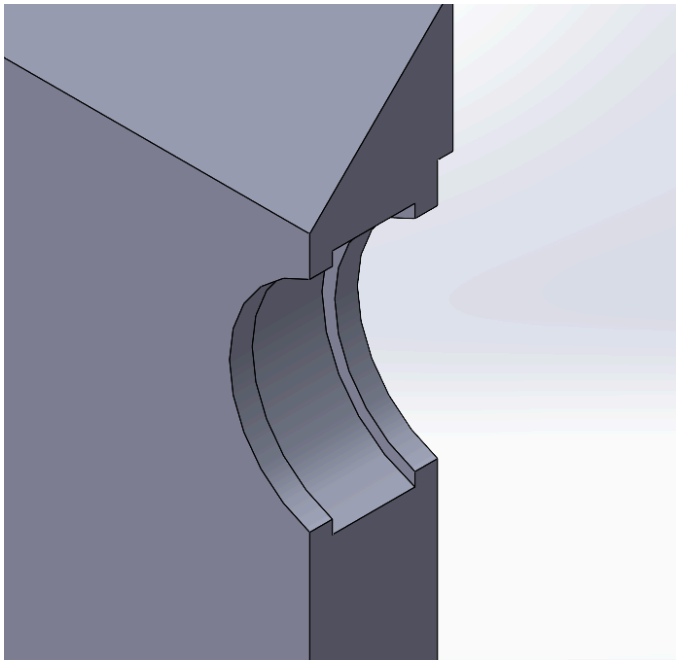

Figure 28: Circular and Midplane Cutout of Outer Shell

The 4th and final component in this design is the perfusion system. This is the same perfusions system mentioned in section 5.16 and is adapted from a Major Qualifying Project completed from 2016-217.

5.5 Testing Methods

Preliminary testing for each design aspect was conducted to determine how well they would perform. The team tested user-friendliness, imageability, perfusion, and autoclavable/sterility. A preliminary version of this device was created so that these tests could be performed.

5.5.1 Perfusion Testing

Perfusion tests were conducted at different flow rates using a PDMS microfluidic system as an analog to a decellularized spinach leaf. The PDMS system had similar dimensions to a leaf punch out within the device (1cm by 1 cm). Our group utilized gravity flow to test the perfusion of the PDMS systems. There were four pieces of the PDMS assembly, two full pieces and two half pieces (one PDMS piece cut in half). The two half pieces were placed on one full PDMS

piece and were separated by 1 mm. This mimicked the cannula tube that is placed into the leaf. The third PDMS piece was placed on top of the two halves, this completed the single-channel microfluidic system. In order to mimic a vascularized system, our group created a channeled PDMS microfluidic system. The only difference was one of the PDMS pieces was cut into fourths instead of halves. The PDMS microfluidic system is seen in Figures 29, 30 and 31 below:

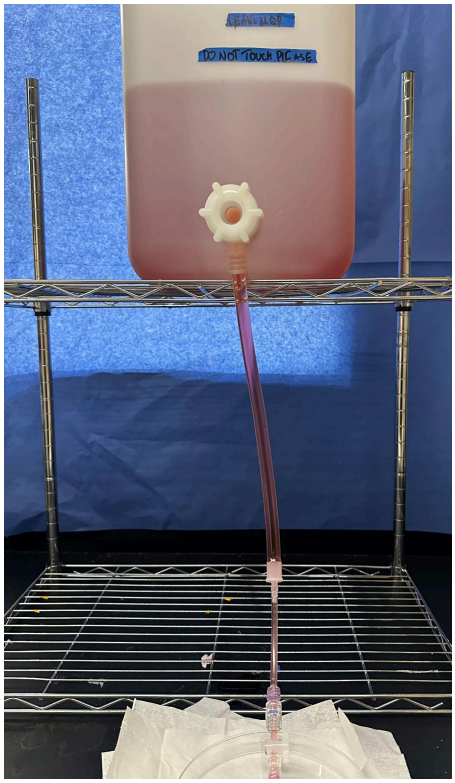

Figure 29: Carboy setup

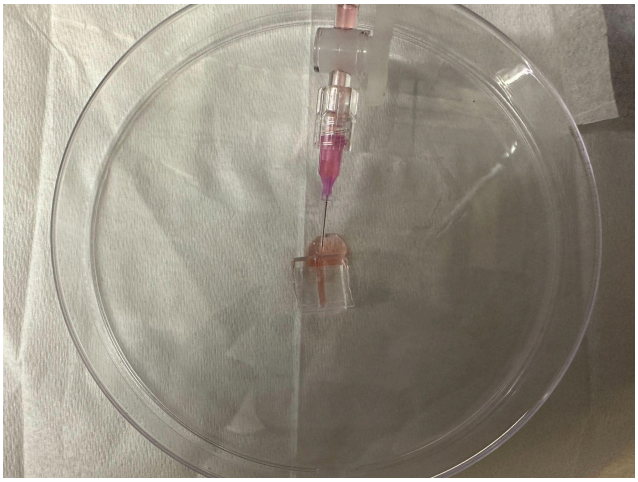

Figure 30: Perfusion of liquid through the single channel PDMS microfluidic system through a cannula

Figure 31: Perfusion of liquid through the multichannel PDMS microfluidic system through a cannula

For the gravity flow setup, the main components were a carboy tank (filled with red dye), 2 different polyethylene size tubings, 1 plastic tube connector, a plastic valve connector, 1 plastic

luer lock connector, a 30 gauge blunt tip needle from BSTEAN ™ , and a polyethylene cannulated tube. All materials besides the 30 gauge needle were found in the lab. The tube that was directly connected to the carboy was 0.0096 m in diameter. It was firmly secured to the nozzle to ensure no leakage. A tube with a diameter of 0.00266 m was attached to the larger tube via a connector. This tube was fixed to a valve connector with the same inner diameter. The valve connector had a luer lock on the end which was used to screw in the 30 gauge needle (diameter 0.0003 m) The needle was inserted into a cannula tube with a diameter of 0.00038 m. Subsequently, the cannula tube was inserted into the PDMS microfluidic system. The entire setup is seen in Figures 30 and 31 above.

Our group conducted three trials in total, each trial found the flow rate for the single-channel system and the multi-channel system. The lengths of the tubes for all the trials remained constant. The large tube had a length of 0.285 m, the medium tube had a length of 0.148 m, the needle had a length of 0.0217 m and the cannula had a length of 0.048 m. The height of the water level in the carboy varied for each trial. The first trial was 0.15 m, the second trial was 0.18 m and the third trial was 0.22 m. The liquid was perfused through the microfluidic systems for 10 minutes. The liquid collected in the petri dish was pipetted into a 5 mL graduated cylinder. The volume was recorded and converted into μL. From the time and volume values, the equation $Q = V/t$ (volume over time) was used to calculate the experimental flow rate. To find the fluid velocity in each tube, the equation $Q = (A)(v)$ was utilized (area over velocity). The example schematic shown in Figure 30 was used as a guide to calculate the experimental pressure drop between each tube.

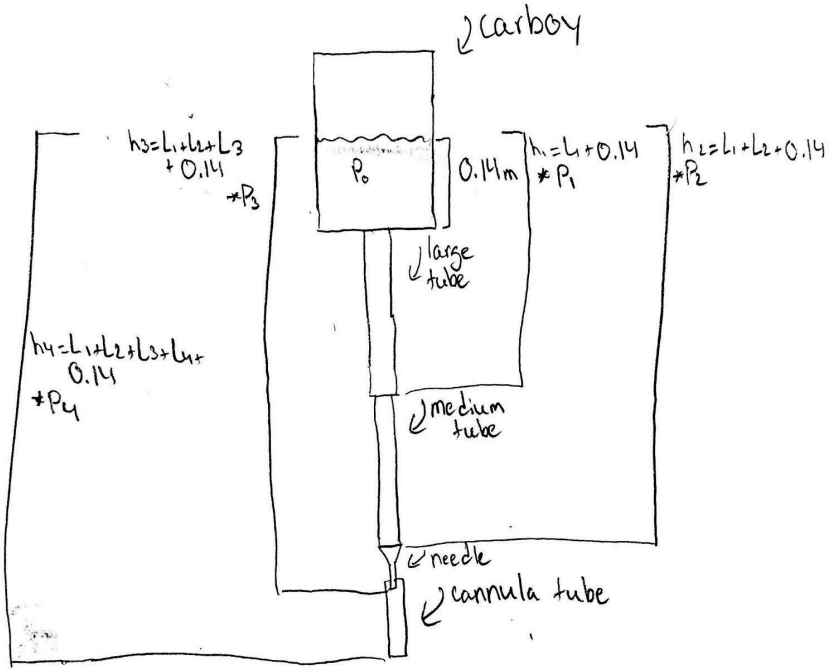

Figure 32: Schematic of perfusion system attached to the carboy.

The perfusion system was broken into four heights: the height of the water in the carboy (0.14 m), h_1 (length of large tube + 0.14 m), h_2 (length of medium tube + h_1), h_3 (length of needle + h_2) and h_4 (length of cannula tube + h_3). Bernoulli's equation $P = P_{atmosphere} + \rho gh$ was utilized to find the pressure in each tube. The height/length from the bottom of each tube to the top of the water level in the carboy was measured using a meter stick. Once the pressure was found, the pressure drop between each component of the system was calculated. There were four pressure drops along the system: ΔP_1 (P_o - P_1), ΔP_2 (P_1 - P_2), ΔP_3 (P_2 - P_3) and ΔP_4 (P_3 - P_4). The total pressure drop across the entire system was calculated by adding all the individual pressure drops. During the pressure drop calculations, the team assumed negligible friction because there was laminar flow throughout the system. The absolute value of the total pressure drop was used to find the theoretical flow rate for each trial. The team used the Hagen-Poiseuille equation, Q =

$\frac{(\Delta Ptotal)(\pi)(rcannula^4)}{(8)(L)(\mu)}$. The team utilized the radius of the cannula because it has the most resistance to pressure. The results are shown in the next section.

5.5.2 User Friendliness Testing

The user fully assembled the device on a clean table to imitate conditions under a fume hood. The components included a silicone o-ring, the top piece, the bottom piece, and the metal mesh. The goal of this test was to see if the new device could be fully assembled faster than the current device. The assembly process could not take longer than 3 minutes because multiple leaf scaffolds must be made in one day. The only tool used was a small metal pry tool, this helped separate the pieces of the device. In the previous design, the user required a screwdriver which made the process more complicated. Limiting the user to one small tool allows them to have more room while assembling the device. The steps to assemble the team's device were:

1. Put the silicone o-ring in the top piece (top piece has the clips)
2. Line up the mesh with the insert on the bottom piece (note: the length of the octagon mesh is different than the width, this means the line-up must be specific)
3. Put the leaf on the bottom piece of the device
4. Clip the top piece and bottom piece together by lining the notches together
5. To disassemble the device, use the pry tool to separate the two pieces

There were 3 trials conducted, the primary user (Bryanna Samolyk) and each person on the team (Aidan, Codey, Ariel and Nishan) recorded their times. Additionally, the team did trials with both the current housing device and the new housing device. The current housing device was used as a reference point. Figure 33 shows the new device before it is built and Figure 34 is an image of the pry tool used to disassemble the device. Figure 35 is an image of the current device before it is built. A table with all the times is shown in the next section.

Figure 33: Image of the new housing device before being assembled with a decellularized leaf. There is a ruler for scaling purposes.

Figure 34: Image of the pry tool used to separate the bottom and top piece of the device.

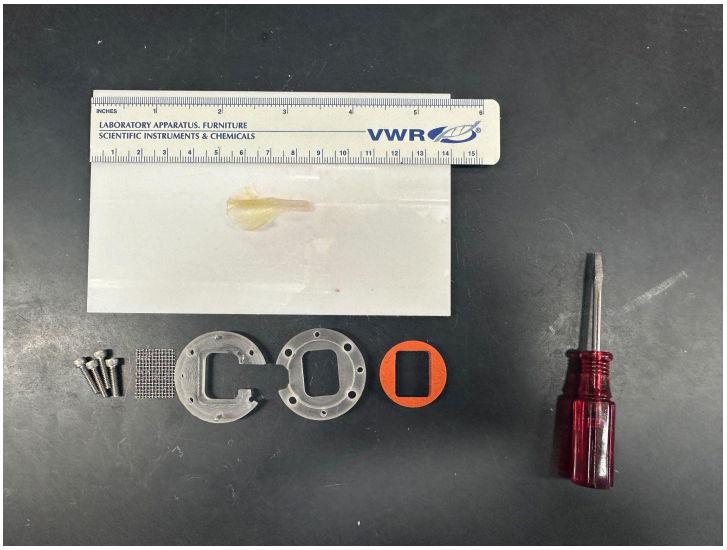

Figure 35: Image of the current housing device before being assembled with a decellularized leaf. There is a ruler for scaling purposes and a screwdriver which was used during the assembly.

5.5.3 Imageability Testing

An important aspect of our design was how well the decellularized leaf could be imaged under a regular microscope. The team tested imageability using an inverted microscope and imaged samples of paper with an X drawn in the center. For the control group, the piece of paper (1cm x 1cm) with the X was placed on the bottom of a 6-well plate. For our experimental group, the paper with the X was placed inside the housing device which sat in a 6-well plate. The images were taken at 4x magnification because higher objectives made the image unclear. The images are shown below:

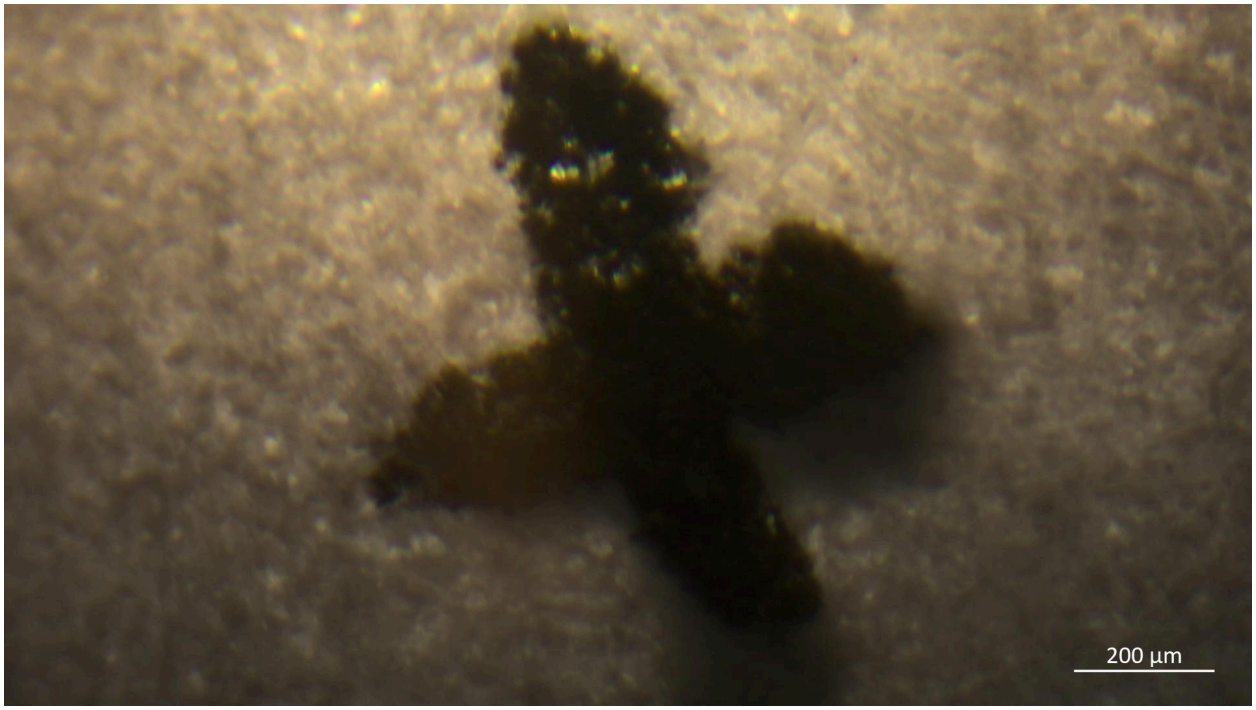

Figure 36: Image of paper with a drawn X at 4x magnification on the bottom of a 6-well plate. It has a scale bar of 200 micrometers.

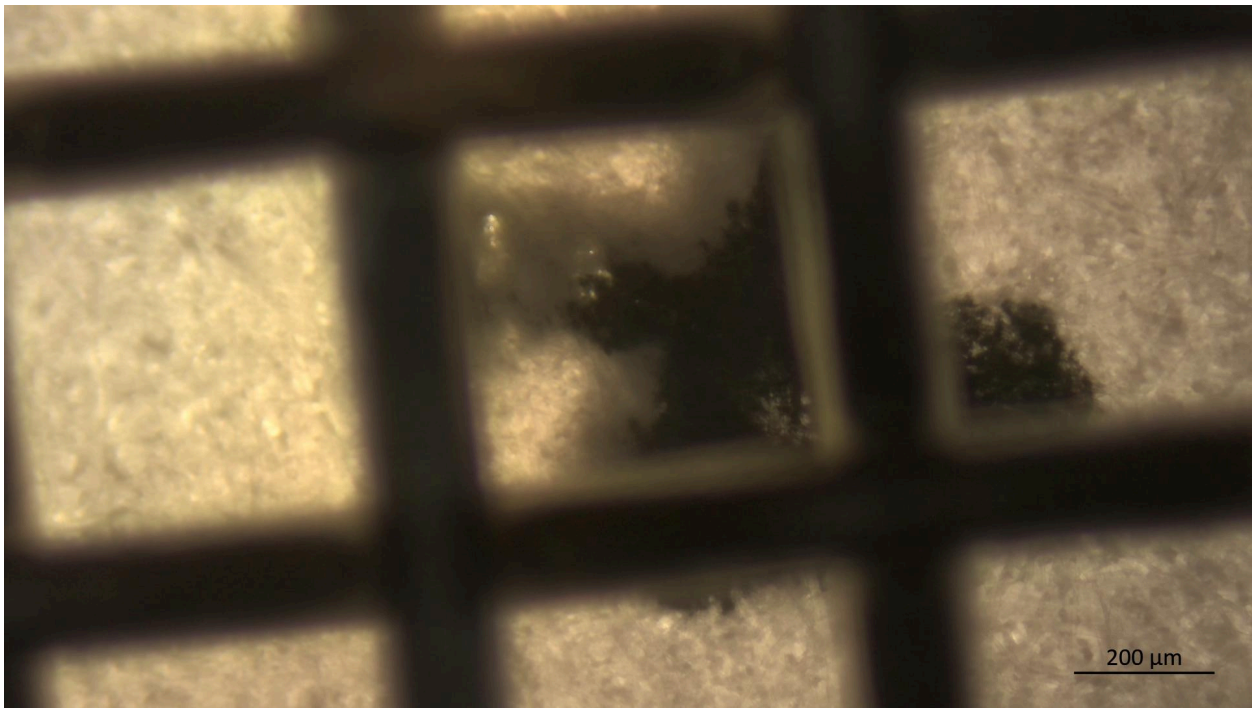

Figure 37: Image of paper with drawn X at 4x magnification in the housing device in a 6-well plate. It has a scale bar of 200 micrometers.

A decellularized spinach leaf was then placed inside the housing device. The team cut the leaf to fit it inside the device. Images were taken on the top and bottom of the leaf at 4x magnification. The results are shown in the next section.

5.5.4 Autoclavable/Sterility Testing

The team's chosen method of sterilization was an autoclave. Autoclaves are commonly used to sterilize lab equipment. They are known as steam sterilizers because they utilize high-temperature boiling water to kill bacteria, fungi, or any other type of harmful material. The goal of this test was to ensure that the device material could withstand the high temperatures of an autoclave (meaning it wouldn't deform). The maximum temperature in an autoclave is usually 121 degrees celsius. The team printed 18 cubes of polypropylene (the device material), each cube had a fill percentage of either 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90%. Before autoclaving the cubes, the width and length were recorded and an image was taken for reference. Subsequently, the team placed the cubes into an autoclave bag and loaded it into the autoclave. After the autoclave ran for 30 minutes at 121 degrees Celsius, the cubes were examined for dimensional changes. Figure 38 is an image of the cubes before being autoclaved.

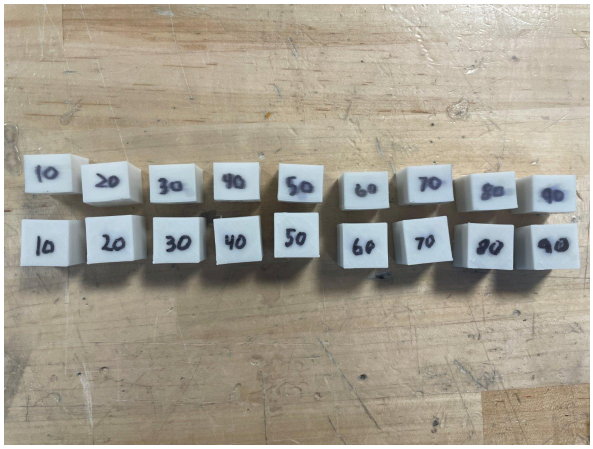

Figure 38: Shows the polypropylene cubes before being autoclaved.

5.5.5 Flip Testing

While the decellularized leaf sits inside the device, keratinocytes are seeded on the top and fibroblasts are seeded on the bottom. In order to seed cells on both sides of the leaf, the device must be flipped. For this reason, the team conducted flip testing as part of the

manufacturing process for skin grafts. The testing took place on a sterilized benchtop with the device attached to the perfusion system. When the leaf finished perfusing, one member of the group disconnected it from the perfusion system and flipped it over with tweezers. A total of 6 trials were conducted, the table is shown in the next section.

6. Results

6.1 Perfusion Testing

The tables for the experimental flow rates, fluid velocity, experimental pressure drops and theoretical flow rates are shown below. For the single channel PDMS device, the channel width was approximately 0.00038 m. The multichannel PDMS device had an opening channel of around 0.00038 m and the smaller channels ranged from 0.00015 m to 0.0002 m.

Table 38: Shows the data collected to calculate the experimental flow rate of the system for each trial. Trial A is for the single PDMS channel piece, Trial B is for the multiple PDMS channel piece.

Experimental Flow Rates						
		Height of Water Level in Carboy (m)	Volume of Liquid Collected (µL)	Time (min)	Flow Rate in µL/min	Flow Rate in m^3/s
Trial 1	A	0.15	200	10	20	3.3×10^{-10}
	B		100	10	10	1.6×10^{-10}
Trial 2	A	0.18	240	10	24	4×10^{-10}
	B		140	10	14	2.3×10^{-10}
Trial 3	A	0.22	300	10	30	5×10^{-10}
	B		160	10	16	2.6×10^{-10}

For Trial 1A and 1B, the carboy was at a height of 0.15 m (measured from the bottom of the carboy to the water level). The single piece PDMS channel (Trial A) had a volume of 200 µL after 10 minutes of perfusing. As a result, it had an experimental flow rate of 20 µL/min (3.3×10^{-10} m^3/s). Here is a sample conversion for the flow rate:

$$\frac{200\ \mu L}{10\ min} = 20\ \mu L/min$$

$$20\ \mu L\ x\ 10^{-9} = 20\ x\ 10^{-9}\ m^3/60\ sec = 3.3\ x\ 10^{-9}\ m^3/s$$

The multiple PDMS channel piece (Trial B) had a volume of 100 µL after the same amount of time. After calculating, the flow rate found was 10 µL/min or 1.67 x 10^{-10} m^3/s. In Trials 2A and 2B, the carboy was at a height of 0.18 m. The single channel piece had a flow rate of 24 µL/min (4 x 10^{-10} m^3/s) and the multichannel piece had a flow rate of 14 µL/min (2.3 x 10^{-10} m^3/s). In Trial 3A and 3B, the carboy was at a height of 0.22 m. The single channel piece had a flow rate of 30 µL (5 x 10^{-10} m^3/s) and the multichannel piece had a flow rate of 16 µL/min (2.6 x 10^{-10} m^3/s). The graph below shows the trend in the flow rates for all three trials:

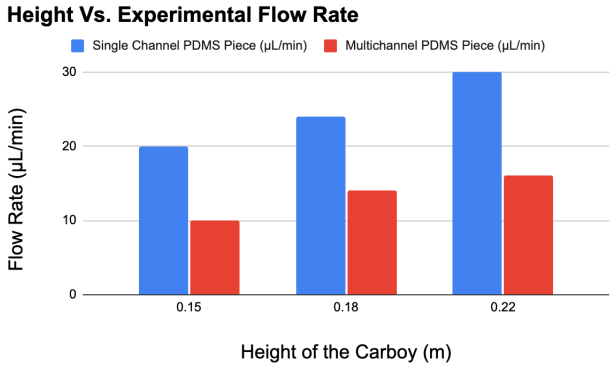

Figure 39: A bar graph showing the effect of height on the experimental flow rate for single and multichannel PDMS pieces.

As shown, both the single channel piece and multichannel piece had the same trend. As the height increased, the flow rate increased. This shows that height and flow rate have a direct relationship.

The team proceeded to calculate the fluid velocity for all the trials. The results are shown by the tables below.

Table 39: Shows the fluid velocity through each tube during trials 1A and 1B

Velocity Results for Trial 1A and 1B			
	Flow Rate (m^3/s)	Cross Sectional Area	Velocity of Fluid in

		of Tube (m^2)	Tubes (m/s)
1A	4×10^{-10}	7.2×10^{-5}	4.6×10^{-6}
		5.6×10^{-6}	6.0×10^{-5}
		7.1×10^{-8}	4.7×10^{-3}
		1.1×10^{-7}	2.9×10^{-3}
1B	1.6×10^{-10}	7.2×10^{-5}	2.3×10^{-6}
		5.6×10^{-6}	3.0×10^{-5}
		7.1×10^{-8}	2.3×10^{-3}
		1.1×10^{-7}	1.5×10^{-3}

Table 40: Shows the fluid velocity through each tube during trials 2A and 2B.

Velocity Results for Trial 2A and 2B			
	Flow Rate (m^3/s)	Cross Sectional Area of Tube (m^2)	Velocity of Fluid in Tubes (m/s)
2A	3.3×10^{-10}	7.2×10^{-5}	5.5×10^{-6}
		5.6×10^{-6}	7.2×10^{-5}
		7.1×10^{-8}	5.6×10^{-3}
		1.1×10^{-7}	3.5×10^{-3}
2B	2.3×10^{-10}	7.2×10^{-5}	3.2×10^{-6}
		5.6×10^{-6}	4.2×10^{-5}
		7.1×10^{-8}	3.3×10^{-3}
		1.1×10^{-7}	2.1×10^{-3}

Table 41: Shows the fluid velocity through each tube during trials 3A and 3B.

Velocity Results for Trial 3A and 3B			
	Flow Rate (m^3/s)	Cross Sectional Area of Tube (m^2)	Velocity of Fluid in Tubes (m/s)

3A	5×10^{-10}	7.2×10^{-5}	6.9×10^{-6}
		5.6×10^{-6}	9.0×10^{-5}
		7.1×10^{-8}	7.1×10^{-3}
		1.1×10^{-7}	4.4×10^{-3}
3B	2.6×10^{-10}	7.2×10^{-5}	3.7×10^{-6}
		5.6×10^{-6}	4.8×10^{-5}
		7.1×10^{-8}	3.7×10^{-3}
		1.1×10^{-7}	2.4×10^{-3}

For Trial 1A, the lowest fluid velocity was 4.6×10^{-6} m/s and the largest fluid velocity was 4.7×10^{-3} m/s. Here is a sample calculation for finding the velocity:

$$v = \frac{Q}{A} = \frac{4\,x\,10^{-10}\,m^2/s}{7.2\,x\,10^{-5}\,m^3} = 4.6 \times 10^{-6} \text{ m/s}$$

For Trial 1B, the lowest velocity was 2.3×10^{-6} m/s and the largest velocity was 3.3×10^{-3} m/s. The carboy had a water level height of 0.15 m for these trials. In Trial 2A, the lowest velocity was 5.5×10^{-6} m/s and the largest velocity was 5.6×10^{-3} m/s. In Trial 2B, the lowest velocity was 3.2×10^{-6} m/s and the largest velocity was 3.3×10^{-3} m/s. These trials had a water level height of 0.18 m. For Trial 3A, the lowest velocity was 6.9×10^{-6} m/s and the largest velocity was 3.3×10^{-3} m/s. For these trials, the carboy had a water level of 0.22 m.

After calculating fluid velocity, the theoretical pressure drops in each tube were calculated. The results are depicted in Table 42.

Table 42: Displays the values for experimental pressure drops along each length of the perfusion system.

Theoretical Pressure Drops					
	ΔP1 (Pa)	ΔP2 (Pa)	ΔP3 (Pa)	ΔP4 (Pa)	Total Pressure Drop Along System (Pa)

Trial 1A/1B	-4254.54	-1447.52	-212.24	-469.96	-6384.27
Trial 2A/2B	-4547.97	-1447.52	-212.24	-469.96	-6677.68
Trial 3A/3B	-4939.19	-1447.52	-215.17	-469.48	-7068.91

For the theoretical pressure drops, the single channel and multichannel pieces had the same values in each trial because the water level heights were the same. In Trial 1A/1B, the total pressure drop was -6384.27 Pa. In Trial 2A/2B, the total pressure drop was -6677.68 Pa. Lastly, in Trial 3A/3B, the total pressure drop was -7068.91 Pa. This trend occurred because pressure drop increases as flow rate increases. Trial 3A/3B had the largest pressure drop because it had the largest flow rate (the water level in the carboy was the highest). Trial 1A/1B had the lowest pressure drop because it had the smallest flow rate (the carboy contained the least amount of water).

The team used the absolute values of the theoretical pressure drops to calculate the theoretical flow rate. The results are displayed in Table 43.

Table 43: Displays the values for the theoretical flow rate for each trial.

Theoretical Flow Rates					
	Height of Water Level in Carboy (m)	Total Pressure Drop (Pa)	Total Length of System (m)	Flow Rate (m^3/s)	Flow Rate (µL/min)
Trial 1A/1B	0.15	6384.27	0.503	1.04×10^{-7}	6240 µL/min
Trial 2A/2B	0.18	6677.68	0.503	1.09×10^{-7}	6540 µL/min
Trial 3A/3B	0.22	7068.91	0.503	1.12×10^{-7}	6720 µL/min

For Trial 1A/1B, the flow rate was 6240 µL/min or 1.04×10^{-7} m^3/s. Here's a sample calculation:

$$Q = \frac{(\Delta Ptotal)(\pi)(rcannula^4)}{(8)(L)(\mu)} = \frac{(6384.27)(\pi)(0.00038m)^4}{(8)(0.503m)(0.001\ Pa*s)} = 1.04\ x\ 10^{-7}\ m^3/s$$

$$1.04\ x\ 10^{-7}\ m^3/s\ x\ \frac{1{,}000{,}000\ \mu L}{m^3} = 104\ \mu L/s\ x\ \frac{60\ s}{min} = 6240\ \mu L/min$$

For Trial 2A/2B, the flow rate was 5540 µL/min or 1.09 x 10^{-7} m^3/s. For Trial 3A/3B, the flow rate was 6720 µL/min or 1.12 x 10^{-7} m^3/s. The data demonstrates that the experimental flow rate values and the theoretical flow rate values had the same trend. The graph below shows the trend in the flow rates for all heights:

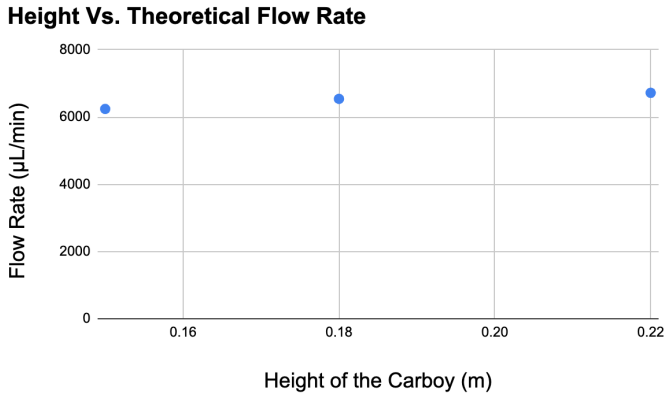

Figure 40: A line graph showing the effect of height on the theoretical flow rate for both microfluidic systems

After calculating the flow rates and pressure drops, the team proceeded to perfuse the leaf with the gravity flow system. The team decided to use the lowest flow rate to not burst the leaf (achieved at 0.15 m). The goal was to confirm that the leaf could be perfused using the setup and not rupture. Figure 41 shows the leaf before being perfused. It's attached to the gravity flow system via shrink wrap.

Figure 41: Shows image of the decellularized leaf attached to the gravity flow system before being perfused.

When the leaf was outside of the housing device, it took approximately 10 minutes for the liquid to fully perfuse through the vasculature. Figure 42 illustrates that the force of gravity flow did not burst the leaf.

Figure 42: Shows an image of the decellularized being perfused after 10 minutes.

The next step was to perfuse the leaf inside the inner housing device. The team proceeded to cut another decellularized leaf so that it could fit properly in the device. The device was then placed into a 6 well plate and connected to the gravity flow perfusion system. Figure 43 shows the leaf in the housing device before being perfused.

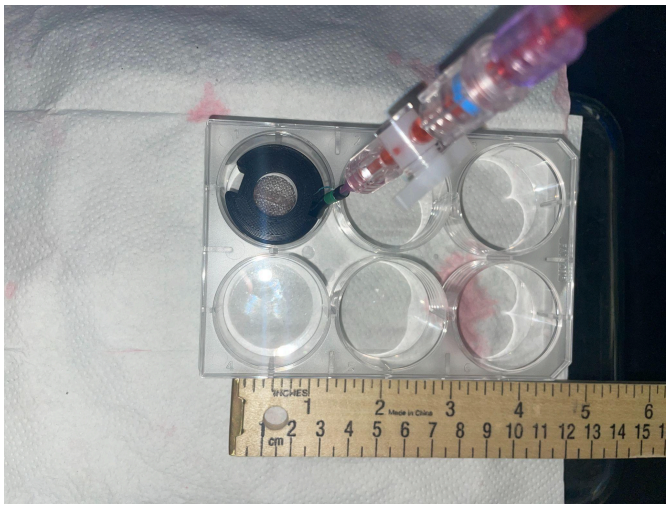

Figure 43: Image of the decellularized leaf before being perfused in the housing device.

After 10 minutes, the team removed the leaf and confirmed that it did not burst from the flow rate/pressure. This is seen in Figure 44.

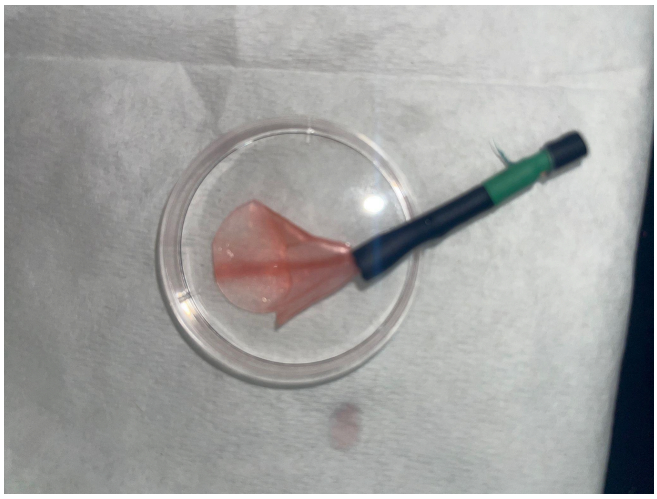

Figure 44: Shows an image of the decellularized leaf perfused after it was in the device for 10 minutes.

As shown, 100% of the leaf was perfused using the gravity flow system and the team's housing device.

6.2 User Friendliness Testing

The following tables display the team's results from testing. Table 44 shows the time it took to assemble the new device. Table 45 shows the time it took the team to assemble the current housing device.

Table 44: Displays the time it took for each group member to assemble the new housing device.

Assembly Time for New Device					
	Primary User's Time (s)	Aidan's Time (s)	Codey's Time (s)	Ariel's Time (s)	Nishan's Time (s)
Trial 1	45.10	10.70	16.60	15.90	15.70
Trial 2	25.12	12.48	14.57	13.87	18.28
Trial 3	18.05	11.81	13.56	11.72	11.23
Average Time (s)	29.42	11.66	14.91	13.83	15.07
Standard Deviation (s)	11.45	0.734	1.264	1.706	2.912

For the primary user (Bryanna Samolyk), her average assembly time was 29.42 seconds. Aidan's average assembly time was 11.66 seconds. Codey's average assembly time was 14.91 seconds. Ariel's average assembly time was 13.83 seconds. Nishan's average assembly time was 15.07 seconds. As shown, on average it took less than 30 seconds for all of us to assemble the device. In addition, everyone's time was within 15 seconds of each other. Figure 45 is an image of the new housing device completely assembled.

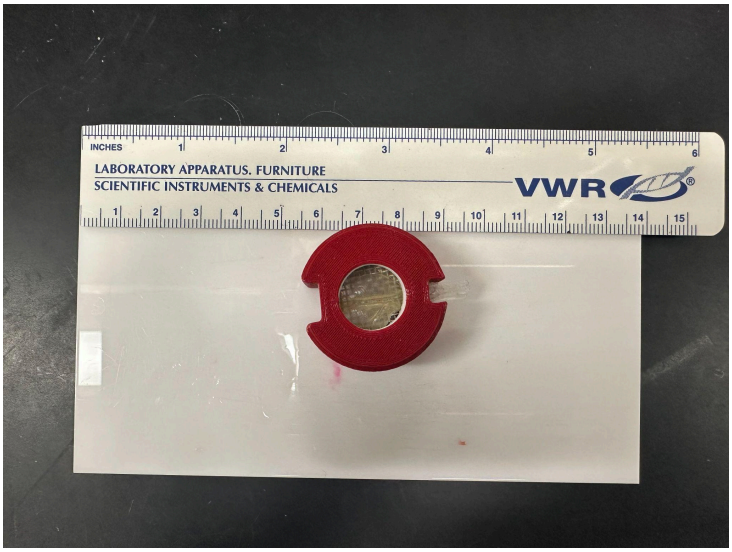

Figure 45: Shows the new housing device completely assembled with the decellularized leaf inside.

Table 45: Displays the time it took for each group member to assemble the previous housing device.

Assembly Time for Current Device					
	Primary User's Time (s)	Aidan's Time (s)	Codey's Time (s)	Ariel's Time (s)	Nishan's Time (s)
Trial 1	112.18	68.22	144.69	90.110	75.47
Trial 2	112.11	58.77	65.430	110.96	75.41
Trial 3	96.750	57.63	67.680	94.800	67.20
Average Time (s)	107.01	61.54	92.600	98.620	72.70
Standard Deviation (s)	7.2573	4.746	36.844	8.9309	3.884

For the primary user (Bryanna Samolyk), her average assembly time was 107.01 seconds. Aidan's average assembly time was 61.54 seconds. Codey's average assembly time was 92.6 seconds. Ariel's average assembly time was 98.62 seconds. Nishan's average assembly time was 72.7 seconds. The data illustrates that it took longer to assemble the previous housing device

than the new housing device. On average, the current device had a time range of nearly 40 seconds. Figure 46 is an image of the current housing device completely assembled.

Figure 46: Shows the current housing device completely assembled with the decellularized leaf inside.

Figure 47 shows a graph comparing the average assembly times for each person between the new device and old device. The standard deviation for each assembly time was calculated using Excel. For the current device, the largest standard deviation (SD) was 36.84 and the smallest standard deviation was 3.88. For the new device, the largest SD was 11.45 and the smallest SD was 0.734. As demonstrated, the current device had an overall larger SD than the new device.

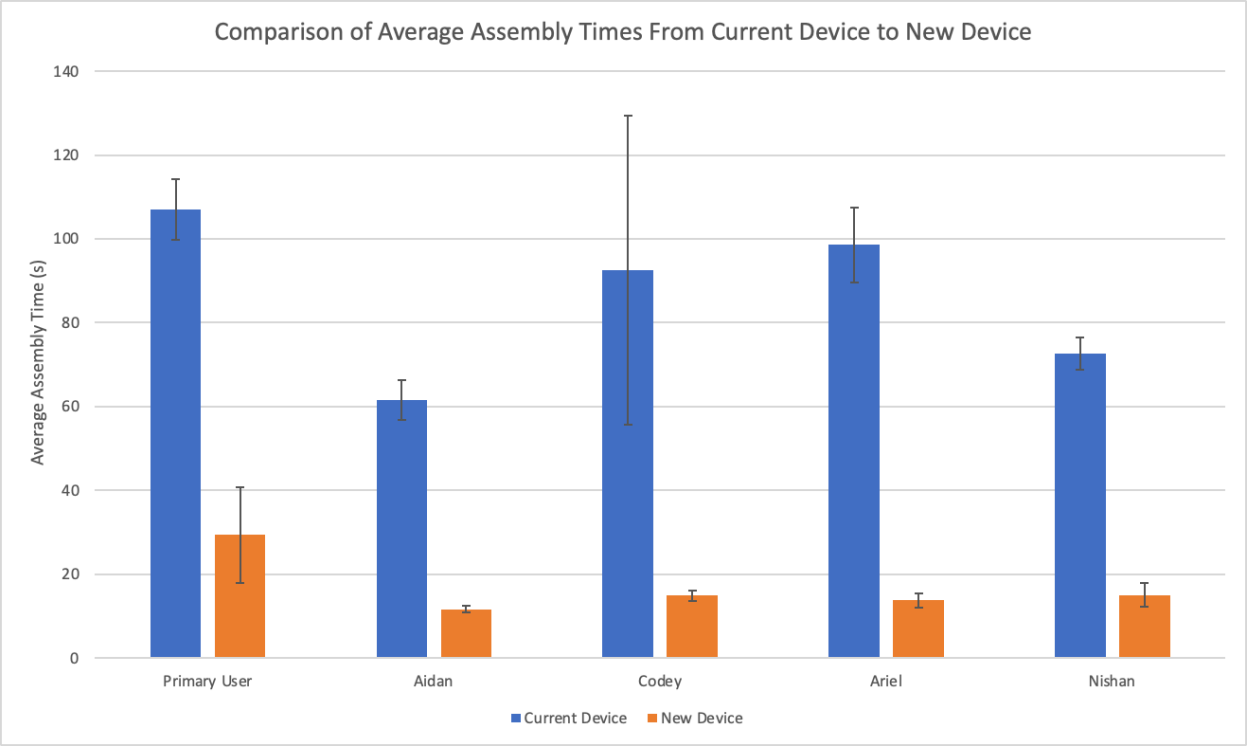

Figure 47: A bar graph that shows the average assembly time for each person with the new device and old device

6.3 Imageability Testing

After imaging the X's on paper, the team imaged a decellularized leaf on the bottom of a 6 well plate under 4x magnification. This was the control group for the test. This is shown in Figure 48:

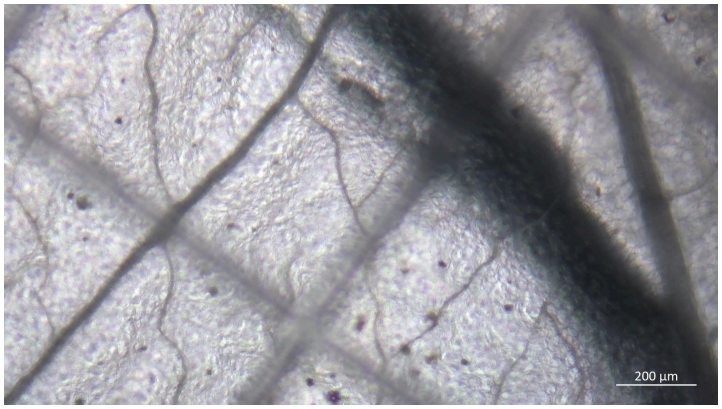

Figure 48: Image of a decellularized leaf on the bottom of a 6 well plate under 4x magnification.

Subsequently, the team imaged the bottom of the decellularized leaf in the housing device in the 6 well plate under 4x magnification. This can be seen in Figure 49:

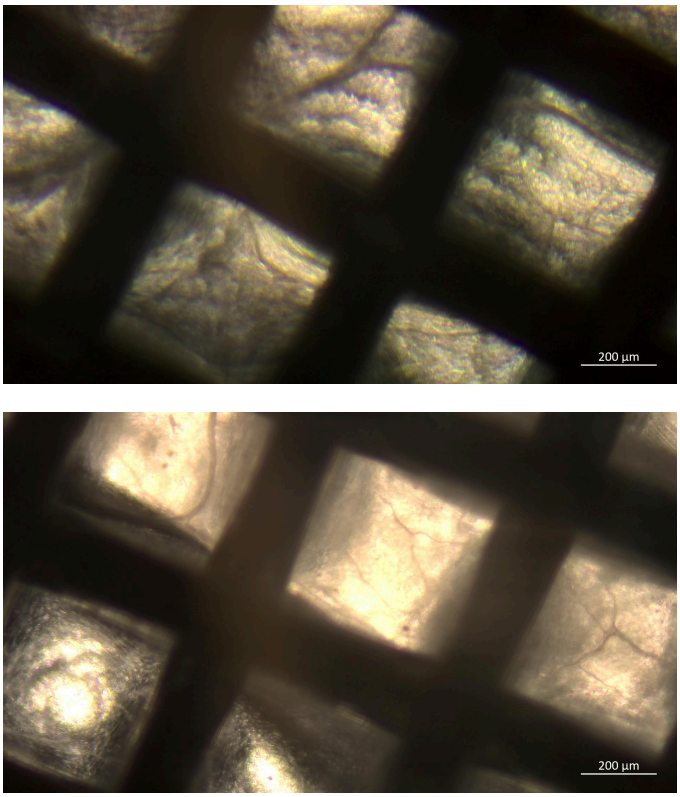

Figure 49: Images of the bottom of the decellularized leaf in the housing device in the 6 well plate under 4x magnification.

Despite the metal mesh occupying most of the image, the vasculature can be clearly seen in both images. Magnifications higher than 4x made the images blurry or the leaf was not visible under the microscope. The next step was imaging the top of the leaf by flipping the device. The results are shown in Figure 50:

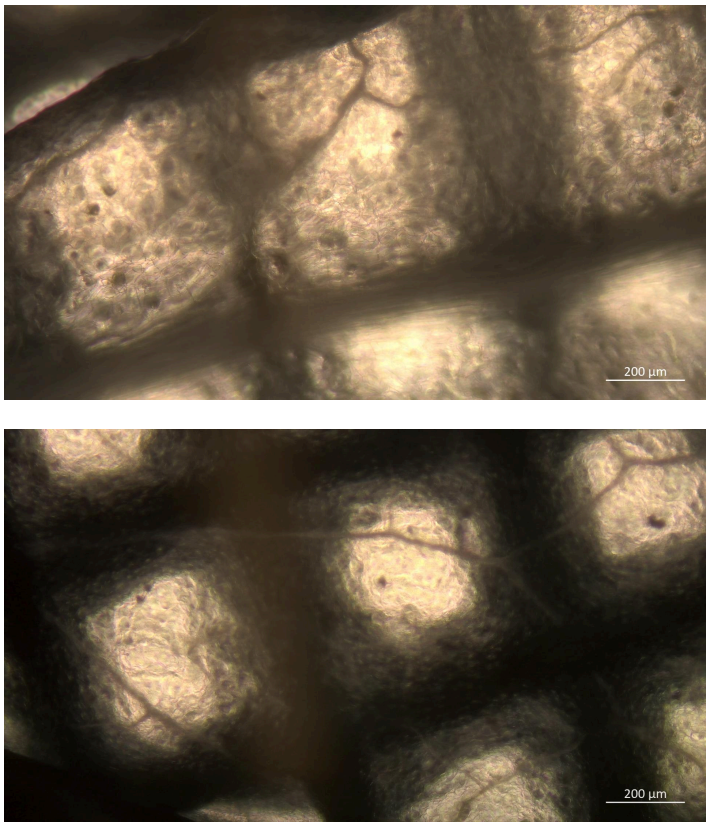

Figure 50: Images of the top of the decellularized leaf in the housing device in the 6 well plate under 4x magnification.

Similar to Figure 48, the vasculature on the top of the leaf can be clearly seen through the mesh. The team only used 4x magnification to obtain better clarity photos. As illustrated, the decellularized leaf can be imaged inside the inner housing device.

6.4 Autoclavability/Sterility Testing

The deformation for each fill percentage is shown in Table 46. The columns on the left show infill percentages, trials and dimensions before sterilization. The columns on the right show the dimension after sterilization as well as the change in dimension between pre and post sterilization.

Table 46: Shows the percent changes in polypropylene cubes after going through one autoclave cycle

Autoclavability Results							
Fill Percent Infill (%)	Cube Sample #	Initial Width (mm)	Initial Length (mm)	Final Width (mm)	Final Length (mm)	Deformation Width (mm)	Deformation Length (mm)
10%	1	20.03	20	19.97	19.96	0.06	0.04
	2	19.96	20.01	19.95	19.96	0.01	0.05
20%	1	20.04	20.03	19.95	20	0.09	0.03
	2	20.09	20.19	20.02	20.11	0.06	0.08
30%	1	19.98	20.03	19.9	19.98	0.08	0.05
	2	20.04	20.17	20.01	20.11	0.03	0.06
40%	1	20	20.03	20	19.97	0	0.06
	2	20.04	19.92	20	19.85	0.04	0.07
50%	1	20.12	20.2	20.01	20.15	0.02	0.05
	2	20.1	20.1	20.08	20.07	0.02	0.03
60%	1	20.07	20.1	20.03	20.08	0.04	0.02
	2	20.07	19.95	20.05	19.93	0.02	0.02
70%	1	20.01	19.97	19.98	19.93	0.03	0.04
	2	20.09	20.02	20.06	20.01	0.03	0.01
80%	1	20.07	19.98	20.03	19.96	0.04	0.02
	2	20.05	19.99	20.02	19.97	0.03	0.02
90%	1	19.98	20	19.96	19.97	0.02	0.03
	2	19.97	20.02	19.96	20.02	0.01	0

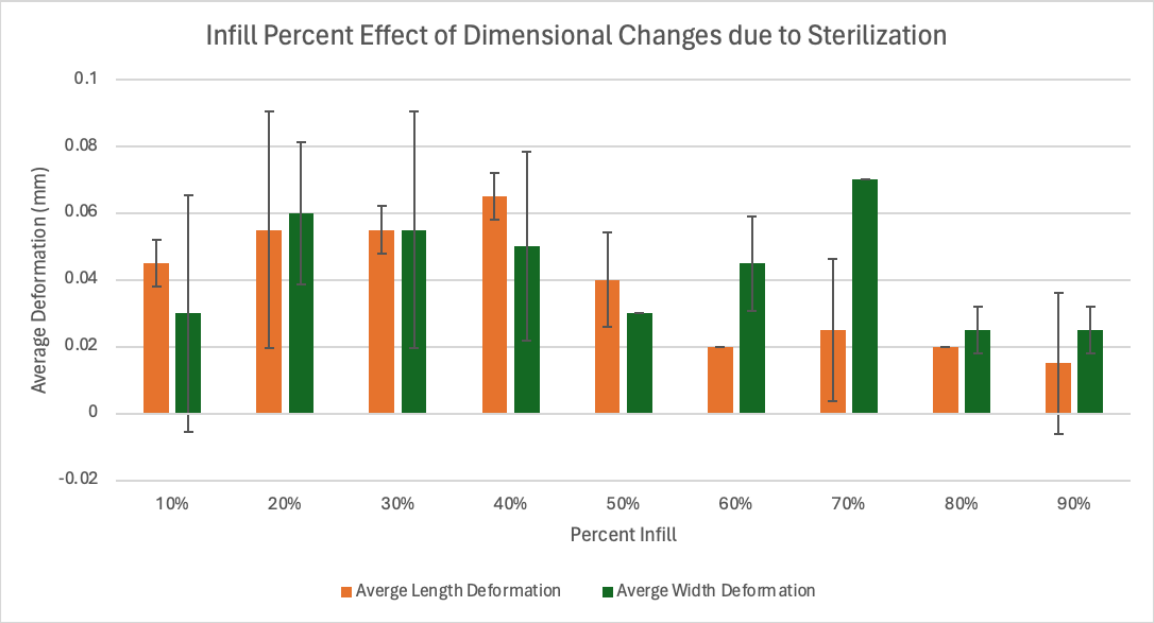

Figure 51: A bar graph that shows the average deformation of the cubes after being autoclaved.

Both 10% fill cube samples experienced less than 0.1 mm in deformation for the length and the width. The length had a standard deviation (SD) of 0.007 and the width had a SD of 0.035. For the 90% fill cube samples, they exhibited less than 0.05 mm in deformation for the length and the width. The length had a SD of 0.02 and the width had a SD of 0.007. As demonstrated from Table 46 and Figure 51, the higher percentage infill cubes had less deformation than the cubes with a lower infill. All the cubes had low standard deviation values.

6.5 Flipping Test

The table below displays our results for the flip test.

Table 47: Shows the results for whether or not the leaf stem broke while flipping the device.

Flip Testing Results	
Trials	**Leaf Stem Break Yes or No**
Trial 1	No
Trial 2	No

Trial 3	No
Trial 4	No
Trial 5	No
Trial 6	No

The decellularized leaf stem did not break for any of the trials while in the device. When the leaf stem was disconnected from the perfusion system and flipped over, the stem was still fully intact. Additionally, the leaf itself was not damaged or crushed during the process.

7. Discussion

7.1 Perfusion Testing Analysis

The goal of this study was to determine the optimal flow rate for leaf perfusion. For the experimental flow rates, the single channel and multichannel flow rate increased as the water level in the carboy increased. This was indicated in both Bernoulli's and Hagen Poiseuille's equations. The single channel flow rate had a more linear progression than the multichannel flow rate. One possibility was that the multichannel PDMS microfluidic system would fall apart in the Petri dish. This was due to human error and the system was extremely fragile. Since it would require reassembly, the opening width may have differed everytime (should have a width of 0.00038 m). In addition, some channels were blocked or had too small of an opening to allow liquid to flow through properly. The multichannel had a lower flow rate than the single channel because there are more pathways for the liquid to perfuse through. Overall, none of the flow rates exceeded 30 μL/min. Since the team utilized gravity flow, a small flow rate is expected. If the team used a syringe pump, then the flow rate would be higher (at least over 100 μL/min).

The theoretical flow rates were larger than the experimental flow rates by a factor of 10^2. The team assumed that the system experienced minor frictional forces and had a laminar flow. For this reason, friction was neglected while calculating the theoretical values. The theoretical flow rates had the same trend as the experimental flow rates. As the height of the water increased, the flow rate also increased. Overall, the flow rates directly correlated to the theoretical pressure drops. As the flow rate increased, the pressure drop along the entire system also increased. This affected the fluid velocity in all the tubes. As the diameter of the tube decreased, the pressures decreased and the fluid velocity increased. The pressures in each tube also decreased as the distance from the bottom of the tube to the water level increased.

The team utilized the flow rate (10 to 20 μL/min) calculated from the multichannel PDMS microfluidic system because it most accurately represented the leaf's vasculature. For this reason, the leaf did not burst during testing (shown in Figure 42). The leaf in Figure 44 perfused less than the leaf in Figure 42 because it had a larger surface area. The leaf in the housing device had to be cut in order for it to properly fit. In addition, some leakage is expected because the

liquid will eventually perfuse out of the leaf. Overall, the device accomplished its goal because the leaf did not rupture during perfusion.

7.2 User Friendliness Testing Analysis

The goal of this study was to compare the assembly times between the team's device and the current device. The new housing device was simpler to assemble than the current housing device. The current housing device had more components because it had screws. Consequently, a screwdriver was also required which made the process more time consuming. This was demonstrated by the results. The lowest average time to assemble the current device was 61.54 seconds. In contrast, the lowest assembly time for the new device was 11.66 seconds. On average, the new device took 5.4 x faster to assemble than the current device. If the user needed six of the team's devices at once, it would take less than two minutes to assemble all of them. For the current device, it could take anywhere between 6-9 minutes. The team's device did not require large assembly tools which allowed for less components. The team utilized a clipping system (instead of a screw system) which made it more user friendly because of its simplicity. As aforementioned, the assembly times between participants for the current device varied up to 40 seconds. The time for the new device varied less than 15 seconds. This demonstrates that most people can assemble the new device in under 30 seconds. One minor issue with the new device was positioning the mesh and the leaf on the bottom piece. However, this could be solved by properly cutting out the mesh and leaf. Overall, the goal was accomplished because it took less than 3 minutes to assemble.

7.3 Imageability Testing Analysis

The goal of this study was to ensure the leaf could be imaged in the inner housing device. Depending on the height (distance away from the lens) of the sample, the images were either clear or indistinct. The decellularized leaf looked clearer on the bottom of the plate because it was closer to the lens. When the leaf was placed in the housing device, the brightness and the height of the magnification lens had to be increased. This is due to the device adding a few millimeters between the sample and the lens/camera. The metal mesh also causes minor obstruction in the device. As a result, it was difficult to obtain an image of the entire leaf

vasculature. The paper sample with the X experienced the same issue. Only part of the X was visible in the image. Overall, the normal control group had clearer images because the samples were on the bottom of the petri dish (closer to the lens).

At higher objectives such as 10X and 20X, it was difficult to image the samples. Under these objectives, the area of focus is larger in comparison to imaging a spinach leaf with keratinocytes and fibroblasts. Vessels in a leaf or two dashes along a surface are much larger than a strand of cells on a surface. As a result, it is harder to image the vessels (for the leaf) and the dashed lines (for the paper) when the magnification increases. It's easier using higher objectives with cells because they are in a smaller area. As you zoom in, you can see more defined images.

7.4 Autoclavability/Sterility Analysis

The goal of this study was to ensure the housing device could withstand autoclave sterilization. When looking at Table X, all percent infill underwent very minimal deformation, as a result there was no clear trend. The higher percent infill cubes had lower percent deformation because they had more material. Most importantly, the results illustrated that the material doesn't melt when autoclaved. This shows that polypropylene is a viable material for the team's device. Higher percent infill is preferred because the locking mechanism/hinges are the thinnest features on the entire device. Overall, the lower infills had more deformity. Consequently, the hinges would be at more of a risk for deformation when going through multiple autoclave cycles.

7.5 Flip Testing Analysis

The goal of this study was to ensure the leaf stem would not break when being flipped inside the housing device. The decellularized leaf stem did not rupture in any of the trials. The stem had enough room in the six well plate because the device fit properly. Everytime the device was flipped, the stem did not bend. It is worth noting that the stem should not be connected to the perfusion system when flipped. The stem could break due to the twisting motion of the tubing. In addition, the user should grab the edge of the device with a tweezer to flip it inside the well plate. They should not grab the cannula of the leaf because it is extremely delicate. The results demonstrated that cells could be seeded on both sides of the leaf without rupturing the leaf stem/leaf during the flipping process.

7.6 Impact Analysis

The following sections will discuss how the team's device will affect these topics: economic, environmental, societal influence, political ramifications, ethical concerns, health and safety issues, manufacturability, and sustainability.

7.6.1 Economic Analysis

Since the outer and inner housing device was composed of polypropylene, it could be completely 3D printed. The filament costs less than $36 per roll, making it affordable. Despite it being cheap to manufacture, the person would still need access to a 3D printer. This should not be a total inconvenience because both devices would be utilized in biomedical engineering labs. Most engineering labs/universities have access to this technology and 3D printers have become more affordable. The only components that are not 3D printable are the steel mesh and silicone ring. These parts are commonly sold on sites such as McMaster, making them affordable.

7.6.2 Environmental Impact

There is a limited environmental impact from the device. The outer housing device should not be disposed of often because it facilitates the perfusion system, the well plate and the inner housing device. As a result, it should not endure excessive wear. The inner device was designed to be reusable. Polypropylene is a durable plastic and can withstand multiple uses. For this reason, the clipping mechanism should not experience excessive degradation. Overall, the minimal disposal of the team's device should not negatively impact the environment.

7.6.3 Societal Influence

The manufacturing process for both devices are cost efficient. Polyethylene filament is cheap and easy to obtain. Also, most biomedical companies and labs have access to 3D printers. For labs/people who do not have direct access to a printer, they can purchase one for as much as $170. This makes the technology and manufacturing process accessible to people in different socioeconomic backgrounds. Lastly, the devices have a simple design, enabling anyone regardless of their education level, to use them in experiments.

7.6.4 Political Ramifications

The design, development and manufacturing of the housing devices would have no political ramifications. The materials used in the design and manufacturing process do not pose any threat or concern to a specific community. If the device is brought to market, it would provide an easier way to culture cells on decellularized leaf scaffolds. Additionally, people can perfuse the leaves with hormones and nutrients by using the team's device. This creates a viable substitute to other skin graft products on the market.

7.6.5 Ethical Concerns

Regarding our device, the matter of ethics arises from the usage of the device, not the device itself. Our main ethical concern centers around the obtainment and utilization of the skin cells used within the device. The source of these cells, human foreskin, requires careful ethical consideration due to concerns regarding informed consent, privacy and misuse. It is essential that the donors fully understand the purpose and potential involvement of their contribution and to provide consent. The main source of these cells come from newborn babies, so avoiding the compromise of human rights may be difficult as they cannot give consent themselves. Furthermore, researchers who use the cells must verify that the cells have been secured and maintained through an ethically sound process.

In addition, while our project primarily focuses on the development of skin scaffolds, it is important to acknowledge the ethical concerns regarding animal testing during every stage of the research process. These concerns ensure that testing minimizes the suffering and distress of the animal as well as justifies the use of the animal in research. It is also important to recognize that serum from these animals are needed to grow cells in the lab too.

Lastly, the discarding of plastic from the housing device or any animal products creates an ethical concern on the environment. For this reason, certain waste should be discarded correctly.

7.6.6 Health and Safety Issues

Regarding health and safety issues, the user must take necessary precautions when handling live human cells (keratinocytes and fibroblasts) while using the device. In addition, it is expected that the user has the proper training to work with human cells. Lastly, during

sterilization of the device, it is important to ensure the proper operation and monitoring of the autoclave equipment. This also includes having the correct training to ensure proper procedures and safety precautions.

7.6.7 Manufacturability

The manufacturing of the device is done using 3D printing. This is an easily scalable method of manufacturing through the purchase of additional printers. 3D printing is an evolving area of technology as well, meaning that manufacturing costs should decrease, and precision of printing should be increased as time progresses. Labor ethics should also not be a problem as 3D printers require minimal maintenance and can be controlled autonomously. This also means that costs can be kept low and expansion of production only requires the purchase of new printers.

7.6.8 Sustainability

In a laboratory setting, our device's design is very sustainable. The clipping system allows for durability and longevity for the user. This enables it to withstand multiple usages while also ensuring it remains functional/effective during a long period of time. Additionally, the use of polypropylene (while not completely sustainable) is a better alternative to other types of plastics. It has a relatively low carbon footprint and does not release toxins. It is also safe for human health because it is biocompatible. Lastly, the lack of multiple components ensures that no spare parts from the device will be left laying around to harm the environment.

8. Conclusions and Recommendations

Based on the tests the team performed, there have been major improvements in comparison to the previous device. During user-friendliness testing, the assembly time was shortened by over a minute proving the device is easy to assemble. This is beneficial if multiple grafts need to be made simultaneously. The added perfusion port in the device enabled the leaf to be perfused in the device without rupturing. The device also provided clear imaging because the user could see the leaf microvessels under the microscope. Lastly the device material had minimal deformation after autoclaving. As a result, the device's function wasn't hindered and it was completely sterilized. This aspect is important for culturing the keratinocytes and epithelial cells.

In terms of future work, the team wants to focus more testing on the leaf itself. Different flow rates would be tested with the leaf inside and outside of the device. This would test the effects of perfusion on the leaf. After confirming the device is sterilizable, the team wants to ensure cells can be seeded on both sides without any contamination. Lastly, imaging of the leaf during perfusion is highly recommended to see up close how the liquid moves throughout the leaf.

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Appendix

Appendix A: Primary Objectives Pairwise Comparison Charts

Designer Primary PCC by Aidan Kaufman

Primary Objective	Enables Facile Culture of Bi-layered Skin Equivalent	Cost Effective	Enables Scaffold Perfusion	Reproducibility	User Friendly	Scalable	Imaging Compatibility	Total
Enables Facile Culture of Bi-layered Skin Equivalent	X	1	1	1	1	1	1	6
Cost Effective	0	X	0	0	0	.5	0	.5
Enables Scaffold Perfusion	0	1	X	.5	0	1	1	3.5
Reproducibility	0	1	.5	X	0	1	.5	3.5
User Friendly	0	1	1	1	X	1	1	5
Scalable	0	0	0	0	0	X	0	.5
Imaging Compatibility	0	1	0	.5	0	1	X	2.5

Designer Primary PCC by Nishan Grandhi

Primary Objective	Enables Facile Culture of Bi-layered Skin Equivalent	Cost Effective	Enables Scaffold Perfusion	Reproducibility	User Friendly	Scalable	Imaging Compatibility	Total
Enables Facile Culture of Bi-layered Skin Equivalent	X	1	1	1	1	1	1	6
Cost Effective	0	X	0	.5	0	1	0	1.5
Enables Scaffold Perfusion	0	1	X	1	.5	1	.5	4
Reproducibility	0	.5	0	X	0	1	0	1.5
User Friendly	0	1	.5	1	X	1	.5	4
Scalable	0	0	0	0	0	X	0	0
Imaging Compatibility	0	1	.5	1	.5	1	X	4

Designer Primary PCC by Ariel Shirzadi

Primary Objective	Enables Facile Culture of Bi-layered Skin Equivalent	Cost Effective	Enables Scaffold Perfusion	Reproducibility	User Friendly	Scalable	Imaging Compatibility	Total

Enables Facile Culture of Bi-layered Skin Equivalent	X	1	1	1	1	1	1	6
Cost Effective	0	X	0	.5	0	.5	0	1
Enables Scaffold Perfusion	0	1	X	1	.5	1	.5	4
Reproducibility	0	.5	0	X	0	1	0	1
User Friendly	0	1	.5	1	X	1	.5	4
Scalable	0	.5	0	.5	0	X	0	1
Imaging Compatibility	0	1	.5	1	.5	1	X	4

Designer Primary PCC by Codey Battista

Primary Objective	Enables Facile Culture of Bi-layered Skin Equivalent	Cost Effective	Enables Scaffold Perfusion	Reproducibility	User Friendly	Scalable	Imaging Compatibility	Total
Enables Facile Culture of Bi-layered Skin Equivalent	X	1	1	1	1	1	1	6
Cost	0	X	0	0	0	.5	0	.5

Effective								
Enables Scaffold Perfusion	0	1	X	1	0	1	0	3
Reproducibility	0	1	0	X	0	1	0	2
User Friendly	0	1	1 +	1	X	1	.5	4.5
Scalable	0	0	.5	0	0	X	0	.5
Imaging Compatibility	0	1	1	1	.5	1	X	4.5

Designer Primary PCC by George Pins

Primary Objective	Enables Facile Culture of Bi-layered Skin Equivalent	Cost Effective	Enables Scaffold Perfusion	Reproducibility	User Friendly	Scalable	Imaging Compatibility	Total
Enables Facile Culture of Bi-layered Skin Equivalent	X	1	.5	1	1	1	.5	5

Cost Effective	0	X	0	0	0	1	0	1
Enables Scaffold Perfusion	.5	1	X	.5	.5	1	.5	4
Reproducibility	0	1	.5	X	0	1	0	2.5
User Friendly	0	1	.5	1	X	1	.5	4
Scalable	0	0	0	0	0	X	0	0
Imaging Compatibility	.5	1	.5	1	.5	1	X	4

Designer Primary PCC by Bryanna Samolyk

Primary Objective	Enables Facile Culture of Bi-layered Skin Equivalent	Cost Effective	Enables Scaffold Perfusion	Reproducibility	User Friendly	Scalable	Imaging Compatibility	Total
Enables Facile Culture of Bi-layered Skin Equivalent	X	1	.75	.75	.75	1	1	5.25
Cost Effective	0	X	0	0	0	0	0	0
Enables Scaffold Perfusion	.25	1	X	1	.75	1	1	5
Reproducibility	.25	1	0	X	.25	1	1	3.5

User Friendly	.25	1	.25	.75	X	1	1	4.25
Scalable	0	1	0	0	0	X	0	1
Imaging Compatibility	0	1	0	0	.0	1	X	2

Designer Primary PCC by Luke Perreault

Primary Objective	Enables Facile Culture of Bi-layered Skin Equivalent	Cost Effective	Enables Scaffold Perfusion	Reproducibility	User Friendly	Scalable	Imaging Compatibility	Total
Enables Facile Culture of Bi-layered Skin Equivalent	X	1	0	.5	.0	1	1	3.5
Cost Effective	0	X	0	0	.5	.5	0	1
Enables Scaffold Perfusion	1	1	X	1	1	1	.5	5,5
Reproducibility	.5	1	0	X	1	.5	1	4
User Friendly	1	.5	0	0	X	1	0	2.5
Scalable	0	.5	0	.5	0	X	0	1
Imaging Compatibility	0	1	.5	0	1	1	X	3.5

Appendix B: Secondary Objectives Pairwise Comparison Charts

Secondary Objectives for Enabling Facile Culture of Bi-layered Skin Equivalent:

Designer Secondary PCC of Enabling Facile Bi-Layerd Skin Equivalent by Adian Kaufman, Codey Battista, Ariel Shirzadi, Nishan Grandhi

Secondary Objectives for Enabling Facile Bi-layered Skin Equivalent	Maintain Long-term Cell Viability in Submerged and Air-Liquid Interface Culture Environments	Easy to Culture Cells on both side of the Scaffold	Total
Maintain Long-term Cell Viability in Submerged and Air-Liquid Interface Culture Environments	X	.5	.5
Easy to Culture Cells on both side of the Scaffold	.5	X	.5

Designer Secondary PCC of Enabling Facile Bi-Layerd Skin Equivalent by George Pins

Secondary Objectives for Enabling Facile Bi-layered Skin Equivalent	Maintain Long-term Cell Viability in Submerged and Air-Liquid Interface Culture Environments	Easy to Culture Cells on both side of the Scaffold	Total
Maintain Long-term Cell Viability in Submerged and Air-Liquid Interface Culture Environments	X	.5	.5
Easy to Culture Cells on both side of the Scaffold	.5	X	.5

Designer Secondary PCC of Enabling Facile Bi-Layerd Skin Equivalent by Bryanna Samolyk

Secondary Objectives for Enabling Facile Bi-layered Skin Equivalent	Maintain Long-term Cell Viability in Submerged and Air-Liquid Interface	Easy to Culture Cells on both side of the Scaffold	Total

	Culture Environments		
Maintain Long-term Cell Viability in Submerged and Air-Liquid Interface Culture Environments	X	1	1
Easy to Culture Cells on both side of the Scaffold	0	X	0

Designer Secondary PCC of Enabling Facile Bi-Layerd Skin Equivalent by Luke Perreault

Secondary Objectives for Enabling Facile Bi-layered Skin Equivalent	Maintain Long-term Cell Viability in Submerged and Air-Liquid Interface Culture Environments	Easy to Culture Cells on both side of the Scaffold	Total
Maintain Long-term Cell Viability in Submerged and Air-Liquid Interface Culture Environments	X	1	1
Easy to Culture Cells on both side of the Scaffold	0	X	0

Secondary Objectives for User Friendliness:

Designer Secondary PCC User Friendliness by Aidan Kaufman

Secondary Objectives for User Friendliness	Minimal/No Tools Required	No Mechanical Components	Minimal Assembly Time	Total
Minimal/No Tools Required	X	1	0	1
No Mechanical Components	0	X	0	0
Minimal Assembly Time	1	1	X	2

Designer Secondary PCC User Friendliness by Codey Battista

Secondary Objectives for User Friendliness	Minimal/No Tools Required	No Mechanical Components	Minimal Assembly Time	Total
Minimal/No Tools Required	X	.5	.5	1
No Mechanical Components	.5	X	.5	1
Minimal Assembly Time	.5	.5	X	1

Designer Secondary PCC User Friendliness by Ariel Shirzadi

Secondary Objectives for User Friendliness	Minimal/No Tools Required	No Mechanical Components	Minimal Assembly Time	Total
Minimal/No Tools Required	X	.5	0	.5
No Mechanical Components	.5	X	0	.5
Minimal Assembly Time	1	1	X	2

Designer Secondary PCC User Friendliness by Nishan Grandhi

Secondary Objectives for User Friendliness	Minimal/No Tools Required	No Mechanical Components	Minimal Assembly Time	Total
Minimal/No Tools Required	X	1	.5	1.5
No Mechanical Components	0	X	.5	.5
Minimal Assembly Time	.5	.5	X	1

Designer Secondary PCC User Friendliness by George Pins

Secondary Objectives for User Friendliness	Minimal/No Tools Required	No Mechanical Components	Minimal Assembly Time	Total
Minimal/No Tools Required	X	.5	0	.5
No Mechanical Components	.5	X	0	.5
Minimal Assembly Time	1	1	X	2

Designer Secondary PCC User Friendliness by Bryanna Samolyk

Secondary Objectives for User Friendliness	Minimal/No Tools Required	No Mechanical Components	Minimal Assembly Time	Total
Minimal/No Tools Required	X	.5	0	.5
No Mechanical Components	.5	X	0	.5
Minimal Assembly Time	1	1	X	2

Designer Secondary PCC User Friendliness by Luke Perreault

Secondary Objectives for User Friendliness	Minimal/No Tools Required	No Mechanical Components	Minimal Assembly Time	Total
Minimal/No Tools Required	X	1	0	1
No Mechanical Components	0	X	0	0
Minimal Assembly Time	1	1	X	2

Secondary Objectives for Enabling Scaffold Perfusion:

Secondary PCC of Enabling Scaffold Perfusion design by Aidan Kaufmann

Secondary Objectives for Enabling Scaffold Perfusion	Drug Delivery	Watertight Cannula (or Port)	Enable Perfusion of Scaffold in Submerged Culture Environment with the Incubator	Perfusion or air/liquid interface	Water tight seeding chamber	Total
Drug Delivery	X	0	0	0	0	0
Watertight Cannula (or Port)	1	X	0	0	0	1
Enable Perfusion of Scaffold in Submerged Culture Environment with the Incubator	1	1	X	.5	1	3.5
Perfusion or air/liquid interface	1	1	.5	X	1	3.5
Water tight seeding chamber	1	1	0	0	X	2

Secondary PCC of Enabling Scaffold Perfusion design by Nishan Grandhi

Secondary Objectives for Enabling Scaffold	Drug Delivery	Watertight Cannula (or Port)	Enable Perfusion of Scaffold in Submerged	Perfusion or air/liquid interface	Water tight seeding chamber	Total

Perfusion			Culture Environment with the Incubator			
Drug Delivery	X	.5	.5	0	0	1
Watertight Cannula (or Port)	.5	X	1	.5	.5	2.5
Enable Perfusion of Scaffold in Submerged Culture Environment with the Incubator	.5	0	X	.5	.5	1.5
Perfusion or air/liquid interface	1	.5	.5	X	.5	2
Water tight seeding chamber	1	.5	.5	.5	X	2.5

Secondary PCC of Enabling Scaffold Perfusion design by Ariel Shirzadi

Secondary Objectives for Enabling Scaffold Perfusion	Drug Delivery	Watertight Cannula (or Port)	Enable Perfusion of Scaffold in Submerged Culture Environment with the Incubator	Perfusion or air/liquid interface	Water tight seeding chamber	Total
Drug Delivery	X	0	0	0	0	0

Watertight Cannula (or Port)	1	X	.5	.5	.5	2.5
Enable Perfusion of Scaffold in Submerged Culture Environment with the Incubator	1	.5	X	0	.5	2
Perfusion or air/liquid interface	1	.5	1	X	.5	3
Water tight seeding chamber	1	.5	.5	.5	X	2.5

Secondary PCC of Enabling Scaffold Perfusion design by Codey Battista

Secondary Objectives for Enabling Scaffold Perfusion	Drug Delivery	Watertight Cannula (or Port)	Enable Perfusion of Scaffold in Submerged Culture Environment with the Incubator	Perfusion or air/liquid interface	Water tight seeding chamber	Total
Drug Delivery	X	0	1	0	0	1
Watertight Cannula (or Port)	1	X	0	1	0	2
Enable Perfusion of Scaffold in	0	1	X	1	0	2

Submerged Culture Environment with the Incubator						
Perfusion or air/liquid interface	1	0	0	X	0	1
Water tight seeding chamber	1	1	1	1	X	4

Secondary PCC of Enabling Scaffold Perfusion design by George Pins

Secondary Objectives for Enabling Scaffold Perfusion	Drug Delivery	Watertight Cannula (or Port)	Enable Perfusion of Scaffold in Submerged Culture Environment with the Incubator	Perfusion or air/liquid interface	Water tight seeding chamber	Total
Drug Delivery	X	0	0	0	0	0
Watertight Cannula (or Port)	1	X	.5	.5	.5	2.5
Enable Perfusion of Scaffold in Submerged Culture Environment with the Incubator	1	.5	X	.5	.5	2
Perfusion or	1	.5	.5	X	0	2

air/liquid interface						
Water tight seeding chamber	1	.5	.5	1	X	3

Secondary PCC of Enabling Scaffold Perfusion design by Bryanna Samolyk

Secondary Objectives for Enabling Scaffold Perfusion	Drug Delivery	Watertight Cannula (or Port)	Enable Perfusion of Scaffold in Submerged Culture Environment with the Incubator	Perfusion or air/liquid interface	Water tight seeding chamber	Total
Drug Delivery	X	0	.75	.75	0	1.5
Watertight Cannula (or Port)	1	X	1	1	.5	3.5
Enable Perfusion of Scaffold in Submerged Culture Environment with the Incubator	.25	0	X	.5	0	.75
Perfusion or air/liquid interface	.25	0	.5	X	0	.75
Water tight seeding chamber	1	.5	1	1	X	3.5

Secondary PCC of Enabling Scaffold Perfusion design by Luke Perreault

Secondary Objectives for Enabling Scaffold Perfusion	Drug Delivery	Watertight Cannula (or Port)	Enable Perfusion of Scaffold in Submerged Culture Environment with the Incubator	Perfusion or air/liquid interface	Water tight seeding chamber	Total
Drug Delivery	X	0	0	1	0	1
Watertight Cannula (or Port)	1	X	1	1	.5	3
Enable Perfusion of Scaffold in Submerged Culture Environment with the Incubator	1	0	X	0	0	1
Perfusion or air/liquid interface	0	0	1	X	0	1
Water tight seeding chamber	1	.5	1	1	X	2.5

Secondary PCCs for Cost Effectiveness:

Secondary Design PCC of Cost Effectiveness by Aidan Kaufman

Secondary Objectives for Cost Effectiveness	Minimize Cost of Starting Materials	Minimize Manufacturing Costs	Total

Minimize Cost of Starting Materials	X	0	0
Minimize Manufacturing Costs	1	X	1

Secondary Design PCC of Cost Effectiveness by Nishan Grandhi

Secondary Objectives for Cost Effectiveness	Minimize Cost of Starting Materials	Minimize Manufacturing Costs	Total
Minimize Cost of Starting Materials	X	1	1
Minimize Manufacturing Costs	0	X	0

Secondary Design PCC of Cost Effectiveness by Ariel Shirzadi

Secondary Objectives for Cost Effectiveness	Minimize Cost of Starting Materials	Minimize Manufacturing Costs	Total
Minimize Cost of Starting Materials	X	.5	.5
Minimize Manufacturing Costs	.5	X	.5

Secondary Design PCC of Cost Effectiveness by Codey Battista

Secondary Objectives for Cost Effectiveness	Minimize Cost of Starting Materials	Minimize Manufacturing Costs	Total
Minimize Cost of Starting Materials	X	0	0
Minimize Manufacturing Costs	1	X	1

Secondary Design PCC of Cost Effectiveness by George Pins

Secondary Objectives for Cost Effectiveness	Minimize Cost of Starting Materials	Minimize Manufacturing Costs	Total
Minimize Cost of Starting Materials	X	.5	.5

Minimize Manufacturing Costs	.5	X	.5

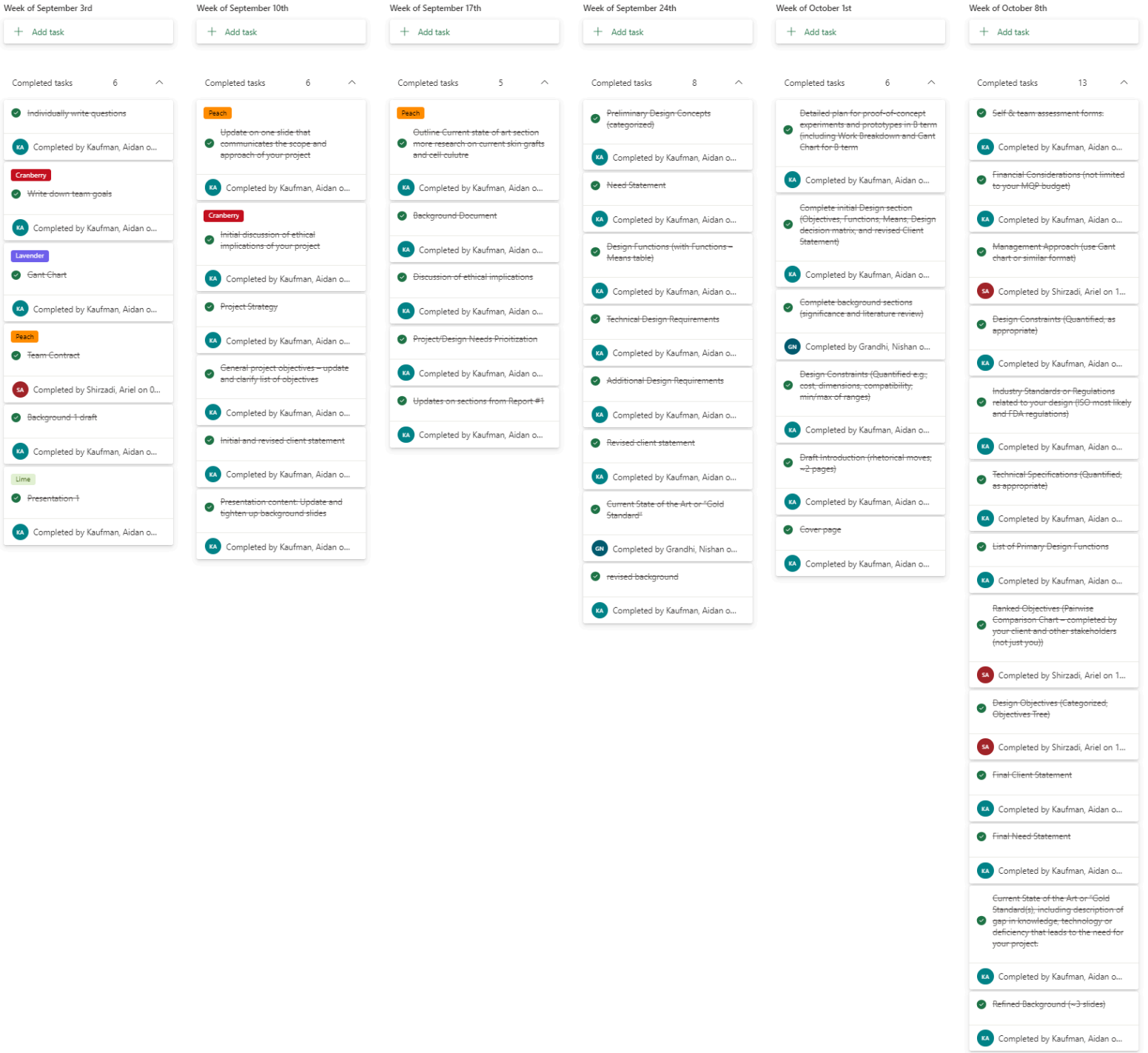
Secondary Design PCC of Cost Effectiveness by Bryanna Samolyk

Secondary Objectives for Cost Effectiveness	Minimize Cost of Starting Materials	Minimize Manufacturing Costs	Total
Minimize Cost of Starting Materials	X	.5	.5
Minimize Manufacturing Costs	.5	X	.5

Secondary Design PCC of Cost Effectiveness by Luke Perreault

Secondary Objectives for Cost Effectiveness	Minimize Cost of Starting Materials	Minimize Manufacturing Costs	Total
Minimize Cost of Starting Materials	X	0	0
Minimize Manufacturing Costs	1	X	1

Appendix C: Weekly Objectives for A term



Week of September 3rd

+ Add task

Completed tasks 6

- ~~Individually write questions~~ — Completed by Kaufman, Aidan o...
- Cranberry: ~~Write down team goals~~ — Completed by Kaufman, Aidan o...
- Lavender: ~~Gant Chart~~ — Completed by Kaufman, Aidan o...
- Peach: ~~Team Contract~~ — Completed by Shirzadi, Ariel on 0...
- ~~Background 1 draft~~ — Completed by Kaufman, Aidan o...
- Lime: ~~Presentation 1~~ — Completed by Kaufman, Aidan o...

Week of September 10th

+ Add task

Completed tasks 6

- Peach: ~~Update on one slide that communicates the scope and approach of your project~~ — Completed by Kaufman, Aidan o...
- Cranberry: ~~Initial discussion of ethical implications of your project~~ — Completed by Kaufman, Aidan o...
- ~~Project Strategy~~ — Completed by Kaufman, Aidan o...
- ~~General project objectives – update and clarify list of objectives~~ — Completed by Kaufman, Aidan o...
- ~~Initial and revised client statement~~ — Completed by Kaufman, Aidan o...
- ~~Presentation content. Update and tighten up background slides~~ — Completed by Kaufman, Aidan o...

Week of September 17th

+ Add task

Completed tasks 5

- Peach: ~~Outline Current state of art section more research on current skin grafts and cell culutre~~ — Completed by Kaufman, Aidan o...
- ~~Background Document~~ — Completed by Kaufman, Aidan o...
- ~~Discussion of ethical implications~~ — Completed by Kaufman, Aidan o...
- ~~Project/Design Needs Prioitization~~ — Completed by Kaufman, Aidan o...
- ~~Updates on sections from Report #1~~ — Completed by Kaufman, Aidan o...

Week of September 24th

+ Add task

Completed tasks 8

- ~~Preliminary Design Concepts (categorized)~~ — Completed by Kaufman, Aidan o...
- ~~Need Statement~~ — Completed by Kaufman, Aidan o...
- ~~Design Functions (with Functions – Means table)~~ — Completed by Kaufman, Aidan o...
- ~~Technical Design Requirements~~ — Completed by Kaufman, Aidan o...
- ~~Additional Design Requirements~~ — Completed by Kaufman, Aidan o...
- ~~Revised client statement~~ — Completed by Kaufman, Aidan o...
- ~~Current State of the Art or "Gold Standard"~~ — Completed by Grandhi, Nishan o...
- ~~revised background~~ — Completed by Kaufman, Aidan o...

Week of October 1st

+ Add task

Completed tasks 6

- ~~Detailed plan for proof-of-concept experiments and prototypes in B term (including Work Breakdown and Gant Chart for B term~~ — Completed by Kaufman, Aidan o...
- ~~Complete initial Design section (Objectives, Functions, Means, Design decision matrix, and revised Client Statement)~~ — Completed by Kaufman, Aidan o...
- ~~Complete background sections (significance and literature review)~~ — Completed by Grandhi, Nishan o...
- ~~Design Constraints (Quantified e.g., cost, dimensions, compatibility, min/max of ranges)~~ — Completed by Kaufman, Aidan o...
- ~~Draft Introduction (rhetorical moves; ~2 pages)~~ — Completed by Kaufman, Aidan o...
- ~~Cover page~~ — Completed by Kaufman, Aidan o...

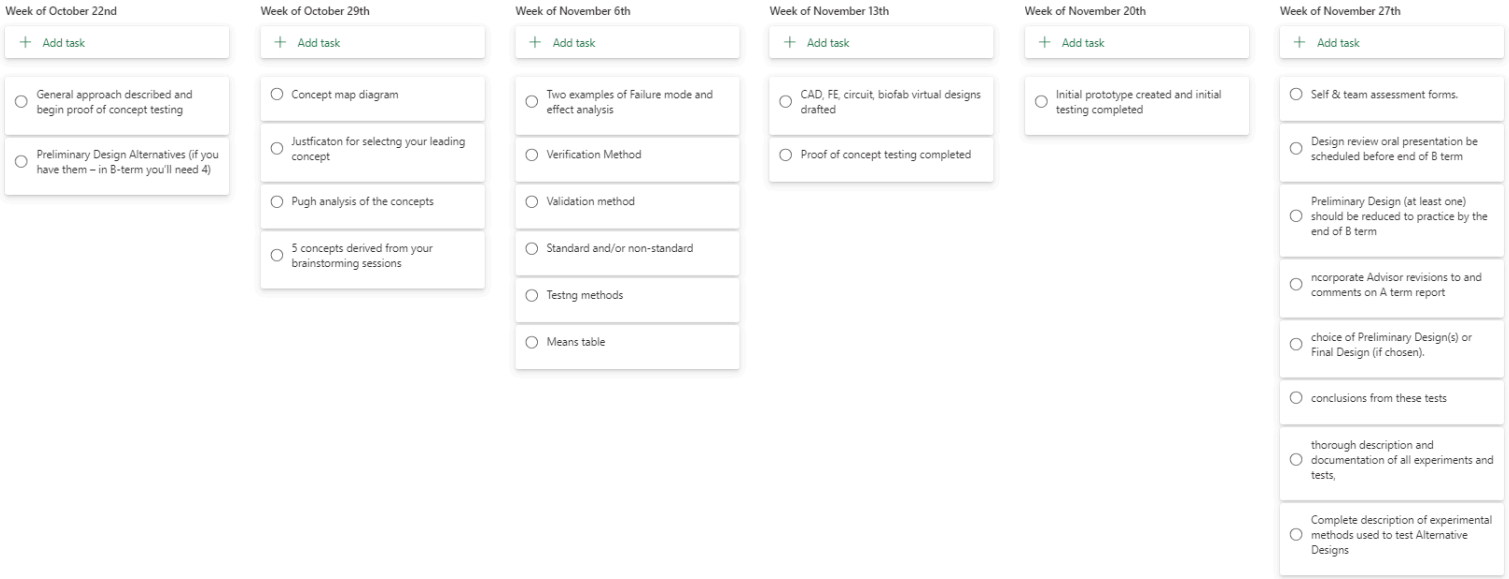
Week of October 8th

+ Add task

Completed tasks 13

- ~~Self & team assessment forms.~~ — Completed by Kaufman, Aidan o...
- ~~Financial Considerations (not limited to your MQP budget)~~ — Completed by Kaufman, Aidan o...
- ~~Management Approach (use Gant chart or similar format)~~ — Completed by Shirzadi, Ariel on 1...
- ~~Design Constraints (Quantified, as appropriate)~~ — Completed by Kaufman, Aidan o...
- ~~Industry Standards or Regulations related to your design (ISO most likely and FDA regulations)~~ — Completed by Kaufman, Aidan o...
- ~~Technical Specifications (Quantified, as appropriate)~~ — Completed by Kaufman, Aidan o...
- ~~List of Primary Design Functions~~ — Completed by Kaufman, Aidan o...
- ~~Ranked Objectives (Pairwise Comparison Chart – completed by your client and other stakeholders (not just you))~~ — Completed by Shirzadi, Ariel on 1...
- ~~Design Objectives (Categorized, Objectives Tree)~~ — Completed by Shirzadi, Ariel on 1...
- ~~Final Client Statement~~ — Completed by Kaufman, Aidan o...
- ~~Final Need Statement~~ — Completed by Kaufman, Aidan o...
- ~~Current State of the Art or "Gold Standard(s), including description of gap in knowledge, technology or deficiency that leads to the need for your project.~~ — Completed by Kaufman, Aidan o...
- ~~Refined Background (~3 slides)~~ — Completed by Kaufman, Aidan o...

Appendix D: Weekly Objectives For B-Term



Appendix E: Rankings for Objectives in Design Decision Matrix

Perfusion:

Sub objective 1: Flow

1: No perfusion through leaf
2: Perfusion rate between 10-100 µL/min with bursting of leaf or lack of perfusion
3: Perfusion rate between 10-100 µL/min without busting of leaf and can perfuse
4: Perfusion rate between 100-300 µL/min with bursting of leaf or lack of perfusion
5: Perfusion rate between 100-300 µL/min without bursting of leaf or and can perfuse

Sub objective 2: Leaking

1: complete leakage, device cannot hold media
2: much leakage, Device can handle media for a short amount of time

3: average leakage, Device needs to be refilled once a day
4: minimal leakage, Device can handle media well with refilling needed every 2-3 days
5: no leakage, Device is completely watertight

User friendliness:

Sub Objective 1: No Mechanical Parts (no gears)

1: Takes more than 10 minutes to assemble or disassemble the device, very difficult to put together in the hood, multiple pieces to sterilize in the autoclave, don't need external tools (ex: screwdriver)
2: Takes more than 8 minutes to assemble or disassemble the device, still very difficult to put together in the hood, multiple pieces to sterilize in the autoclave, don't need external tools (ex: screwdriver)
3: Takes more than 5 minutes to assemble or disassemble the device, more of an inconvenience to put together in the hood, some pieces to sterilize in the autoclave, don't need external tools (ex: screwdriver)
4: Takes less than 3 minutes to assemble or disassemble the device, moderately easy to put together in the hood, not many pieces to sterilize in the autoclave, don't need external tools (ex: screwdriver)
5: Takes less than 2 minutes to assemble or disassemble the device, very easy to put together in the hood, not many pieces to sterilize in the autoclave, don't need external tools (ex: screwdriver)

Sub Objective 2: Open Cavity to Seed Cells

1: Very difficult to seed cells, there's no openings available, can't seed cells from the top or bottom of the device
2: Has a small opening to seed cells, however, need to maneuver the device in different ways in order to properly seed cells
3: Has large enough opening for a pipette tip to seed cells, however, still need to maneuver the device a bit to seed all the cells
4: Has large opening, minimal movement of device to seed cells
5: Has large opening and can seed cells without moving the device at all

Sub Objective 3: Method of Sterilization
1: Need to replace each part because sterilizing isn't doable (ex: some porous materials can't be sterilized)
2: Can't use the same sterilization method for all the parts, depending on the material, some parts can't go into the autoclave (ex: not all plastics are autoclavable)
3: Can't use the same sterilization method for all parts, however, most of the parts are autoclavable but need to be put on a special setting
4: One piece of the device can't be autoclaved, however all the other pieces can be autoclaved
5: The entire device can be autoclaved (one sterilization method for the entire device)

Imageability:

Sub Objective 1: Transparent Imaging

1: Material is opaque
2: Material is less than 80% transparent
3: Material is between 80-85% transparent
4: Material is between 85-95% transparent
5: Material is at least 95% transparent

Sub Objective 2: Clear Imaging

1: Poor resolution at 200 microns
2: Scaffold can be magnified 200 microns with average resolution, harder to pinpoint some features on the scaffold
3: Scaffold can be magnified at 200 microns with excellent resolution, can pinpoint most features on the scaffold
4: Scaffold can be magnified at 100 microns with average resolution, harder to pinpoint some features on the scaffold
5: Scaffold can be magnified at 100 microns with great resolution, harder to pinpoint most features on the scaffold

Appendix F: Decision Matrices

			Sliding System			Hinge System			Peg System			Magnetic System			Zip Ties		
	Constraint	Weight %	Score	Normalized score	Weighted Sum	Score	Normalized score	Weighted Sum	Score	Normalized score	Weighted Sum	Score	Normalized score	Weighted Sum	Score	Normalized score	Weighted Sum
	Biocompatabilty		Y			Y			Y						Y		
	Sterilty/Autoclave		Y			Y			Y			Y			N		
	Cell Seeding		Y			Y			Y			Y			Y		
	Scaffold Access		Y			Y			Y			Y			Y		
	Downstream Compata		Y			Y			Y			Y			Y		
	Time		Y			Y			Y			Y			Y		
	Cost		Y			Y			Y			Y			Y		
	Manufacturabilty		Y			Y			Y			Y			Y		
	Longevity		Y			Y			N			Y			N		
	Bilayer		Y			Y			Y			Y			Y		
	Watertight		Y			Y			N			Y			Y		
	Objectives																
O1	Imageabili	30		2	60		2	60									
O	Transparent Imaging		5	1		5	1										
O	Clear Imaging		5	1		5	1										
O2	Perfusion	35.4		1.4	49.56		2	70.8									
O	Clamping/Flow		2	0.4		5	1										
O	Leaking		5	1		5	1										
O3	User Frienc	34.6		3	103.8		2.4	83.04									
O	No Mechincal Parts		5	1		2	0.4										
O	Open Cavity to Seed C		5	1		5	1										
O	Method of Sterilzation		5	1		5	1										
					213.36			213.84									

Image shows the scores for user friendliness design elements

	Screws			Metal Latch			Plastic Clip			Hose Clamps			Anchor Screws			Alligator Clip through pins	
Score	Normalized score	Weighted Sum	Score	Normalized score	Weighted Sum	Score	Normalized score	Weighted Sum	Score	Normalized score	Weighted Sum	Score	Normalized score	Weighted Sum	Score	Normalized score	Weighted Sum
Y			Y			Y			Y			Y			Y		
Y			Y			Y			Y			Y			Y		
Y			Y			Y			Y			Y			Y		
Y			Y			Y			Y			Y			Y		
Y			Y			Y			Y			Y			Y		
Y			Y			Y			Y			Y			Y		
Y			Y			Y			Y			Y			Y		
Y			Y			Y			Y			Y			Y		
Y			Y			N			N			Y			Y		
Y			Y			Y			Y			Y			Y		
Y			Y			Y			Y			Y			N		
	2	60		2	60								0.4	12			
5	1		5	1								1	0.2				
5	1		5	1								1	0.2				
	2	69.2		1.4	48.44								2	69.2			
5	1		2	0.4								5	1				
5	1		5	1								5	1				
	2.2	77.88		2.8	99.12								2	70.8			
1	0.2		4	0.8								4	0.8				
5	1		5	1								5	1				
5	1		5	1								1	0.2				
		207.08			207.56									152			

Image shows the scores for user friendliness design elements

	Alligator Clip through pins	
Score	Normalized score	Weighted Sum
Y		
Y		
Y		
Y		
Y		
Y		
Y		
Y		
Y		
Y		
N		

Image shows the scores for user friendliness design elements

	Direct Injection System			Screw in Direct injection system	
Score	Normalized score	Weighted Sum	Score	Normalized score	Weighted Sum
Y			Y		
Y			Y		
Y			Y		
Y			Y		
Y			Y		
Y			Y		
Y			Y		
Y			Y		
Y			Y		
Y			Y		
Y			Y		
	2	60		2	60
5	1		5	1	
5	1		5	1	
	1.6	55.36		2	69.2
5	1		5	1	
3	0.6		5	1	
	3	106.2		3	106.2
5	1		5	1	
5	1		5	1	
5	1		5	1	
		221.56			235.4

Image shows the scores for the perfusion system design elements

	Capillary action/continuous gravity flow	
Score	Normalized score	Weighted Sum
Y		
Y		
Y		
Y		
Y		
Y		
Y		
Y		
Y		
Y		
Y		
	2	60
5	1	
5	1	
	1	34.6
2	0.4	
3	0.6	
	3	106.2
5	1	
5	1	
5	1	
		200.8

Image shows the scores for the perfusion system design elements

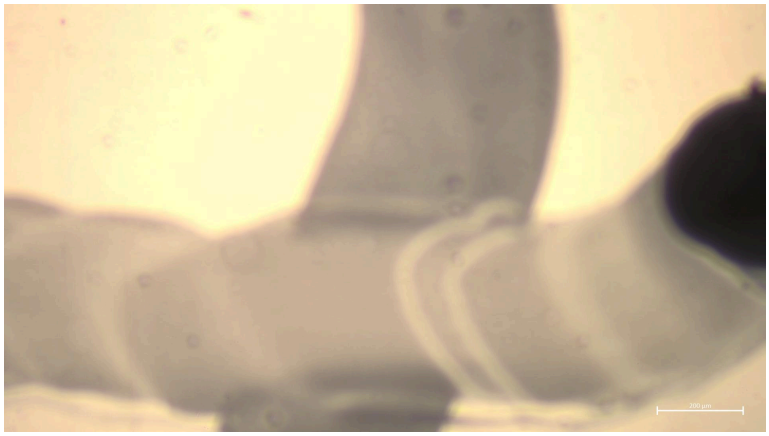
	open cavity			glass screen			metal screen			nylon mesh	
Score	Normalized score	Weighted Sum	Score	Normalized score	Weighted Sum	Score	Normalized score	Weighted Sum	Score	Normalized score	Weighted Sum
Y			Y			Y			Y		
Y			Y			Y			N		
Y			Y			Y			Y		
Y			Y			Y			Y		
Y			Y			Y			Y		
Y			Y			Y			Y		
Y			Y			Y			Y		
Y			Y			Y			Y		
Y			Y			Y			Y		
N			Y			Y			Y		
Y			Y			Y			Y		
				0.8	24		2	60			
			2	0.4		5	1				
			2	0.4		5	1				
				1.2	41.52		2	69.2			
			1	0.2		5	1				
			5	1		5	1				
				3	106.2		3	106.2			
			5	1		5	1				
			5	1		5	1				
			5	1		5	1				
					171.72			235.4			

Image shows the scores for imageability design elements

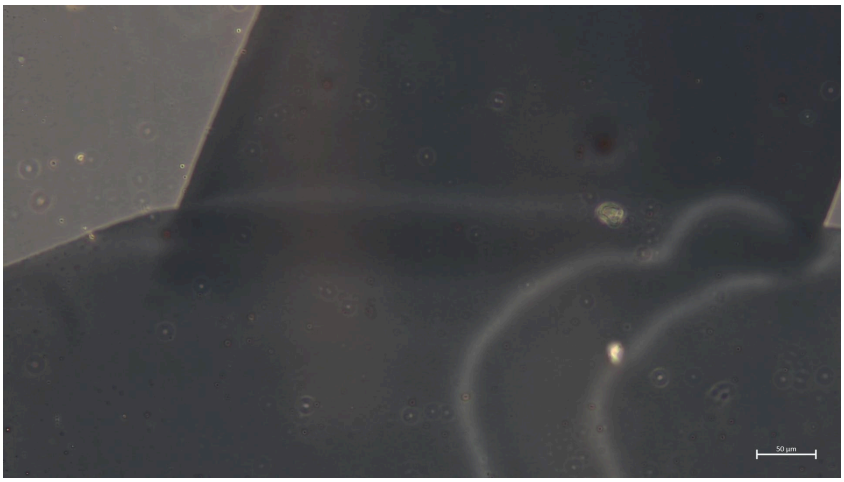
	removal from deivce	
Score	Normalized score	Weighted Sum
Y		
Y		
Y		
Y		
Y		
Y		
Y		
Y		
Y		
Y		
Y		
	2	60
5	1	
5	1	
	2	69.2
5	1	
5	1	
	3	106.2
5	1	
5	1	
5	1	
		235.4

Image shows the scores for imageability design elements

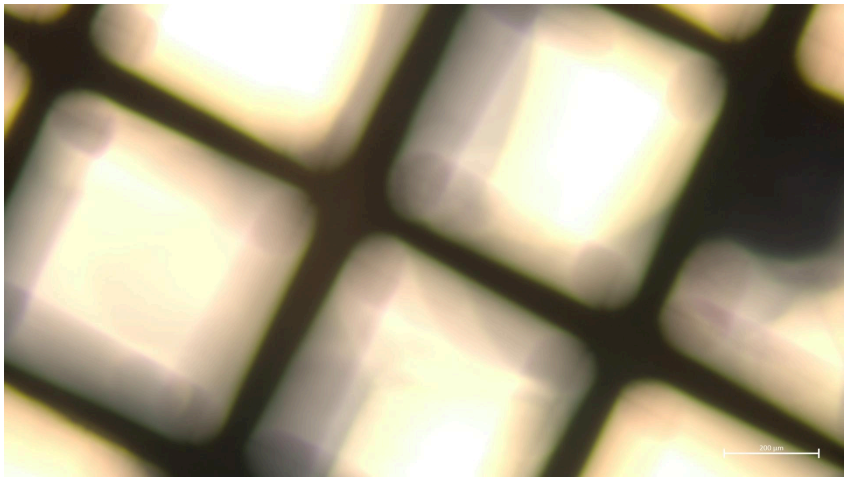
Appendix G: Image Testing Images



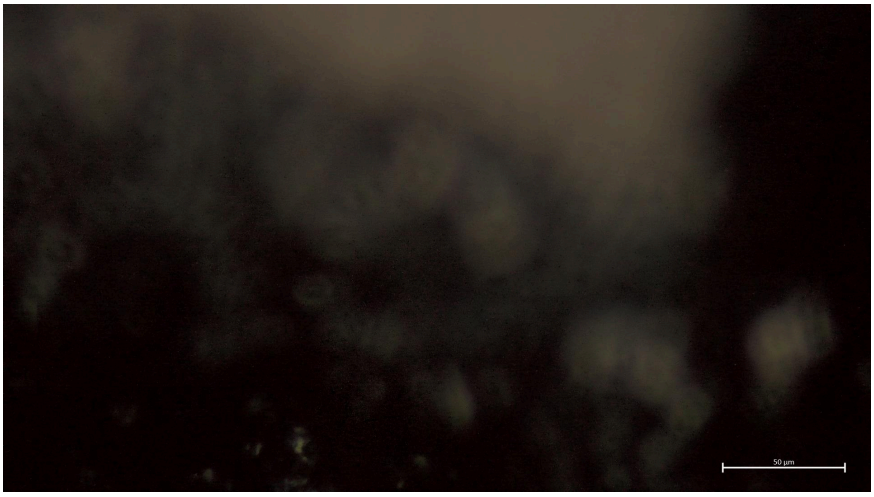
4X clear slide control group



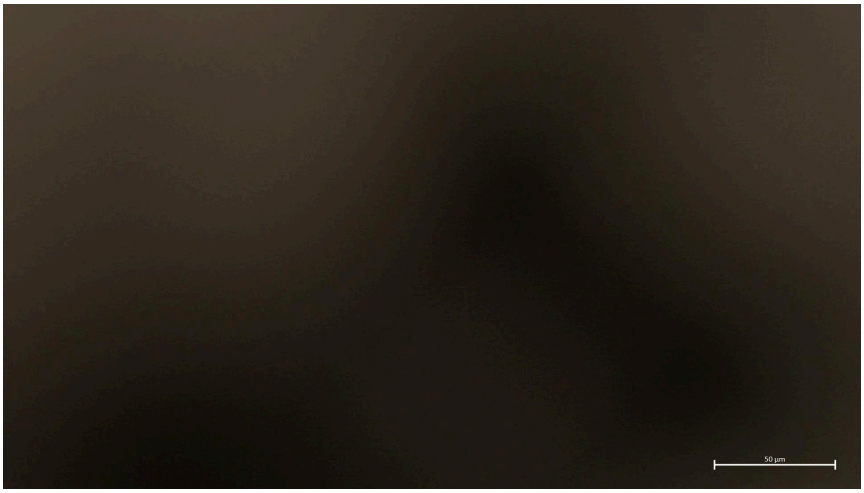
10X clear slide control group



4X clear slide in device

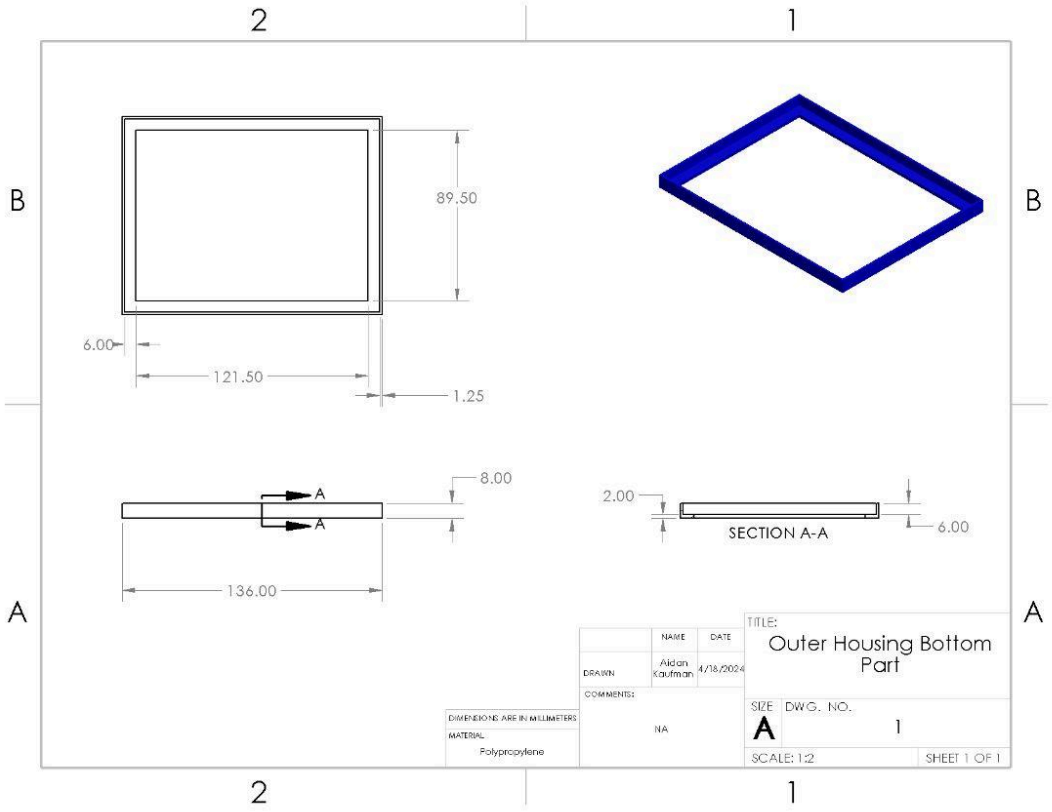


20X magnification of paper with X

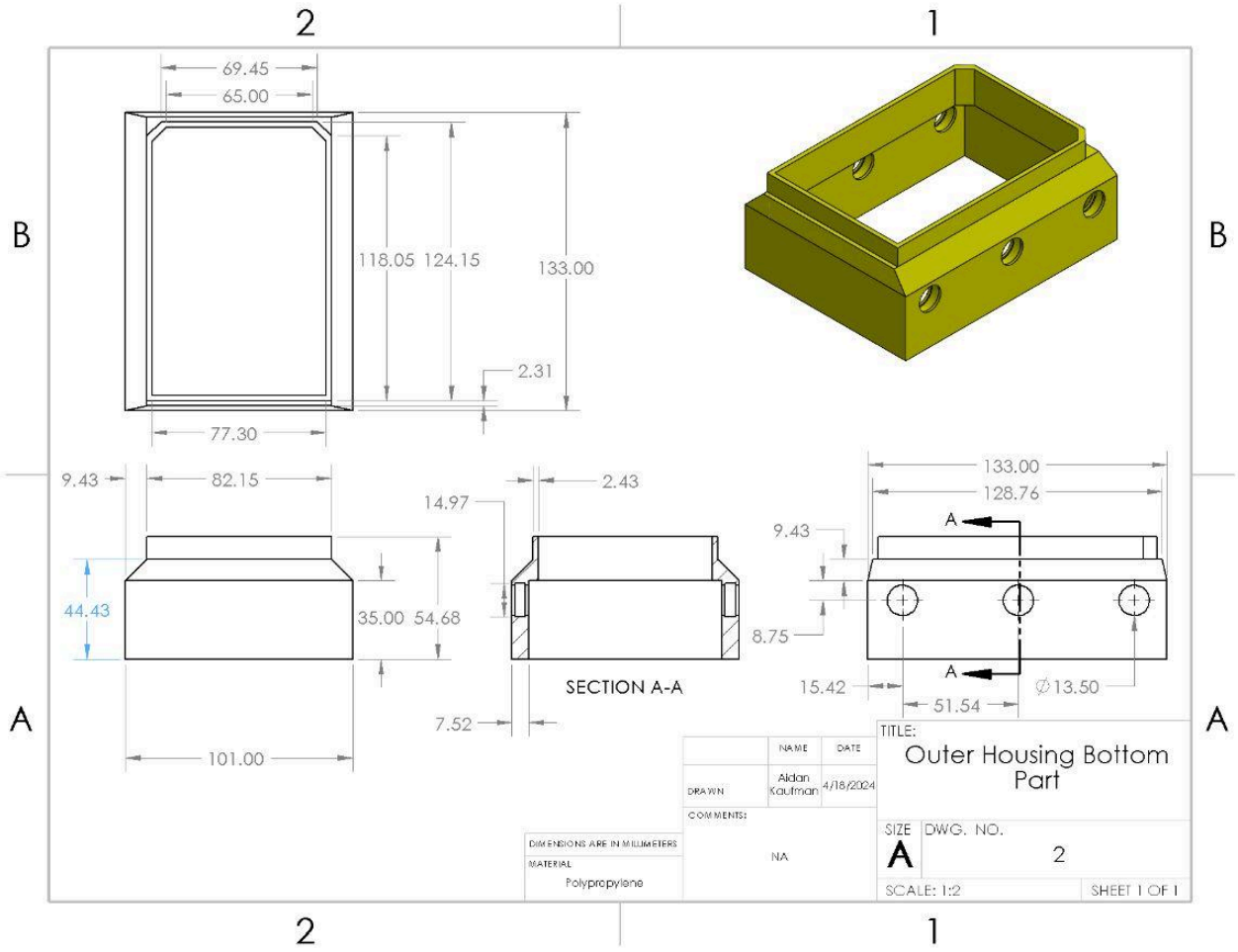


10X magnification with paper with X in device

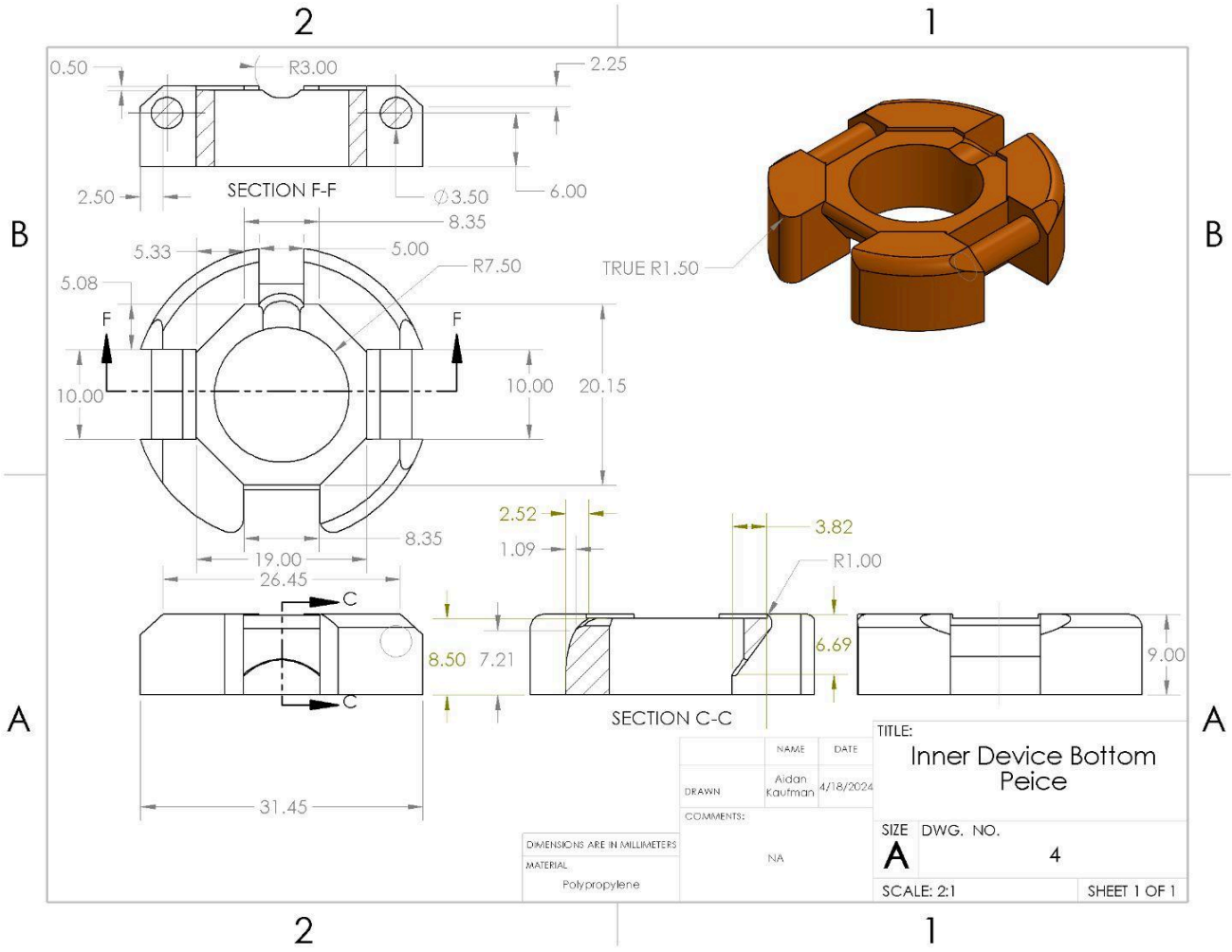
Appendix H: Soldiworks Dimensional Drawings



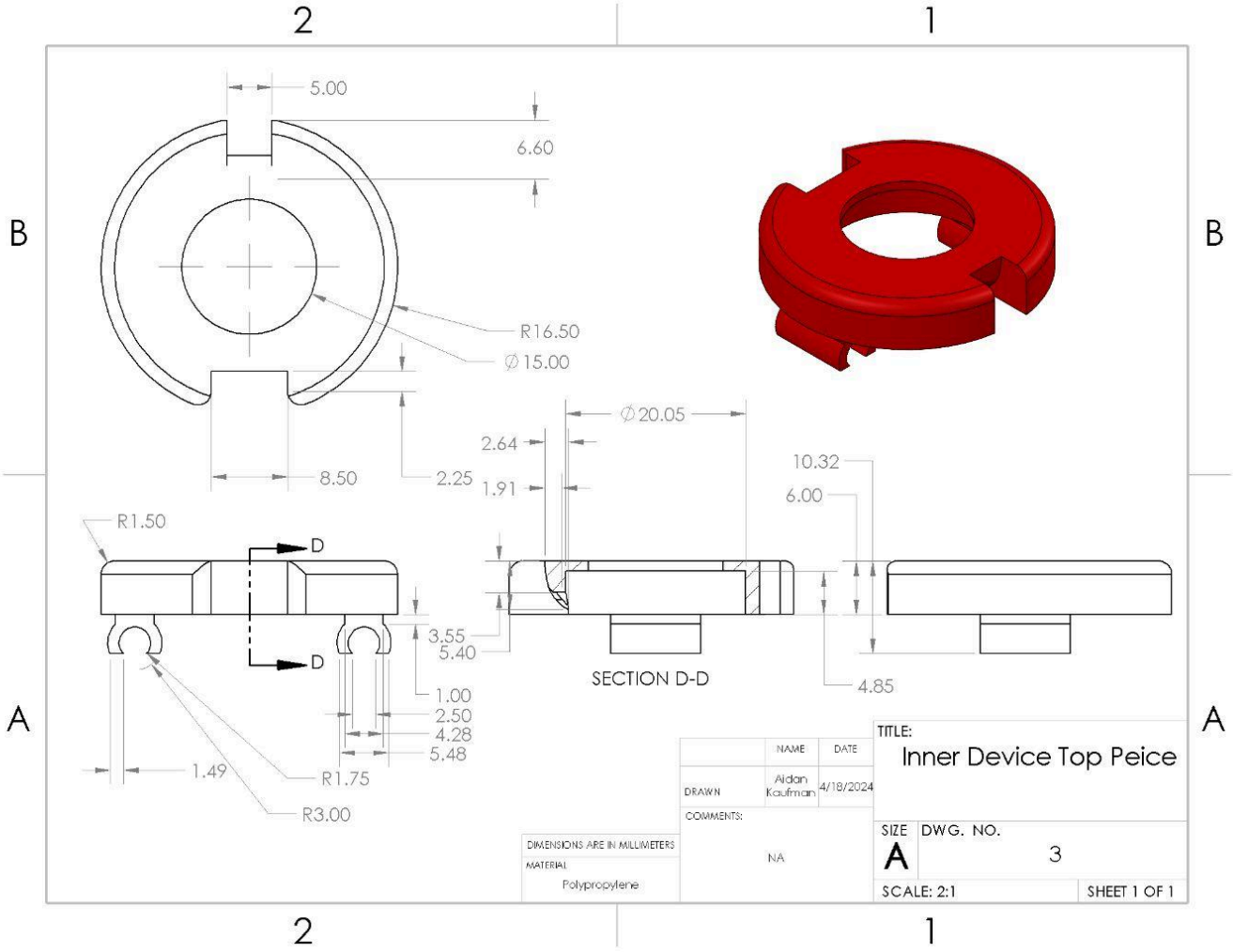
Outer Housing Bottom Part Dimensional Drawing



Outer Housing Bottom Part Dimensional Drawing



Outer Housing Bottom Part Dimensional Drawing



Outer Housing Bottom Part Dimensional Drawing

Appendix I: Perfusion Testing Data

Trial 1A (this trial had PDMS strips with one channel)											
	Length of Tubes (m)	Inner Diameter of Tubes (m)					Time For Collecting Water (min)			Calculations for Velocity without Cannula Tube	Note: this flow rate was

														found without the cannula, flow rate will change with the cannula included in the set up
Big Tube	0.13853	0.0096					30 min				Flow Rate (m^3/min)	Cross-Sectional Area (m^2)	Velocity (m/min)	
Medium Tube	0.148	0.00266								Big Tube	0.00000028	0.000000723	0.0039	

Needle	0.0217	0.0003								Medium Tube	0.00000028	0.000000055	0.05	
Cannula Tube	0.04805	0.00038								Needle	0.00000028	0.00000000007	3.96	
	Height of Tubes (m)	Volume without Cannula Attachment (uL)	Time without Cannula Attachment (min)	Flow Rate without Cannula Attachment (uL/min)	Volume with Cannula Attachment (uL)	Time with Cannula Attachment (min)	Flow Rate with Cannula Attachment (uL/min)			Length A (m)	Length B	Length C	Length D	Length E	Length F
Carboy/big tube to ground	0.29	8400	30	280	1800	10	180			0.138	0.000671	0.148	0.019	0.02217	0.005506
Medium tube to ground	0.16	8400	30	280	1800	10	180								
Needle to ground	0.015	8400	30	280	1800	10	180								
Cannula to ground	0	0	0	0	1800	10	180								

Height	Density (kg/m^3)	Viscosity (u)	Length	Inner Diameter of Tubes (m)											
0.29			0.13853	0.0096											
0.16			0.1478	0.00266											
0.015			0.0217	0.0003											
0	997	0.001	0.04805	0.00038											
	Flow Rate (m^3/sec)	Cross-Sectional Area (m^2)	Velocity (m/sec)												
Big Tube	0.000000003	0.0000723456	0.00004146762208												
Medium Tube	0.000000003	0.0000055543 46	0.0005401175944												
Needle	0.000000003	0.00000007065	0.04246284501												
Cannula Tube	0.000000003	0.000000113354	0.02646576213												
Trial 1B (this trial had PDMS strips with multiple channels)															

	Length of Tubes (m)	Inner Diameter of Tubes (m)													
Big Tube	0.13853	0.0096													
Medium Tube	0.148	0.00266													
Needle	0.0217	0.0003													
Cannula Tube	0.04805	0.00038													
	Height of Tubes (m)	Volume with Cannula Attachment (uL)	Time with Cannula Attachment (min)	Flow Rate with Cannula Attachment (uL/min)											
Carboy/big tube to ground	0.29	2200	10	220											
Medium tube to ground	0.16	2200	10	220											
Needle to	0.015	2200	10	220											

ground														
Cannula to ground	0	2200	10	220										
Height	Density (kg/m^3)	Viscosity (u)	Length	Inner Diameter of Tubes (m)										
0.29			0.13853	0.0096										
0.16			0.1478	0.00266										
0.015			0.0217	0.0003										
0	997	0.001	0.04805	0.00038										
	Flow Rate (m^3/sec)	Cross-Sectional Area (m^2)	Velocity (m/sec)											
Big Tube	0.000000000366666667	0.0000723456	0.00005068264921											
Medium Tube	0.000000000366666667	0.000005554346	0.0006601437265											
Needle	0.00000	0.00000007065	0.05189903279											

	0.000366666667													
Cannula Tube	0.000000000366666667	0.000000113354	0.0323470426											
Trial 2A (this trial had PDMS strips with single channel)														
	Length of Tubes (m)	Inner Diameter of Tubes (m)												
Big Tube	0.3575	0.0096												
Medium Tube	0.148	0.00266												
Needle	0.0217	0.0003												
Cannula Tube	0.04805	0.00038												
	Height of Tubes (m)	Volume with Cannula Attachment (uL)	Time with Cannula Attachm	Flow Rate with Cannula Attachment (uL/min)										

			ent (min)											
Carboy/big tube to ground	0.53	1500	10	150										
Medium tube to ground	0.19	1500	10	150										
Needle to ground	0.04	1500	10	150										
Cannula to ground	0	1500	10	150										
Height	Density (kg/m^3)	Viscosity (u)	Length	Inner Diameter of Tubes (m)										
0.53			0.13853	0.0096										
0.19			0.1478	0.00266										
0.04			0.0217	0.0003										
0	997	0.001	0.04805	0.00038										
	Flow Rate (m^3/sec)	Cross-Sectional Area (m^2)	Velocity (m/sec)											

Big Tube	0.00000000025	0.0000723456	0.00003455635173											
Medium Tube	0.00000000025	0.0000055543 46	0.00045009799 53											
Needle	0.00000000025	0.0000000 07065	0.03538570418											
Cannula Tube	0.00000000025	0.0000000 113354	0.02205480177											
Trial 2B (this trial had PDMS strips with multiple channels)														
	Length of Tubes (m)	Inner Diameter of Tubes (m)												
Big Tube	0.3575	0.0096												
Medium Tube	0.1478	0.00266												
Needle	0.0217	0.0003												
Cannula Tube	0.04805	0.00038												
	Height of	Volume with	Time with	Flow Rate with										

	Tubes (m)	Cannula Attachment (uL)	Cannula Attachment (min)	Cannula Attachment (uL/min)										
Carboy/big tube to ground	0.53	1000	10	100										
Medium tube to ground	0.19	1000	10	100										
Needle to ground	0.04	1000	10	100										
Cannula to ground	0	1000	10	100										
Height	Density (kg/m^3)	Viscosity (u)	Length	Inner Diameter of Tubes (m)										
0.53			0.13853	0.0096										
0.19			0.1478	0.00266										
0.04			0.0217	0.0003										
0	997	0.001	0.04805	0.00038										
	Flow Rate	Cross-Sectional	Velocity (m/sec)											

	(m^3/sec)	Area (m^2)												
Big Tube	0.0000000001666666667	0.0000723456	0.00002303756782											
Medium Tube	0.0000000001666666667	0.0000055434 6	0.0003000653302											
Needle	0.0000000001666666667	0.0000000706 5	0.02359046945											
Cannula Tube	0.0000000001666666667	0.0000001133 54	0.01470320118											
Trial 3A (this trial had PDMS strips with single channel)														
	Length of Tubes (m)	Inner Diameter of Tubes (m)												
Big Tube	0.285	0.0096												

Medium Tube	0.148	0.00266												
Needle	0.0217	0.0003												
Cannula Tube	0.04805	0.00038												
	Height of Tubes (m)	Volume with Cannula Attachment (uL)	Time with Cannula Attachment (min)	Flow Rate with Cannula Attachment (uL/min)										
Carboy/big tube to ground	0.47	1700	10	170										
Medium tube to ground	0.18	1700	10	170										
Needle to ground	0.04	1700	10	170										
Cannula to ground	0	1700	10	170										
Height	Density	Viscosity (u)	Length	Inner Diameter										

	(kg/m^3)			of Tubes (m)										
0.47			0.13853	0.0096										
0.18			0.1478	0.00266										
0.04			0.0217	0.0003										
0	997	0.001	0.04805	0.00038										
	Flow Rate (m^3/sec)	Cross-Sectional Area (m^2)	Velocity (m/sec)											
Big Tube	0.000000000266666667	0.0000723456	0.00003686010852											
Medium Tube	0.000000000266666667	0.000005554346	0.0004801045284											
Needle	0.000000000266666667	0.00000007065	0.03774475112											
Cannula Tube	0.000000000266666	0.000000113354	0.02352512189											

	6667													
Trial 3B (this trial had PDMS strips with multiple channels)														
	Length of Tubes (m)	Inner Diameter of Tubes (m)												
Big Tube	0.285	0.0096												
Medium Tube	0.148	0.00266												
Needle	0.0217	0.0003												
Cannula Tube	0.04805	0.00038												
	Height of Tubes (m)	Volume with Cannula Attachment (uL)	Time with Cannula Attachment (min)	Flow Rate with Cannula Attachment (uL/min)										
Carboy/big tube to ground	0.47	400	10	40										
Medium tube	0.18	400	10	40										

to ground														
Needle to ground	0.04	400	10	40										
Cannula to ground	0	400	10	40										
Height	Density (kg/m^3)	Viscosity (u)	Length	Inner Diameter of Tubes (m)										
0.47			0.13853	0.0096										
0.18			0.1478	0.00266										
0.04			0.0217	0.0003										
0	997	0.001	0.04805	0.00038										
	Flow Rate (m^3/sec)	Cross-Sectional Area (m^2)	Velocity (m/sec)											
Big Tube	0.00000000066666667	0.0000723456	0.000009215027129											
Medium Tube	0.00000000066666	0.000005554346	0.0001200261321											

	66667													
Needle	0.0000000000666666666667	0.00000007065	0.009436187782											
Cannula Tube	0.0000000000666666666667	0.00000011335	0.005881280472											

Perfusion Testing Data

Theoretical Pressure Drop Calculations																										Experimental Pressure Drop Calculations									
Trial 1A (Single Channel)																										Trial 1A (Single Channel)									

Q (m^3/s)	u (pa*s)	LA	LB	LC	LD	LE	LF	r1	r2	r3	r4	v1	v2	v3	v4	Delta P for LA (Hagen Poisuelle) (Pa)	Delta P for LB	Delta P for L1 (Bernouli) (Pa)	Total Pressure Drop Along L1	Delta P for LC	Delta P for LD	Delta P for L2	Total Pressure Drop Along L2	Delta P for LE	Delta P for LF	Delta P for L3	Total Pressure Drop Along L3		Density (kg/m^3)	Gravity (m/s^2)	h1	h2	h3	F1	F2	F3	Delta F for L1	Delta F for L2	Delta P for L Total (Pa)
0.0000000003	0.001002	0.138	0.00671	0.0148	0.019	0.0217	0.00506	0.0048	0.00133	0.00015	0.00019	0.00004146762208	0.0005401175944	0.004246284501	0.02646576213	0.001990964205	0.01642348323	0.0001447137186	0.01855916116	0.03622467241	287.4338287	0.8995979382	288.3696513	328.2796886	29.7361972	-0.5502256641	357.4656601		997	9.8	0.29	0.16	0.015	2835.20878	1564.25312	146.64873	1270.955636	1417.60439	2688.56005
Trial 1B (Multiple Channels)																													Trial 1B (Multiple Channels)										
Q (m	u (p	LA	LB	LC	LD	LE	LF	r1	r2	r3	r4	v1	v2	v3	v4	Delta	Delta P	Delta P	Total Pr	Delta	Delta	Delta	Total Pr	Delta P	Delta P	Delta P	Total Pr		Density	Gra	h1	h2	h3	F1	F2	F3	Del	Del	Delt

^3/s)	a*s)															P for LA (Hagen Poisuelle) (Pa)	for LB	for L1 (Bernouli) (Pa)	essure Drop Along L1	P for LC	P for LD	P for L2	essure Drop Along L2	for LE	for LF	for L3	essure Drop Along L3		(kg/m^3)	vity (m/s^2)							taPF for L1	taPF for L2	aP for L Total (Pa)
0.000000003666666667	0.001002	0.138	0.00671	0.0148	0.019	0.0217	0.00506	0.0048	0.00133	0.00015	0.00019	0.00005068264921	0.0006601437265	0.005189903279	0.0323470426	0.002433400695	0.02007314617	0.0002161772833	0.02272272415	0.04427459961	351.3080129	1.343843834	352.6961313	401.2307305	36.34424102	-0.8219420414	436.7530295		997	9.8	0.29	0.16	0.015	2835.20878	1564.25312	146.64873	1270.95566	1417.60439	2688.56005
Trial 2A (Single Channel)																													Trial 2A (Single Channel)										
Q (m^3/s)	u (pa*	LA	LB	LC	LD	LE	LF	r1	r2	r3	r4	v1	v2	v3	v4	Delta P for L	Delta P for LB	Delta P for L1 (B	Total Pressure	Delta P for	Delta P for	Delta P for L2	Total Pressure	Delta P for LE	Delta P for LF	Delta P for L3	Total Pressure		Density (kg/m^3)	Gravit	h1	h2	h3	P1	P2	P3	DeltaPF	DeltaPF	DeltaPf

	s)															A (Hagen Poisuselle) (Pa)		ernouli) (Pa)	Drop Along L1	LC	LD		Drop Along L2				Drop Along L3			y (m/s^2)							for L1	for L2	or L Total
0.0000000025	0.001002	0.3575	0.00671	0.0148	0.019	0.0217	0.00506	0.0048	0.00133	0.00105	0.00119	0.00003455635173	0.0004500979953	0.03538570418	0.02205480177	0.004298126228	0.01368623603	0.0001004956379	0.01808485789	0.03018722701	239.5281906	0.6247207904	240.1830986	273.5664072	24.78016433	-0.3821011556	297.9644703		997	9.8	0.53	0.19	0.04	5181.58846	1857.55058	391.06328	3324.03788	1466.4873	4790.52518
Trial 2B (Multiple Channels)																													Trial 2B (Multiple Channels)										
Q (m^3/s)	u (pa*s)	LA	LB	LC	LD	LE	LF	r1	r2	r3	r4	v1	v2	v3	v4	Delta P for LA (Hagen	Delta P for LB	Delta P for L1 (Bernouli)	Total Pressure Drop Alon	Delta P for LC	Delta P for LD	Delta P for L2	Total Pressure Drop Alon	Delta P for LE	Delta P for LF	Delta P for L3	Total Pressure Drop Alo		Density (kg/m^3)	Gravity (m/	h1	h2	h3	F1	F2	F3	Delta F for	Delta F for	Delta P for LT

																Poisuelle)(Pa)		(Pa)	g L1				g L2				ng L3			s^2)							L1	L2	otal
0.000000000166666666667	0.001002	0.3575	0.00671	0.0148	0.019	0.0217	0.00506	0.0048	0.00133	0.00015	0.00019	0.00002303756782	0.0003000653302	0.02359046945	0.01470320118	0.002865417485	0.09124157352	0.0004466472796	0.01203423957	0.020124818	159.6854604	0.2776536846	159.9832389	182.3776048	16.52010956	-0.1698227358	198.7278916		997	9.8	0.53	0.19	0.04	5181.58846	1857.55058	391.06328	3324.03788	1466.4873	4790.52518
Trial 3A (Single Channel)																													Trial 3A (Single Channel)										
Q (m^3/s)	u (pa*s)	LA	LB	LC	LD	LE	LF	r1	r2	r3	r4	v1	v2	v3	v4	Delta P for LA (Hagen Poisuell	Delta P for LB	Delta P for L1 (Bernouli) (Pa)	Total Pressure Drop Along L1	Delta P for LC	Delta P for LD	Delta P for L2	Total Pressure Drop Along L2	Delta P for LE	Delta P for LF	Delta P for L3	Total Pressure Drop Along L3		Density (kg/m^3)	Gravity (m/s^2)	h1	h2	h3	F1	F2	F3	Delta P for L1	Delta P for L2	Delta P for Total

																e) (Pa)																							
0.0000000000266666666667	0.001002	0.285	0.00671	0.0148	0.019	0.0217	0.00506	0.0048	0.00133	0.00015	0.00019	0.00003686010852	0.0004801045284	0.03774475112	0.02352512189	0.003654910135	0.01459865176	0.001143417036	0.0183679036	0.03219970881	255.4967367	0.7107934327	256.2397298	291.8041676	26.43217529	-0.4347462037	317.8015967		997	9.8	0.47	0.18	0.04	4594.99354	1759.78476	391.06328	2835.20878	1368.72148	4203.93026
Trial 3B (Multiple Channels)																													Trial 3B (Multiple Channels)										
Q (m^3/s)	u (pa*s)	L A	L B	L C	L D	L E	L F	r1	r2	r3	r4	v1	v2	v3	v4	Delta P for LA (Hagen Poisuelle) (Pa)	Delta P for LB	Delta P for L1 (Bernouli) (Pa)	Total Pressure Drop Along L1	Delta P for LC	Delta P for LD	Delta P for L2	Total Pressure Drop Along L2	Delta P for LE	Delta P for LF	Delta P for L3	Total Pressure Drop Along L3		Density (kg/m^3)	Gravity (m/s^2)	h1	h2	h3	F1	F2	F3	Delta F for L1	Delta F for L2	Delta P for L Total

0.000000000066666666667	0.001002	0.285	0.00671	0.0148	0.019	0.0217	0.00506	0.0048	0.00133	0.00015	0.00019	0.000009215027129	0.0001200261321	0.00943618778	0.005881280472	0.0009137275338	0.003649662941	0.000007146356474	0.004570536831	0.008049927202	63.87418416	0.04442458954	63.92665868	72.95104191	6.608043822	-0.02717163773	79.5319141		997	9.8	0.47	0.18	0.04	4594.99354	1759.78476	391.06328	2835.20878	1368.72148	4203.93026

Perfusion Testing Calculations

Appendix J: Autoclave Testing Data

Percent Infill	Original Width (mm)	Original Length (mm)	Original Height (mm)	Post Autoclave Width (mm)	Post Autoclave Length (mm)	Post Autoclave Height (mm)	Deformation Width (mm)	Deformation Length (mm)	Deformation Height (mm)	Percent Deformation Width	Percent Deformation Length	Percent Deformation Height	Average Deformation Length	Average Deformation Width	Percent Infill	Standard Deviation Length	Standard Deviation Width
10% #1	20.03	20	16.76	19.97	19.96	16.73	0.06	0.04	0.03	0.29955067 4	0.2	0.178997 6134	0.045	0.03	10%	0.007071067812	0.03535533906
10% #2	19.96	20.01	19.93	19.95	19.96	19.9	0.01	0.05	0.03	0.0501002004	0.2498750625	0.150526844	0.055	0.06	20%	0.03535533906	0.02121320344
20% #1	20.04	20.03	16.8	19.95	20	16.73	0.09	0.03	0.07	0.4491017964	0.149775337	0.4166666667	0.055	0.055	30%	0.007071067812	0.03535533906

20% #2	20.09	20.19	20	20.03	20.11	19.95	0.06	0.08	0.05	0.2986560478	0.396235 7603	0.25	0.065	0.05	40%	0.007071 06781 2	0.02828427125
30% #1	19.98	20.03	16.9	19.9	19.98	16.85	0.08	0.05	0.05	0.4004004004	0.249625 5617	0.295857 9882	0.04	0.03	50%	0.014142 13562	0
30% #2	20.04	20.17	20.01	20.01	20.11	19.95	0.03	0.06	0.06	0.1497005988	0.297471 4923	0.299850 075	0.02	0.045	60%	0	0.01414213562
40% #1	20	20.03	16.92	20	19.97	16.9	0	0.06	0.02	0	0.299550 674	0.118203 3097	0.025	0.07	70%	0.021213 20344	0
40% #2	20.04	19.92	20.13	20	19.85	20.05	0.04	0.07	0.08	0.1996007984	0.351405 6225	0.397416 7909	0.02	0.025	80%	0	0.00707106781 2
50% #1	20.12	20.2	16.94	20.1	20.15	16.9	0.02	0.05	0.04	0.0994035 7853	0.247524 7525	0.236127 5089	0.015	0.025	90%	0.021213 20344	0.00707106781 2
50% #2	20.1	20.1	19.98	20.08	20.07	19.96	0.02	0.03	0.02	0.0995024 8756	0.149253 7313	0.100100 1001					
60% #1	20.07	20.1	16.92	20.03	20.08	16.87	0.04	0.02	0.05	0.1993024 415	0.099502 48756	0.295508 2742					
60% #2	20.07	19.95	19.94	20.05	19.93	19.9	0.02	0.02	0.04	0.0996512 2073	0.100250 6266	0.200601 8054					
70% #1	20.01	19.97	16.9	19.98	19.93	16.83	0.03	0.04	0.07	0.1499250 375	0.200300 4507	0.414201 1834					
70% #2	20.09	20.02	20.01	20.06	20.01	19.94	0.03	0.01	0.07	0.1493280 239	0.049950 04995	0.349825 0875					

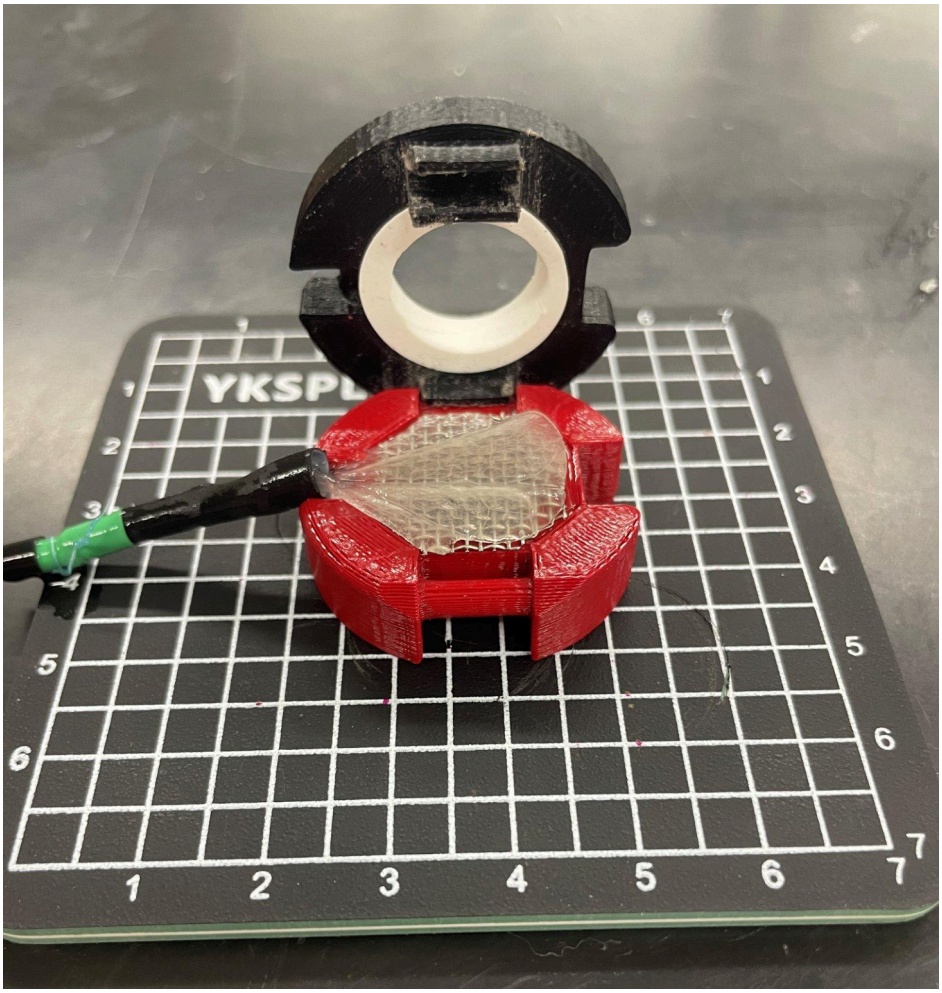
80 % #1	20.07	19.98	16.91	20.03	19.96	16.89	0.04	0.02	0.02	0.1993024415	0.100100101	0.1182732111					
80 % #2	20.05	19.99	19.96	20.02	19.97	19.93	0.03	0.02	0.03	0.1496259352	0.100050025	0.150300 6012					
90 % #1	19.98	20	16.81	19.96	19.97	16.77	0.02	0.03	0.04	0.100 1001 001	0.15	0.23 7953 599					
90 % #2	19.97	20.02	20.03	19.96	20.02	20.02	0.01	0	0.01	0.050 0751 1267	0	0.04 9925 1123 3					

Autoclave Testing Data

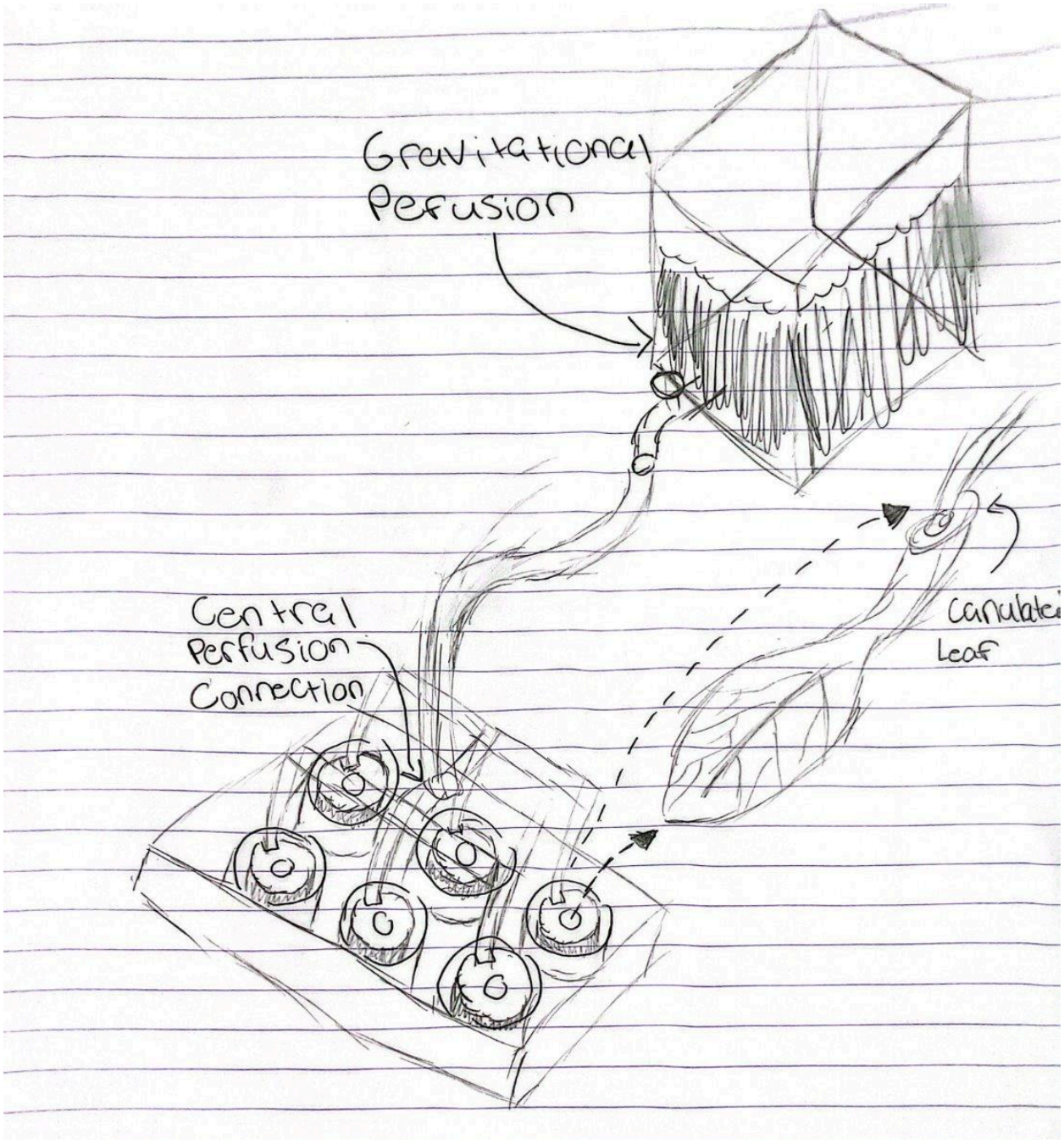
Appendix K: Bill of Materials

Component Name	Size / Quantity	Origin of Purchase	Manufacturer	Part Number
PLA filament	1.75mm (1kg)	Amazon	ELEGOO	N/A
Polypropylene filament	1.75mm (750g)	3DJake	Fiberology	FL-PP-NATURAL-175-750
Silicone Sheet	12”x12”x$^{3}/_{16}$”	McMaster Carr	McMaster Carr	86045K81
T316 Stainless Steel Mesh	36”x6’ 20 Mesh count	TWP Inc.	TWP Inc.	020X020T0160
Heat Shrink Tubing	1 Count (580 pcs)	Amazon	Ginsco	N/A
Polyethylene Tubing	10’ (ID 3/16”, OD 5/16”)	McMaster Carr	McMaster Carr	93334T34
Female Luer Lock	⅛” Diameter	Amazon	MEETOOT	NA
Blunt Needle Tip with Luer Lock	50 pack of 30 Gauge ½”	Amazon	BSTEAN	NA

Appendix L: Decellularized Leaf in Assembled Inner Device



Appendix M: Ideation Drawing



Appendix O: 3D Printer Settings