Point-of-Care Scaffold for Tissue Reconstruction

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Acknowledgment

We would like to thank our project advisors Professor Page from Worcester Polytechnic Institute and Dr. Giatsidis from University of Massachusetts Medical school for their guidance throughout this past year. Professor Page was helpful in revisions of brainstorming ideas, providing advice and feedback, and helping us with our engineering designs and iterations. Dr. Giatsidis was crucial in helping us understand the main need for this device and with extensive knowledge on the subject of adipose tissue and breast reconstruction.

We would also like to thank Professor Mensah from Worcester Polytechnic Institute for his guidance in the beginning stages of our MQP project and providing feedback on written aspects of the project in BME 4300. As well as Jyotsna Patel in the Gateway Labs at Worcester Polytechnic Institute for teaching the team how to properly perform cryosectioning and histology staining.

For assistance with testing our device on human lipoaspirate we would like to thank Dr. Fujimaki and Nicholas Jarostchuk, who were critical when testing our prototype, ran our device with ease, and gave essential feedback.

Finally, we would also like to thank Lisa Wall for all of her help in purchasing materials and helping us find and make use of everything available to us on campus for prototyping. As well as Andrew Leverone for all of his help while printing multiple iterations of our design in 3D resin, giving feedback on how it can be improved, and finally printing our final filtering compartment.

Executive Summary

Abstract

This project aimed to create a single-use closed system for decellularizing lipoaspirate in under an hour to produce a scaffold for breast tissue reconstruction. This design was inspired by the novel mechanical technique to decellularize adipose tissue by Zhang et al. The decellularization and filtration techniques used in the prototype were verified through multiple iterations of testing with a carrot lipoaspirate surrogate. The final design was able to blend and decellularize lipoaspirate fat from the patient and filter through a mesh to remove waste liquid presumed to be composed of blood, oil, and tumescent fluids to obtain a 49.69% yield of presumptive ECM content, but due to limitations histology could not be performed to verify the composition.

Introduction

Breast cancer is the second most common cancer diagnosis among women, which usually requires a complete breast removal or a mastectomy [2]. After a mastectomy, 40% of women undergo breast reconstruction, with the most common methods being: artificial implants, autologous free flap reconstruction, autologous fat grafting, and cadaver scaffolds [2]. The focus of our device will be to work with adipose tissue. Adipose tissue is a loose fibrous connective tissue that is packed with many cells called adipocytes [9]. As shown in **Figure 1**, each adipocyte is filled with a single large droplet of triglyceride, and the cells are tightly packed, leaving little room for the extracellular matrix (ECM), resembling a sponge [9].

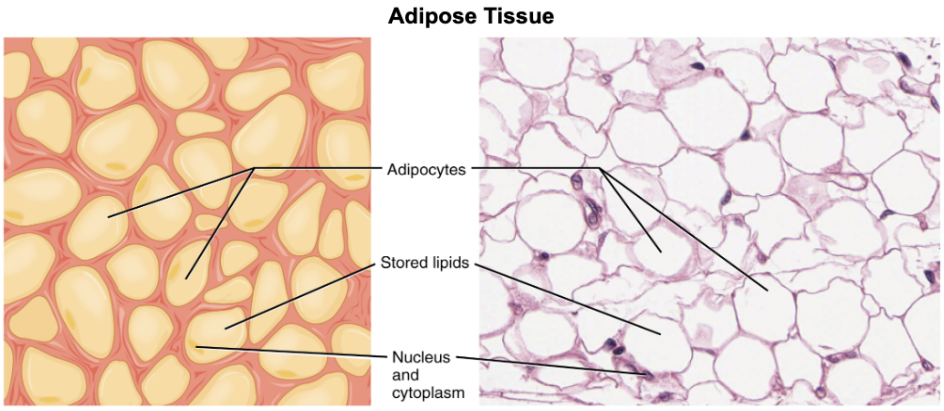

Figure 1: Structure of Adipose Tissue [9].

Currently, the most promising method for breast reconstruction without cell death is cadaver-derived scaffolds. The scaffolds undergo a multiple-step chemical treatment to completely rid the ECM scaffold of cadaver immunogenic cellular components [4]. However, the problem with this method is that it takes a long time to manufacture and is a costly treatment [4]. Therefore, our group researched a method to decellularize a patient's own adipose tissue in the operating room quickly and safely. Through research, we found a method that decellularizes adipose tissue from liposuction through mechanical blending, mesh filtration, and centrifugation, to create a product called Adipose Collagen Fragments, also known as ACF. This product is very beneficial as it is biocompatible, autologous, and contains on the ECM components of adipose tissue to create a scaffold that allows for adipocyte migration. This product has only been created in the lab and not yet used in the OR. Upon finding this method, our team went about to find a way to replicate similar aspects of this method in a closed device for use in the OR to create a scaffold for breast reconstruction on patient’s who have undergone a mastectomy.

Methods

The team focused primarily on two goals: remove the water, blood, and oil from the lipoaspirate, and disrupt the cells to decellularize the tissue.

Methods for Decellularization

Many ideas were brought up and we debated which method would be the most ideal for the users to decellularize tissues with the time restriction of liposuction surgery. Based on this, we found blending to be our best route, and a Nutribullet blender was used for all testing and in our final prototype, as shown in **Figure 2** below.

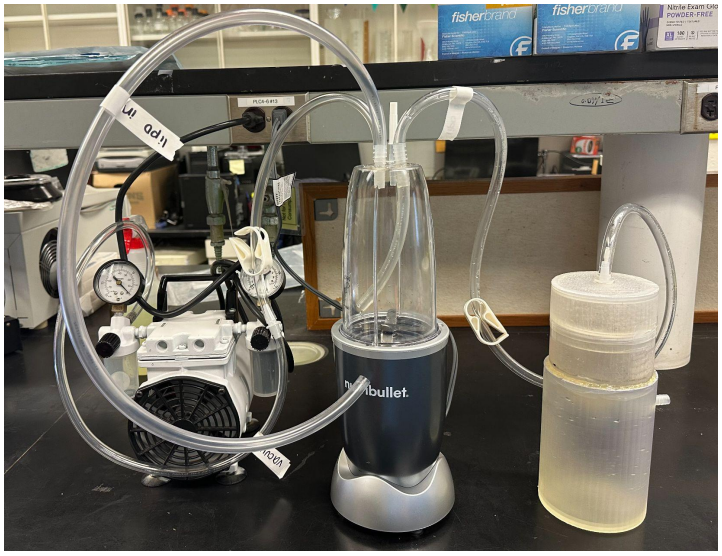

Figure 2: Final Prototype Setup.

Methods for Filtration of Blood, Oil, and Waste Liquids

To remove the majority of the blood, oil, and remaining waste liquids from the decellularized tissue, different types of mesh filters were tested to find which would be the best for our design. Additionally, the added step of decanting was performed before mesh filtration to eliminate the majority of blood and oil in the canister.

Methods for Prototyping

The prototypes were first modeled on SolidWorks CAD software. Then, most device components were 3D printed using resin, making the device disposable and robust enough to hold the human tissue and filter out the waste without any leaks. Finally, all the components were assembled and glued by hand using UV resin.

Design Validation and Verification Protocol

To test whether the components of our system were properly decellularizing and filtering the lipoaspirate, the team performed verification tests with a carrot lipoaspirate surrogate mixture composed of 5% fibers (carrots), 10% oil (vegetable oil), and 85% aqueous solution (DPBS), based on previously researched values [4].

Protocol for Testing Methods of Decellularization

To determine if the prototype properly decellularized samples of boiled carrots and DPBS were taken before and after blending. These samples were cryosectioned and H&E stained to visualize the cell membranes, as shown in **Figure 3**. The results were quantified visually to assess the structure of the cell walls and as expected the cell walls had burst and were adequately broken.

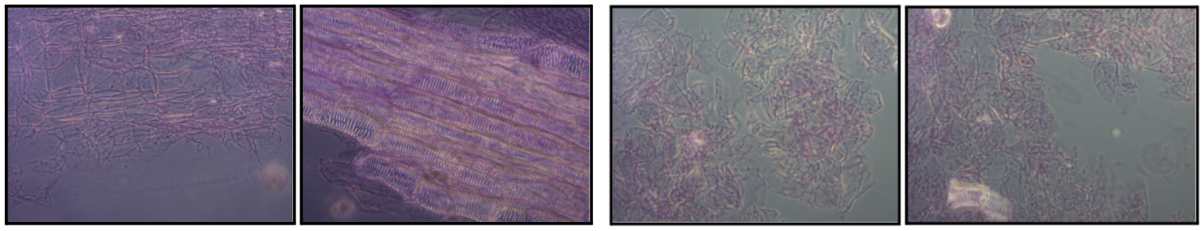

Figure 3: Histology stained images of control carrot under a 20X objective on the left. Histology stained images of blended carrots under a 20X objective on the right.

Protocol for Testing the Methods for Filtration of Blood, Oil, and Tumescent Liquids

To determine if the prototype successfully removed water, oil, other liquid and debris, the team performed microscope observations on samples of the liquid waste and final product of blended carrots, DPBS, and oil after filtration through the mesh filter, with a representative image of the solid product removed from the carrot lipoaspirate surrogate shown in **Figure 4**. Visual observations were also made of the liquid waste and solid product in their flasks and meshes, as shown in **Figure 5**.

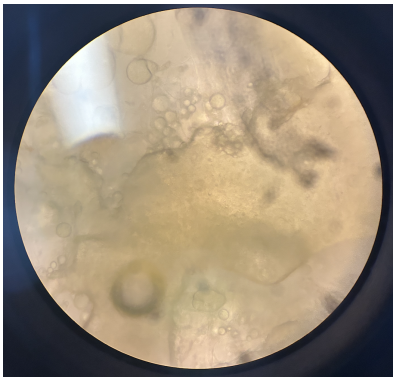

Figure 4: Solid product removed under a 40X objective. As shown there are oil droplets as depicted by the small circular droplets, water not shown in the field, and solid fibers shown by the large yellow orange sections.

Figure 5: Liquid waste removed on the left and solid product above the mesh filter on the right.

Protocol for Testing and Verifying the Prototype

After verifying the protocol, the team tested the prototype with human lipoaspirate at the University of Massachusetts Medical School. The device was verified by weighing the starting

lipoaspirate material, the filter's contents, and the waste container's content. The ratio of the filtered content was taken and compared to the theoretical ratio of ECM in adipose tissue to determine a percent yield of 49.69%.

However, verification methods outside of weighing the final products still need to be done. An H&E staining of the composition of the lipoaspirate material before blending and after blending, filtrate material (waste), and retentate material (final ECM product) will be performed after another round of testing.

Results

The results of testing the prototype under the simulation lipoaspirate and human lipoaspirate are summarized below.

Results of Validation Tests

From the decellularization validation tests, the images in **Figure 3** showed that the control had strong and intact cell walls. In contrast, the cell walls were broken apart and thin in the blended images, as if no liquid was inside the cell walls. The filtration tests visually showed that a large amount of oil was being removed from the carrot lipoaspirate surrogate; a significant amount was seen on top of the liquid waste canister in **Figure 4** on the left. The solid product, as shown on the right of **Figure 4**, was dry but oily to the touch, and under a microscope, small oil droplets could be seen within the fibers retained, as shown in **Figure 5**.

Filtration Results

Qualitative results for the prototype are portrayed in **Figure 6**. The quantitative analysis of ECM content is summarized in **Table 1**.

Figure 6: Filtered waste and ECM from adipose tissue. On the left is an image of the waste sample with big chunks caught in the prefilter. In the middle is the presumptive ECM content caught in the main mesh filter. On the right is the filtered out waste presumptively containing oil, tumescent liquid, and blood.

Table 1. Percent Yield of ECM Calculation

Weight of lipoaspirate material before blending and filtering	572g
Weight of content in waste container	306g
Weight of content in filter	16g
Weight of content left in blender	250g
ECM Content in Tissue	(572g - 250g) * 0.10 = 32.2g
Percent Yield of ECM	16g/32.2g = 50%

Discussion

Carrot, oil, and DPBS testing worked well with our device. However, when the verification testing with human lipoaspirate was performed, it was observed that the carrot surrogate did not fully act as human tissue would and was not a perfect surrogate.

The device was tested with human lipoaspirate tissue and once blended the tissue did not decant. Due to this, everything had to be filtered through at once instead of decanting out most of the oil from the adipocytes which should have stayed to the top of the mixture. Due to the

inability to decant and lack of histology, the ECM quantity collected at the end of filtration is unknown.

Based on our data, for future testing we recommend that future teams run more human lipoaspirate tissue through the device and perform histology on every step of the process such as lipoaspirate before blending, after blending, after the prefilter, after the main filter, and the waste pulled out to ensure the device decellularized adipose tissue and collects the desired ECM while filtering out oil, blood, tumescent fluids, and other potential contaminants.

Abstract

Breast cancer is the second most common cancer diagnosis among women [1]. A mastectomy is usually performed to remove the diseased area, and breast reconstruction is performed afterward for aesthetics [2]. Methods of reconstruction consist of: artificial implants, autologous fat grafting, free-flap reconstruction and cadaver-derived scaffolds. This project aimed to create a single-use closed system for decellularizing lipoaspirate in under an hour to produce a scaffold for breast tissue reconstruction. This device would replace the current autologous fat grafting method, where the patient's fat is processed and adipocytes are injected into the breast, since this method lacks proper blood supply only 30-70% of adipocytes survive leading to unpredictable aesthetic outcomes [3]. This design was inspired by the novel mechanical technique to decellularize adipose tissue by Zhang et al. The decellularization and filtration techniques used in the prototype were verified through multiple iterations of testing with a carrot lipoaspirate surrogate. The final design was able to blend and decellularize lipoaspirate fat from the patient and filter through a mesh to remove waste liquid presumed to be composed of blood, oil, and tumescent fluids to obtain a 49.69% yield of presumptive ECM content, but due to limitations histology could not be performed to verify the yield composition.

Chapter 1: Introduction

Breast cancer is the second most common cancer diagnosis among women and the second most common cause of cancer death in the United States due to late diagnosis [1]. Surgery is usually required for breast cancer, a mastectomy is when the entire breast is removed and a lumpectomy is when only the cancerous tumor is removed while saving the rest of the breast tissue [2]. After a mastectomy, 40% of women undergo breast reconstruction [2]. The most common reconstruction surgeries available are artificial implants, fat grafting, and cadaver scaffolds, each with a list of complications, including rejection, cell death, and price.

Currently, no on-the-spot, point-of-care device can take a patient's tissue and decellularize it to turn it into a scaffold for new cell growth and soft tissue repair. Therefore, our group researched a method to decellularize a patient's own adipose tissue in the operating room quickly and safely. Through research, we found a method that decellularizes adipose tissue from liposuction through mechanical blending, mesh filtration, and centrifugation, to create a product called Adipose Collagen Fragments, also known as ACF [3]. This product is very beneficial as it is biocompatible, autologous, and contains on the ECM components of adipose tissue to create a scaffold that allows for adipocyte migration. This product has only been created in the lab and not yet used in the OR. Upon finding this method, our team went about to find a way to replicate similar aspects of this method in a closed device for use in the OR to create a scaffold for breast reconstruction on patient's who have undergone a mastectomy. This device is needed to efficiently deliver a biocompatible tissue-engineered adipose-derived extracellular matrix based scaffold to the damaged area to repair soft tissue by creating a microenvironment similar to the body's tissue architecture to promote host integration and implant vascularization.

If properly executed, our device would be able to replace the current gold standard, cadaver-based scaffolds. The device will decellularize a patient's adipose tissue, eliminating the possibility of biological rejection. It will be available at a lower cost than the current gold standard of cadaver-based scaffolds because many processes are required to decellularize cadaver tissue that will no longer be needed. Since the device will be patient-specific; there is no risk of foreign DNA contamination if complete decellularization is not achieved. With the help of the device, patients will receive a healthy, non-rejectable way of healing and growing new cells to replace lost breast tissue, which is more efficient and biocompatible than other solutions such as implants and cheaper than cadaver scaffolds.

1.1 Project Goals

1. Design a device for adipose tissue decellularization, water and oil removal, and ECM retrieval after liposuction
2. Provide patients and physicians with a quick FDA approval for commercial use.

Chapter 2: Literature Review

This chapter reviews the need and background for the project including female breast physiology and anatomy, information of breast cancer, and soft tissue reconstruction. Another aspect of the project that needs to be reviewed is the liposuction procedure, lipoaspirate material, and separation methods to obtain the extracellular matrix. The prior art for current treatments for breast reconstruction and separation of extracellular matrix from lipoaspirate is also discussed, with an emphasis on obtaining adipose collagen fragments.

2.1 Female Breast Physiology and Anatomy

Soft tissue is found all over the body and serves to connect, protect, and support other tissues and organs in the body. They include muscles such as the heart, fat, blood vessels, nerves, tendons, and the tissues surrounding the bones and joints [4]. Adipose tissue is a common connective soft tissue found under the skin, called subcutaneous fat, between the muscles, called visceral fat, within the bone marrow, and surrounding many internal organs [5]. Adipose tissue is primarily known for storing and releasing energy and providing insulation. However, recently, it has been discovered that it is also an active organ in the endocrine system [6]. It secretes several important bioactive factors, such as leptin, which is important in regulating energy supply and demand through hunger and satiety signals [7]. It can also respond to insulin by converting excess blood sugar to lipids and storing them away for future use [7].

Because adipose tissue is a loose fibrous connective tissue, it is packed with many cells called adipocytes [8]. Each adipocyte cell is filled with a single large droplet of triglyceride . As this occupies most of the volume of the cell, its nucleus and other cellular components are pushed to the edge. In addition, because the adipocytes are tightly compact, there leaves little room for the extracellular matrix [8]. Adipose tissue can resemble a sponge; a representative image of adipose tissue can be seen below in **Figure 1**.

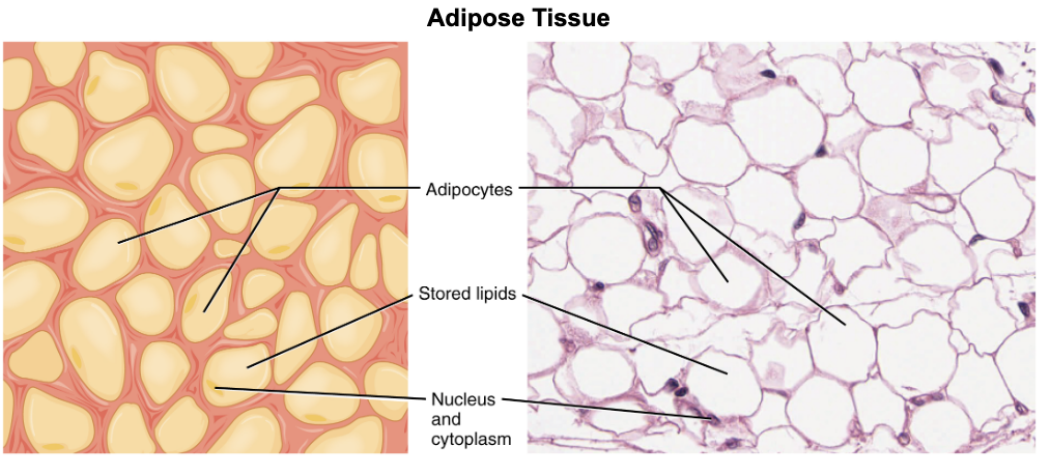

Figure 1: Structure of Adipose Tissue [8]

The primary cells that compose adipose tissue are called adipocytes. There are primarily two types of adipocytes, white and brown adipocytes [9].

The most common adipocyte is white adipose tissue (WAT). WAT is critical for energy storage. It stores fat in the form of triglycerides inside the cytoplasmic lipid droplets, which helps to maintain free fatty acid levels in the blood [9]. Additionally, different localizations of WAT have different roles in the body. A dysfunction in adipocyte function leads to obesity and can lead to breast cancer. Dysfunctional adipose tissue can secrete excessive fatty acid, cholesterol, triglycerides, hormones, and adipokines that are linked to metabolic dysfunction, insulin resistance, and inferior outcome in cancer treatment [10]. Fatty acids are the major source of ATP in a tumor, therefore, high levels of free fatty acids, cholesterol, glycerol, and triglycerides lead to accumulation which lead to breast tumor initiation, development, and migration.

The breast is an organ with a structure composed of lobes, lobules, ducts, adipose tissue and fibrous tissue. Its unique structure is conducive to its main function of producing milk for lactation. Each breast consists of 15-20 sections called lobes that connect to lobules that end in bulbs, where the milk is produced, which connect to the ducts that lead to the nipple [11]. Adipose tissue and fibrous tissue fill the space between lobules and ducts. The pectoralis muscle

lies under each breast, atop the ribcage [11]. Each breast also contains blood vessels and vessels that carry lymph nodes [11]. The breast tissue is encircled by a thin layer of connective tissue called fascia. The deep layer of this fascia sits atop the pectoralis muscle, and the superficial layer sits just under the skin [11]. The male breast structure is nearly identical to the female breast but lacks the specialized lobules since males do not produce milk. Most breast cancers arise from the cells forming the lobules and terminal ducts; therefore, breast cancer is more common in women. The anatomy of the female breast is shown below in **Figure 2**.

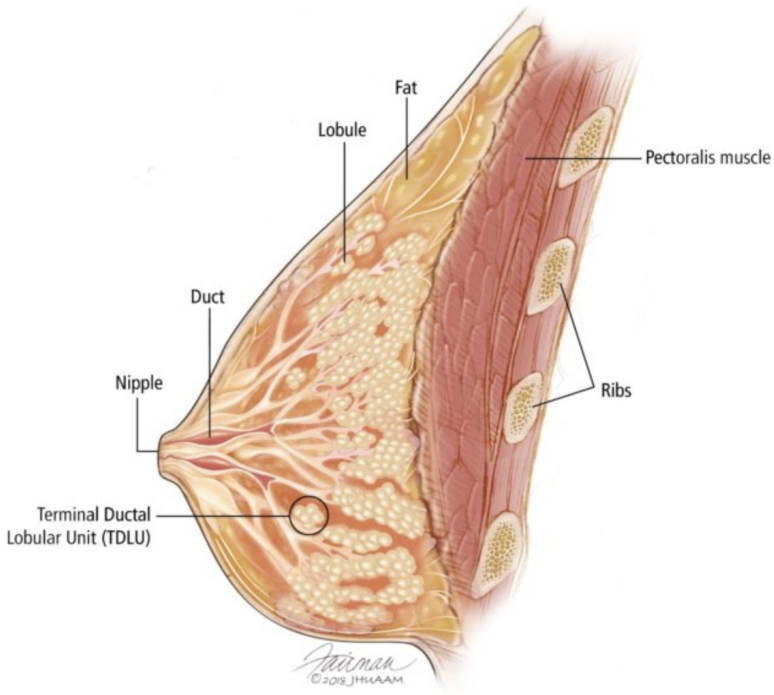

Figure 2: Female breast anatomy [12]

2.2 Breast Cancer Disease state

Breast tissue damage can occur due to the weakening or disruption of the muscle or connective tissue [13]. This damage can be caused by a wide range of internal and external factors. One of the simplest methods of tissue damage is through physical trauma, either blunt

force trauma or penetrating trauma [14]. Minor trauma can often be treated through natural processes, but more serious forces causing the tissue to be crushed or torn can easily cause disabilities and permanent damage [14].

Breast cancer is a common disease that leads to a need for tissue reconstruction methods [15]. The two most common kinds of breast cancer are invasive ductal carcinoma and invasive lobular carcinoma. Invasive ductal carcinoma is when the cancer cells begin in the ducts and then grow into other parts of the breast tissue. Invasive lobular carcinoma is when the cancer cells begin in the lobules and then spread to the surrounding tissue [15].

2.3 Breast Cancer Clinical Presentation

Breast cancer is a common diagnosis for women in the United States, with 30% of women developing it yearly [16]. It develops and presents differently for everyone, however, the most common symptom presented is a lump or mass on the breast. A study which examined the presenting symptoms of women with breast cancer found that 1,922 women out of 2,316 patients presented with a breast lump [17]. The next two common symptoms they found were nipple abnormalities and breast pain, with at least 13% of patients presenting with either or both of these symptoms. Additional, less common, symptoms they found were breast skin abnormalities, swelling, infection, and back pain. Moreover, the study also encountered patients who did not develop a mass on their breast but developed symptoms of fatigue, weight loss, and a lump on their neck. Though this is not common, a patient can bring up a concern to their doctor and find out they have breast cancer without having a mass.

2.4 Clinical Outcome

After a patient is diagnosed with breast cancer, different treatments are offered based on the stage of cancer, location, and factors such as a person's overall health. Once a patient suspects a tumor or a change in their health, it is crucial to seek medical attention, as a prolonged diagnosis can lead to worsened conditions. Studies have shown that the longer it takes to diagnosis can lead to a lower than a five-year chance of survival and may lead to a poorer experience of care [17]. For example, another study concluded that a women's survival rate for breast cancer after five years was 72% for those who refused surgery and 87% for those who accepted surgery [18]. While the ten-year survival rate for those who declined surgery decreased to 36%, and those who accepted surgery decreased to 75%. Furthermore, refusing proper treatment can lead to infection, degraded tissue integrity, impacted tissue function and spread of cancer [19].

2.5 Economic Impact

Many women have a BRCA gene mutation and are at a high risk of developing breast cancer [20]. A preventative surgery called a double mastectomy is usually recommended to ensure the patients will not develop breast cancer later in their lives. The estimated national average cost to have a double mastectomy surgery is about $32,500 [21]. The recovery period after that will take around four weeks. Based on the average weekly earnings in 20A, unpaid time off would mean the patient would lose approximately $4,164 in income [22]. After recovering from a double mastectomy, patients may choose to get breast implants. The average cost of a double breast reconstructive surgery is about $100,000 [23]. They can also choose to go with a free flap reconstruction which can be priced around $25,000 to $50,000 per breast [22]. If they do not have the money for methods like this, they can choose fat grafting as a reconstructive

method, which can cost between $2,500 to $7,000 [24]. There is a reconstructive method that is still currently being tested that could have much greater reconstructive properties, however for a small area the starting price is $1,100 and increases as size demand increases [25]. After surgeries, recovery time can vary from six to eight weeks [23]. Being out of work for another eight weeks unpaid will cost the patient another $8,328 [22]. With an unfortunate diagnosis, the patient could end up with bills totaling upwards of $145,000.

2.6 Soft Tissue Reconstruction

Breast reconstruction, a type of soft tissue reconstruction, aims to help restore one or both breasts to an almost normal shape, size, symmetry, and appearance after mastectomy or lumpectomy procedures [26]. When the adipose tissue is removed due to breast cancer through a mastectomy or lumpectomy, it can be treated through tissue reconstruction. Soft tissue reconstruction is the replacement of destroyed tissue with living tissue, usually through surgery. Tissue reconstruction consists of regeneration and repair or (wound healing) [11]. During regeneration, specialized tissue is replaced by the proliferation of surrounding undamaged cells. During repair, lost tissue is replaced by granulation tissue which then matures into scar tissue. Tissue reconstruction is vital for wound coverage by providing stem cells, growth factors, and vascular supply [12]. Autologous adipose tissue is commonly used in tissue reconstruction [13]. Adipose tissue is abundant in multipotent stem cells, similar to those found in the bone marrow, and demonstrates self-renewal, unlimited proliferation capacity, and pro-angiogenic capacity and can promote epithelial cell differentiation and neovascularization [13].

Breast reconstruction could occur immediately after a mastectomy procedure or be scheduled after a patient has healed from a mastectomy [26]. Reconstruction can fall into two categories: either implant-based or graft reconstruction. Implant reconstruction uses breast

implants to help form a new breast, while graft reconstruction uses the patient's tissue from another part of the body to form a new breast [26]. Using a patient's tissue will be a focal point for the device since the hope is to decellularize the tissue to create an injectable scaffold that will help increase cell proliferation without the usual random cell death associated with fat grafting methods. In theory, the scaffold we aim to create will be composed of isolated extracellular matrix and its proteins without any cell remnants or other contaminants. The extracellular matrix is composed of an array of multidomain macromolecules organized in a cell/tissue-specific manner, providing a structural scaffold via a network of protein-protein interactions as well as protein-proteoglycan interactions [27]. It has many growth factors and bioactive molecules which control cell proliferation, adhesion, and apoptosis [26]. Major components of the extracellular matrix include elastin, collagen, proteoglycans, and cell-binding glycoproteins [27]. The extracellular matrix connects to cells through integrins, syndecans, and other receptors [28]. One of the goals for the device is to break the connection of cells to the extracellular matrix to isolate the matrix by itself for scaffold production. By doing so, the decellularized extracellular matrix can be utilized as a scaffold to promote cell proliferation for breast reconstruction.

2.7 Decellularized Scaffolds

Decellularized scaffolds are made by decellularizing adipose tissues to obtain their extracellular matrices. The decellularization process removes immunogenic cellular components from the tissue structure while allowing the biological activity and integrity of the extracellular matrix (ECM) to allow space to grow and lessen the chance of rejection [29]. An example can be seen in **Figure 3** below, with adipose tissue scaffolding. The normal newly collected adipose tissue is on the left, showing how fatty and full the tissue is. The decellularized tissue is shown on the right; it is very thin and has much less mass than the tissue on the right. In the microscopic

views, you can see how the adipose tissue is full of cells, and in the scaffold, you can see that there are minimal cells left behind, and only the tissue structure is left.

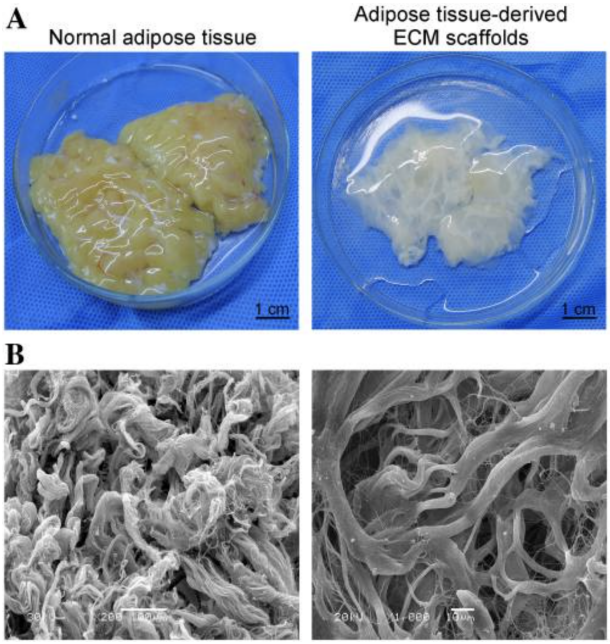

Figure 3: Adipose tissue pre and post decellularization [29]. Freshly collected adipose tissue is shown on the left hand side in section A, there is yellow fat within the ECM that creates the bulk of the mass within the tissue, in the left hand side of section B there is lots of tissue and cells within the ECM. A decellularized sample can be seen in the right hand side of the figure. In the right hand side in section A, the ECM is no longer yellow with fat and is much less dense than the original tissue. In the right hand section in section B, the ECM is empty with just the scaffold and shape of the ECM left behind, there are no more cells or tissue within the ECM.

2.8 Tumescent Liposuction

Liposuction is a surgical procedure that involves removing fat from the body. When weight is gained, the fat cells increase in size and volume. Liposuction can be used to reduce the number of fat cells in a specific area to improve the contours of the body and to reduce the appearance of excess fat, but it is not a weight-loss procedure [30]. General areas of the body that can be treated with liposuction include the face, chin, neck, arms, breasts, abdomen, hips, thighs, and buttocks [30]. Liposuction is typically performed under general anesthesia, so the patient is put under during the 1-2 hour long procedure. As with any major surgery and

undergoing anesthesia, there are many risks that can occur such as bleeding or a reaction to the anesthesia. Other outcomes can include contour irregularities, fluid accumulation, numbness, internal puncture, fat embolisms, kidney and heart problems, and lidocaine toxicity [30].

Tumescent liposuction is the most common type of liposuction in which the surgeon injects a sterile solution which aids in fat removal, lidocaine as a pain reliever, and epinephrine to cause the blood vessels to constrict, shown below in **Figure 4**. Then a cannula is inserted into the body through small incisions with a criss-cross sectioning technique to separate the adipose tissue from the fibrous stroma [31]. The cannula is able to remove excess fat from the desired areas of the body in the intermediate fat layer by using suction powered by a vacuum pump, as shown below in **Figure 5** [30]. The original cannula was sharp and has one hole, but after many iterations the preferred cannulas are blunt with one or multiple holes placed at the tip [32]. Blunt cannulas are used to lower the risk of puncturing the deep spaces beyond the pockets of fat to lower the risk of blood loss [32]. Different sized cannulas are used for different purposes, for example a large diameter cannula is used for more stromal disruption and fat separation but they can cause more indirect trauma and blood loss. As previously mentioned, the fat is pulled from the body by means of a vacuum pump, however for smaller amounts of fat or more delicate areas of the body a manual syringe may be preferred [32].

Lipoaspiration produced lipoaspirate composed of an aqueous solution, oil, and ECM. From fat that was liposuctioned from mice, the amount of aqueous solution in unfiltered fat is about 15% fat and 85% aqueous solution [33]. From a study that derived the ECM for skin filling, they noted that from 50mL of Coleman fat, they were able to remove 4.91mL of adipose collagen fragment (ACF) which is essentially the ECM [3]. This would give a ratio of about 10% ECM in the Coleman fat. These components would add up to be about 10% ECM (fibers), 5%

oil, and 85% aqueous solution, which in the context of lipoaspirate would be the solution used to assist in liposuction and blood and other liquid waste, that make up lipoaspirate.

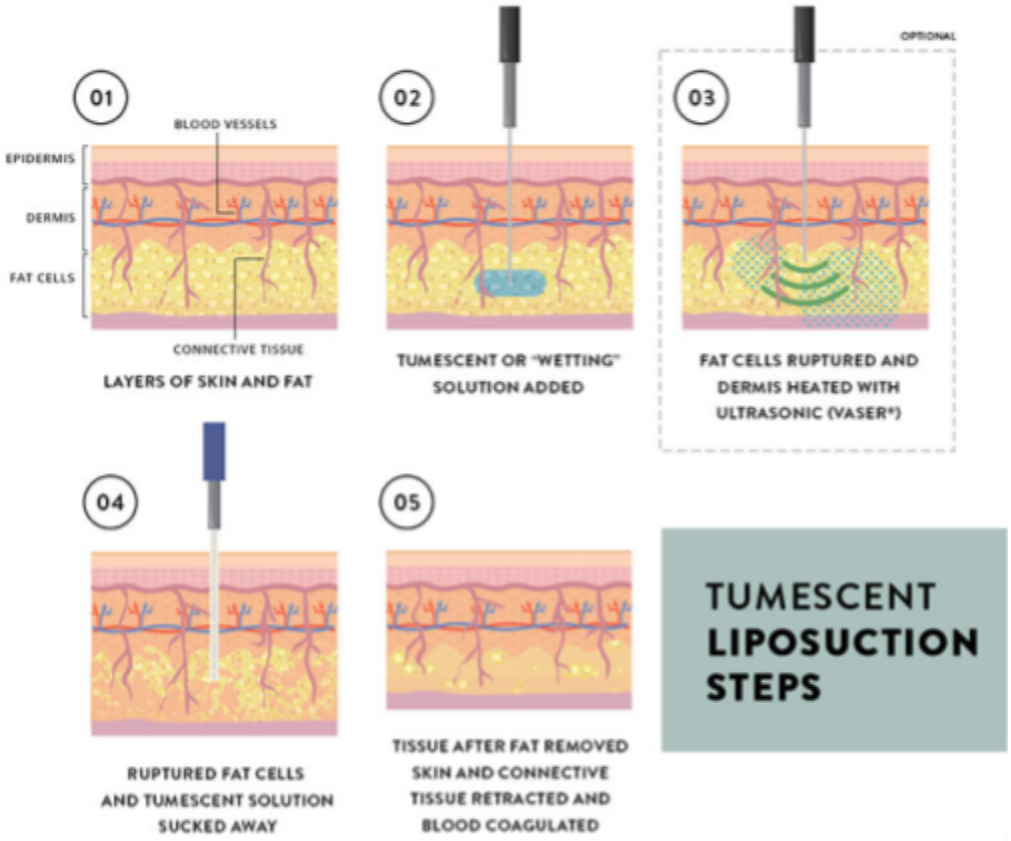

Figure 4: The liposuction procedure uses a metal cannula, inserted below the skin and above the muscle to aspirate the fat cells to thin the body and give the appearance of smoother skin [34].

Figure 5: Liposuction pump machine. This figure shows the liposuction pump machine with the parts and pumps of the machine [35]. The canisters that are attached to the right side of the machine collect the lipoaspirate. The bottom of the machine is the vacuum pump that powers the suction of the cannula. There is a pedal attached to the pump that allows for control of the machine.

2.9 Separation Methods

The most commonly used methods to prepare fat grafts are sedimentation, filtering, washing, and centrifugation. Fat processing is necessary because lipoaspirate contains adipocytes, collagen fibers, blood, and debris, shown in **Figure 6** below [34]. These elements can cause inflammation at the recipient site which can cause detrimental reactions for the fat graft.

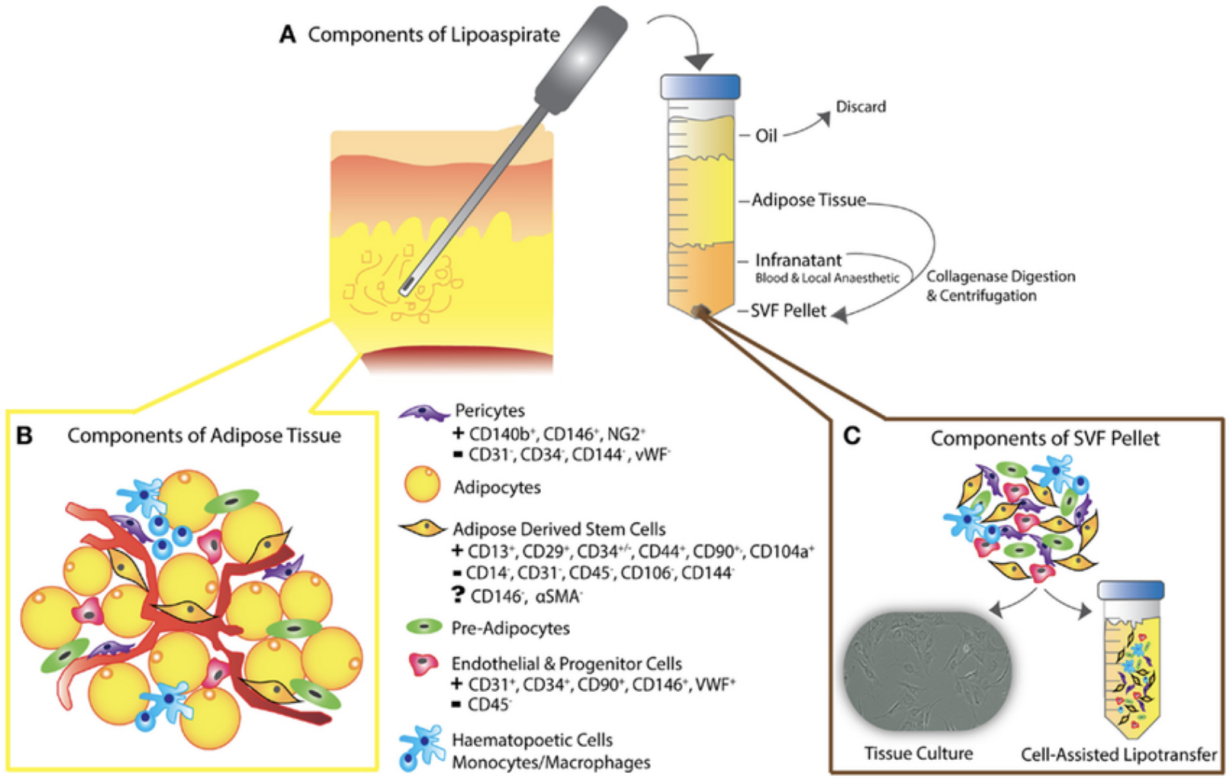

Figure 6: Components of lipoaspirate that need to be filtered out from the final product of the device [36].

As previously mentioned, each adipocyte is filled with a large droplet of triglyceride, and around the adipocyte is the extracellular matrix fiber component. When the cell membrane is disrupted, the adipocyte will burst, exposing the triglyceride and leaving behind the fibrous component of the extracellular matrix. These oil and fibrous components, as well as the blood and tumescent liquid, will need to be separated in order to obtain a pure extracellular matrix component to be used as a scaffold for tissue reconstruction purposes.

Although centrifugation and washing have proven to be effective in separating the components of lipoaspirate, the device is intended for clinical use, for immediate use in the OR. Therefore, a faster, single-use, closed system filtration technique is needed. Therefore, we explored the ideas of tangential flow filtration, mesh filtration, decantation, and bead filtration for the device, which can be made single-use and would be quick and easy to use.

2.9.1 Tangential flow filtration

Tangential flow filtration, also known as cross-flow filtration, is a process of separation widely used in bio-pharmaceutical and food industries [37]. Its mechanism of action is that fluid is passed parallel to the filter, rather than being pushed through a membrane perpendicularly, shown in **Figure 7** below. This method is preferred for its continuous filtration and that it prevents clogging [37]. The permeate is put off to the side while the retentate is recycled back to the feed.

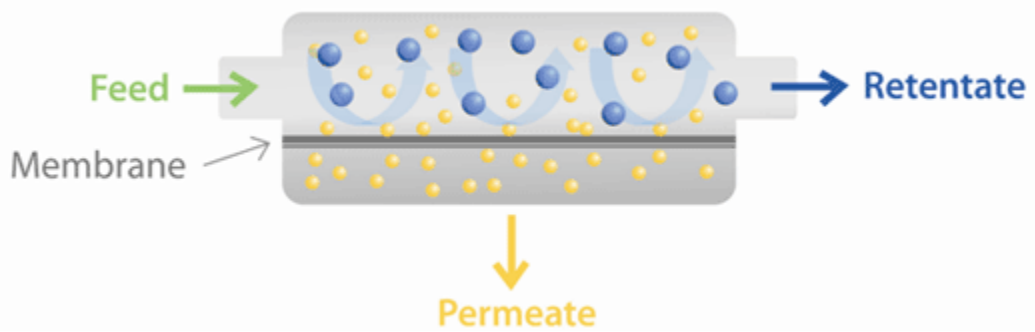

Figure 7: Schematic for simple tangential flow filtration [37]. Feed goes in and the permeate gets filtered out through the membrane and the retentate is kept and filtered back through until there is a substantial decrease in volume.

Tangential flow filtration is typically used in concentration and diafiltration processes. In concentration processes, the idea is to increase the concentration of a solution by removing fluids while keeping the solute molecules. This process is done by selecting a filter significantly smaller than the solute molecules to allow for a higher retention of solute molecules.

In diafiltration processes, the goal is to separate small and large particles, without altering the overall concentration by washing out solvents with another buffer. In the scope of this project, tangential flow filtration could be used on lipoaspirate to filter out blood, oil, and tumescent liposuction liquids, while concentrating the extracellular matrix component.

2.9.2 Mesh filters

Mesh filters are woven metal wire or plastic strands that capture larger particles that cannot pass through the holes in the mesh, shown below in **Figure 8** [38]. Mesh filters are categorized as surface filtration methods because the particles are caught on the surface of the mesh. In the U.S, the size of the mesh is standardized in the US Standard Mesh number. This number gives the number of openings per inch of mesh, so the larger the number, the more openings in the mesh per inch, and the smaller the holes are. Mesh size is the size of the actual holes in the mesh, and this number is usually given in microns.

For this device, the mesh filters catch the ECM, while the blood, oil, and water of the adipose tissue pass through. This would require appropriate mesh size that could catch the ECM. According to Zhang et al, adipose collagen fragments can be obtained from liposuction fat through mesh filtration using 0.25-0.15mm mesh size [3].

Figure 8: Examples of mesh filters [39].

2.9.3 Decantation

Decantation is a process that separates heterogeneous or immiscible liquids of different densities or to separate solid particles from a liquid. Decantation occurs under the effect of Earth's gravity. In terms of liposuction adipose tissue, decantation would be used to separate the oil and water and blood from the solid, the layers of lipoaspirate can be seen below in **Figure 9**. Decantation can also be sped up through centrifugation. When centrifuging lipoaspirate material, the top layer is the oil from the triglycerides, the middle layer is the extracellular matrix fibrous component, and the bottom layer is the blood and tumescent liposuction liquids.

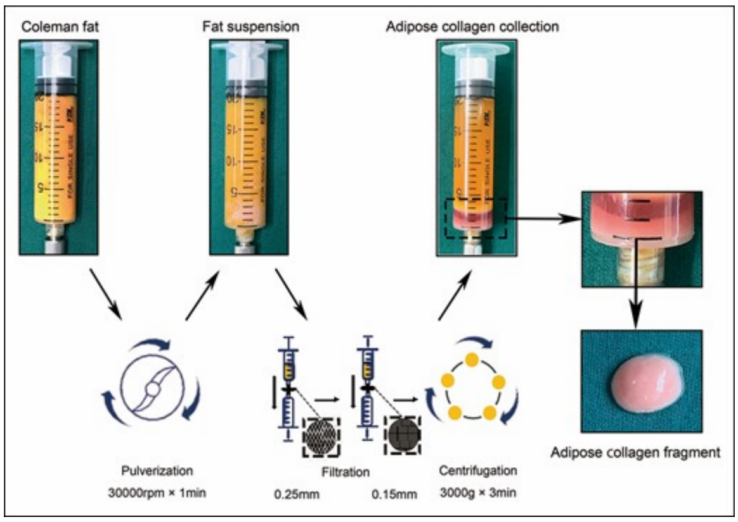

Figure 9: Decanted lipoaspirate [3]

2.9.4 Bead filtration

Bead filters are in closed vessels, filled with thousands of plastic or glass beads. These beads form a layer through which water is pushed through, typically with a pressure pump, while solid particles remain stuck in the beads, similar to the bioreactors in **Figure 10**.

Typically standard bead media is used for bead filtration and it is aimed to filter biological and mechanical material. Most of these beads are made of low density polyethylene

[40]. The beads allow water to pass through but trapping solids and the polyethylene allows an attachment area for nitrifying bacteria. Bead filters are considered bio-clarifiers, meaning they can perform biofiltration and clarification in a single unit.

Clarification is known as the process of removing solids from water. Bead filters are widely used to filter aquatic systems, so the particles they're aimed to target are particles less than 100 microns, bacteria, algae, and clay. There are four mechanisms through which the beads remove solids. The first mechanism is when particles greater than 100 microns are subjected to physical straining. Particles that are 50 -100 microns are filtered through settling. Particles that are 5-50 microns are removed by interception, a process caused by collisions between the particle and the bead media surface. Lastly, particles less than 20 microns are removed through bioabsorption.

Figure 10: Glass bead filtration bioreactors [41].

2.10 Prior Art

After undergoing a mastectomy, it is common for patients to undergo breast reconstructive surgery. Many procedures can be performed to reshape and rebuild breast tissue after a mastectomy or lumpectomy. This section will delve into the different methods of reconstruction.

2.10.1 Breast Implants

Breast implants are one of the most common ways to reconstruct a breast after a mastectomy and are popular as a form of breast enhancement. There are two different breast implant filling materials, silicone and saline, however the rubber shell is the same material, polysiloxane, with examples of each shown in **Figure 11** [42]. The implant's primary purpose is to help reconstruct the breast(s) post-surgery, with the average cost for implants being about $4,516 without other related expenses [26]. They are a good option for patients looking to reconstruct their breasts within a shorter clinical time frame with fewer high-risk surgeries and are widely available as there is an unlimited supply on the market. However, there are several risks associated with the surgery. A primary concern is the rupturing of the device inside the body. The filling leaking from the device could remain within the scar tissue or move beyond the breast, resulting in adverse reactions in areas other than the breast, which could be harmful if left untreated [43].

Figure 11: Saline vs silicone breast implants with saline on the left and silicone on the right [42]. Saline is less expensive, leaves a shorter scar as it is easier to place in the body as it is softer, the volume can be adjusted during surgery, but it can show ripples through the skin. SIlicone feels more natural with less of a risk of rippling and it is more stiff so it requires a larger scar when inserted.

2.10.2 Free Flap

A "free flap" reconstruction is when a section of tissue, skin, or muscle is grafted, with the original blood vessels intact, from one part of the body and attached to another part of the body to reconstruct new features. The flap method is used to rebuild structures of the face, breast, or any other body structure and is a main type of reconstruction for mastectomy patients. This type of surgery is microvascular, as the blood vessels of the transplanted tissue need to be reconnected to ensure a smooth transition of the flap [23]. Due to the fact that it is living tissue, it looks and seems more natural as it can change based on body physiology, like gaining or losing weight [23]. The free flap is usually taken from these three areas, shown in **Figure 12**. The procedure lasts around 4-6 hours, with a recovery time of 6-8 weeks [44]. There would be scarring in the area where the graft was taken as well as along the implantation site. Without insurance, the flap reconstruction is priced around $25,000 to $50,000 or more per breast, or $50,000 to $100,000 for both.

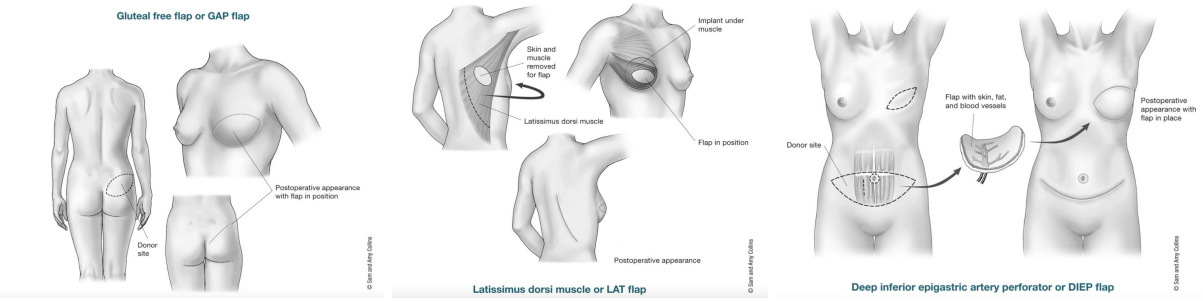

Figure 12: Free flap sections of skin, muscle, and fat taken from the buttocks, latissimus dorsi, and stomach respectively, used for breast reconstruction [23].

2.10.3 Autologous Fat Transplant (Fat Grafting)

Fat grafting is the process of taking adipocytes obtained from liposucted adipose tissue from one area of the body and moving it to another to reconstruct or enhance the area. Autologous fat grafts are used for reconstructive surgeries such as scar treatments and aesthetic plastic surgery such as breast augmentation, rhinoplasty, and facial rejuvenation. Fat transplants can cost anywhere between $2,500 to $7,000, depending again on the type of reconstruction [24].

Fat-grafting benefits are that fat is a readily available tissue that does not trigger an immune response or cause significant donor site morbidity when harvested and that adipose tissue has the potential for proliferation and regeneration. However, one of the main dilemmas when grafting fat tissue is unpredictable resorption, around 30-70% [24], which can lead to needing multiple injections and poor aesthetic outcome, shown in **Figure 13** below. There are minimal donor site complications, mainly caused by liposuction, such as bruising.

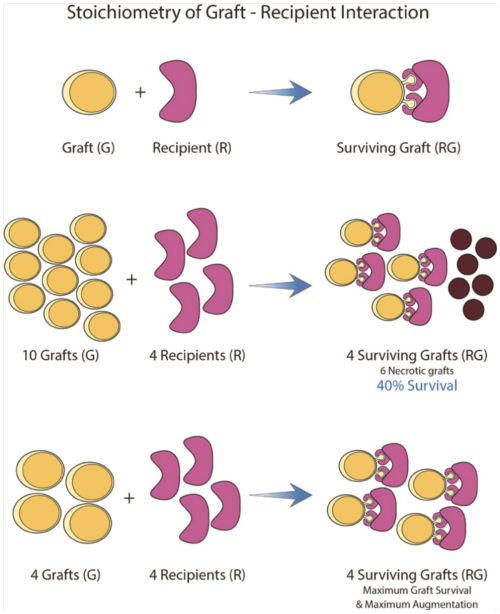

Figure 13: Unpredictable implantation, survival, and growth after grafting [24]. The first row shows how the fat cell is grafted. The second row shows that there are not as many recipient areas that are able to receive the fat grafts showing that 6/10 fat grafted cells die and undergo necrosis. The last row shows how only 4/10 of the grafts survive. This process is unpredictable as it is unknown how many blood vessels are able to receive the fat grated cells and maintain life.

2.10.3.1 Coleman fat

Sydney R. Coleman coined a technique for fat grafting in 1994 to decrease the traumatic handling of fat during liposuction. The main principles of his design include harvesting parcels of fat with a blunt cannula, refinement with centrifugal to remove nonliving components, and aliquoting the fat back into the body for tissue reconstruction, as shown in **Figure 14**. The original process begins with manual lipoaspiration under low pressure, then the fat is centrifuged for 3 minutes at 3400 rpm, and reinjected into the patient [45].

This technique was the original gold standard for liposuction and lipofilling but has been modified many times. The results of this technique and other previously mentioned fat grafting techniques have variable results with resorption rates in the 30-70% range, so optimization is required [45].

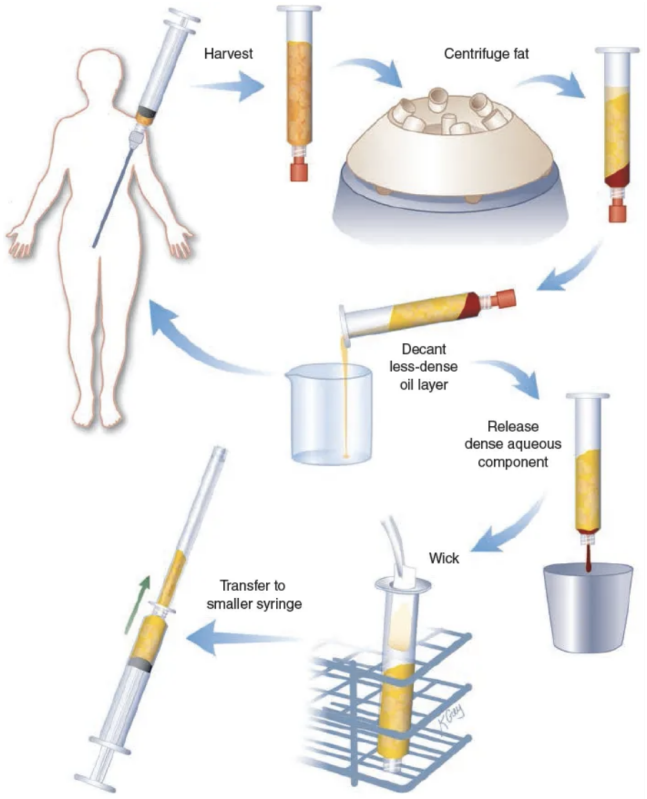

Figure 14: The Coleman technique outlining the main steps of harvesting, refinement, and purposeful placement [46].

2.10.3.2 Revolve

A common brand of autologous fat grafting is the Revolve System, shown below in **Figure 15**. The Revolve system is an advanced adipose system device that can be integrated with the normal canister used to collect lipoaspirate during a liposuction procedure [47]. It is used to aspirate and harvest the lipoaspirate to filter and separate the adipocytes from the rest of the components of lipoaspirate such as water, oil, blood, etc. for transfer of adipocytes to other locations in the body for aesthetic body shaping. The process of using the Revolve system starts with harvesting fat from the patient. The fat is harvested by connecting the vacuum port to the waste container to provide enough suction to pull the lipoaspirate through the device [48]. The smallest volume that can be processed through the Revolve is 100mL and the largest volume is 350mL [48]. Next the lipoaspirate needs to be washed, filtered, and turned into a concentrate.

The system needs to be removed from the patient and prepped for washing. The lipoaspirate is washed with Lactated Ringer's Solution at 37-39°C while rotating the handle to turn the blades inside the system [48]. The solution, along with the waste of the lipoaspirate, needs to be removed through the mesh using the vacuum source. Lastly, the concentrated adipocytes need to be reinjected back into the patient. Transferring is done by connecting multiple syringes to the patient port, connected with a luer lock, and shaking the canister to move the adipocytes to the port to remove as many adipocytes as possible [48]. The syringes filled with adipocytes can be used to insert them back into the patient in the desired locations.

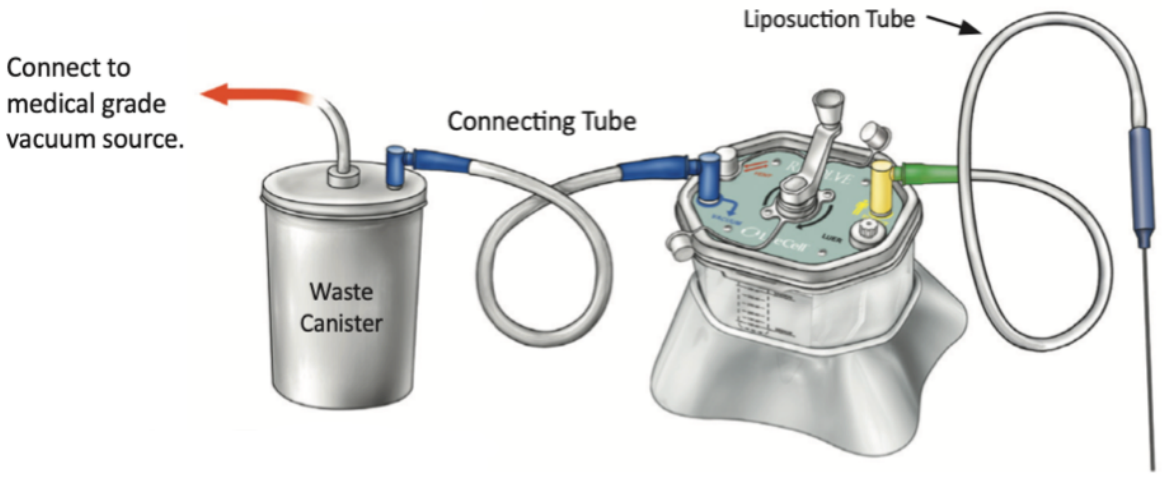

Figure 15: Revolve system components include the waste container that connects to the vacuum source, the Revolve system, and the liposuction tube connected to the cannula. The Revolve system contains a manually operated blade to mix together the solution and fat cells to remove all water and waste to leave only behind the fat cells that get caught between the mesh [48].

2.10.4 Cadaver Derived Scaffolds (Renuva)

Cadaver-derived scaffolds are constructed by decellularizing adipose tissue, from a donor or cadaver, in a freeze-thaw cycle that leaves behind a powdered scaffold of adipose tissue that needs to be rehydrated before use. Renuva Allograft Adipose Matrix, the commercial name for cadaver-derived scaffolds, is comprised of adipose tissue which is intended for the replacement of damaged or inadequate adipose tissue matrix in areas of the body where native fat would exist

[49]. Renuva is processed to preserve the extracellular matrix that contains collagen, protein, and growth factors [50]. The resulting matrix scaffold is used as an alternative to autologous fat transfer that provides the framework to support cellular repopulation and vascularization at the injection site [49]. The benefits of this scaffold are that it can be used to restore volume in the face, hands, and body in a short in-office procedure that does not require undergoing anesthesia and has a short unspecified recovery time [51]. This scaffold is injected into the skin, where fat would be originally, to serve as a scaffold to allow for cell integration and regrowth, as shown in **Figure 16** below. There is no specific price for this procedure for breast reconstruction. The total cost depends on the volume of the area being treated, the amount of product required, and the number of sessions recommended. The cost for a small area starts at $1100 and goes up based on the factors previously mentioned [25].

Possible adverse reactions of using human adipose tissue are anaphylaxis, local infections, systemic infection, discoloration of the skin at the injection site, possible swelling, tenderness, redness, bruising, pain, or irritation at the injection site [49]. Renuva also cannot be used to replace large volumes. However, the graft retention was high and there was no cell death. Through studies the adipose tissue-derived ECM scaffold promoted adhesion and growth of cells by surrounding cells.

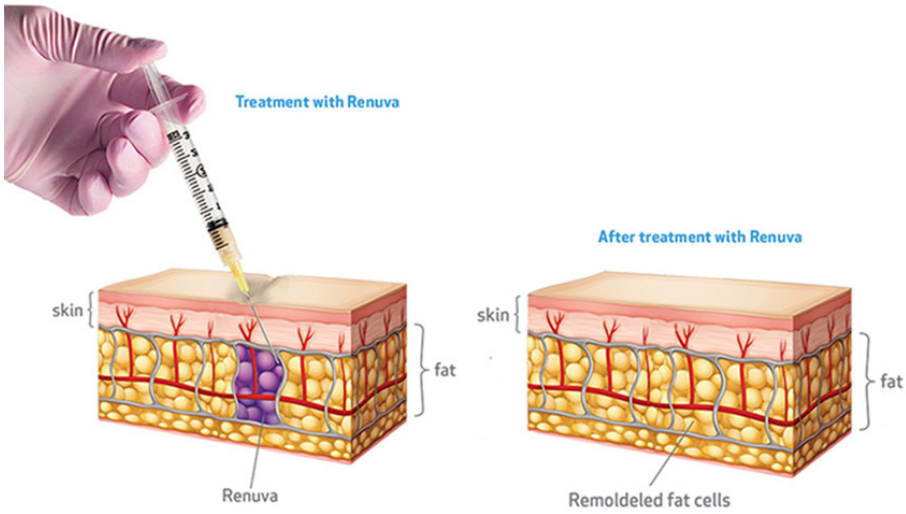

Figure 16: Injection of Renuva underneath the skin to allow for cell integration into the scaffold [52].

2.10.5 Adipose-Derived Injectable Scaffold

Adipose-derived injectable collagen scaffolds are a biomaterial that can be used in many medical procedures. These types of scaffolds are made from the collagen, or extracellular matrix, of adipose tissue. The extracellular matrix is processed to form a scaffold that can be injected back into the body to support tissue regeneration by migration of adipocytes to the location of the scaffold. The scaffold is used in reconstructive surgery to repair or replace damaged or missing tissue. These scaffolds can be made from the same adipocytes of the patient being treated and, therefore, will not be rejected. Since the main component is a scaffold, there will be promising integration of adipocytes for complete grafting.

2.10.5.2 Adipose Collagen Fragment (ACF)

A new technique, formed off the basis of the Coleman technique, was developed as a novel mechanical processing technique for extracting only the ECM components of adipose tissue. The adipose tissue-derived ECM components are called “adipose collagen fragments” and

it serves as a scaffold for adipocyte migration by providing natural binding domains [53]. ACF contains bioactive components that have a therapeutic effect on wound healing and collagen synthesis.

This type of scaffold is prepared with fresh lipoaspirate, which is centrifuged at 1,200g for 3 minutes (to generate a similar result to that of Coleman fat) [53]. The fat is washed twice with sterilized saline and homogenized for 60s in a special made ACF extractor. This fat suspension is loaded into 20mL syringes and filtered through unidirectional filters of size 0.25-0.15mm mesh and centrifuged again at 3,000g for 3 minutes [53]. Through this study from an original volume of 50mL of Coleman fat, they were able to remove 4.91mL of adipose collagen fragment (ACF) which is essentially the ECM [3]. This would give a ratio of about 10% ECM in the Coleman fat or lipoaspirate.

The result is shown below in **Figure 17**, the ACF can be seen as the pinkish-white substance in the top left corner of the figure. ACF is full of collagen, throughout the whole substance, shown in **Figure 18**, and when compared to Coleman fat, it is a better scaffold for adipocyte migration. ACF also does not facilitate the process of cell death, as there are no cells that are transferred back into the body. The device will take inspiration from the fat processing techniques for obtaining ACF, as the goal is to obtain the extracellular matrix component from lipoaspirate to create a scaffold for tissue regeneration.

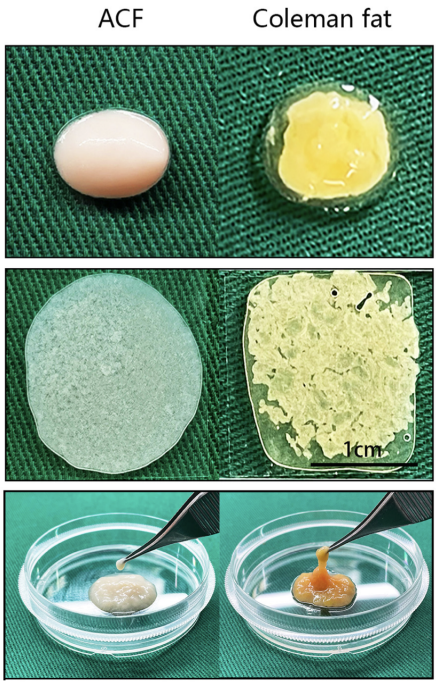

Figure 17: Coleman fat vs Adipose Collagen Fragment (ACF). These are two types of derivatives made from adipose tissue. This figure shows the consistencies of each type of derivative. As shown on the left side of the figure, ACF is a pinkish-white, homogenous, and viscous semifluid substance with a smooth texture and no cells leftover in the solution. On the right side of the figure shows Coleman fat, it has collagen components with less adipocytes contained within the scaffold than normal adipose tissue, it is able to be picked up with tweezers and is a solid like substance [53].

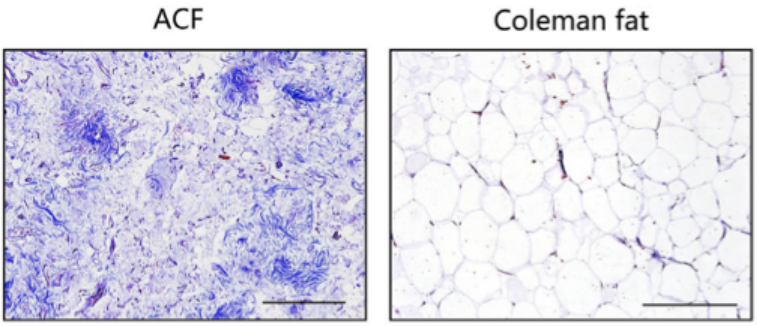

Figure 18: Histology staining of Coleman fat vs Adipose Collagen Fragment (ACF). This figure shows the masson's stain histology staining of ACF and Coleman fat, showing the collagen shown in a purple -blue color. As shown on the left side of the figure, the collagen components of ACF are seen throughout the whole field of view, almost the whole substance is made of collagen fragments. On the right side of the image, the collagen fragments of Coleman fat are dispersed and spread apart similar to the structure of adipose tissue [53].

2.10.6 Competitive Advantage

Table 1: Competitive Advantage Table of Prior Art

	IMPLANTS	FLAP RECONSTRUCTION	FAT GRAFTING (COLEMAN FAT/REVOLVE)	CADAVER DERIVED SCAFFOLDS (renuva)	ADIPOSE COLLAGEN FRAGMENT	OUR DEVICE
PRICE	$1,000 - $12,000	$25,000 - $100,000	Average $9300	< $1,100 depending on volume	Not on market yet	Estimated $550 for device
LENGTH OF PROCEDURE	1-2 hours	4-6 hours	Liposuction 3-4hrs Device 10mins during liposuction procedure time	6-8 hours	Not on market yet	Liposuction 3-4hrs Device 1hr during liposuction procedure time
LENGTH OF RECOVERY	1-2 weeks	6-8 weeks	2-6 weeks Similar to fat grafting	2-3 weeks	Not on market yet	2-6 weeks Similar to fat grafting
SCARRING	Minimal Scarring	Considerable Scarring	Minimal Scarring But cell death causes uncertain results	Minimal Scarring	Minimal Scarring	Minimal Scarring
REJECTION?	Sometimes	No	No But cell death occurs	No	No	No
PATIENT SPECIFIC	Sometimes, depends on the material being implanted	Yes	Yes	No	Yes	Yes

After comparing all 5 competitors and our potential device, a competitive advantage table was compiled into **Table 1**, considering the price, length of procedure, length of recovery, if scarring occurs, the aesthetic rating (based on scarring and how well it matches the surrounding area), and if the product is patient specific. It is shown that cadaver derived scaffolds are the best option for recovery time, minimal scarring, and though it is not patient specific it does not get rejected by the body. Second would be fat grafting because it is similar to cadaver derived scaffolds and though it is patient specific, it does not adhere to the body and integrate completely every time it is used. The flap reconstruction procedure is well known and reliable but it causes notable scarring and has long recovery times. As for implants, they are also well known, reliable, and more customizable but they can sometimes fail within the body and need intervention surgery to remove the implant if something goes wrong.

While considering ACF, the device aims to obtain a similar final product in a short

amount of time but keeping the process in a closed system. With that being said, the device will take inspiration from this process in aspects such as mesh filter size and blending time.

The hope for the device is that it will be cheaper than all other similar options such as cell free scaffolds and cadaver derived scaffolds. It should be relatively quick to be used as a point-of-care machine as well as patient specific to avoid rejection, leave minimal scarring, and have a better integration rate of cells than fat grafting.

Chapter 3: Project Strategy

3.1 Initial Client Statement

To provide patients requiring tissue reconstruction better opportunities for and easier access to the best care by transforming the current standards in reconstructive surgery through the development of the first point-of-care derived scaffold for tissue repair.

3.1.1 Objectives

The objectives for our device are based upon our two main goals. Our first objective was to test ways based on prior literature to break down adipocytes obtained by liposuction while removing water and oil in order to retrieve the ECM of the tissue. Additionally the entire process must take place under an hour for the device to be able to use during a liposuction procedure. We also want to ensure the device is relatively small and easy to use. That way it can easily fit into an operating room and anyone in the room can use the device. Finally our last objective was to create a closed system device for a sterile injection of the scaffold into the patient.Additionally, to ensure we meet the goal of having a quick FDA approval, we want to ensure the device uses biocompatible methods.

3.1.2 Constraints

Our device has to be able to be used in an operating room and this within itself brings constraints. Everything in an OR needs to be sterile meaning the device should be a closed system and should be single use. This will ensure every patient has a 100% sterile device. Since the device is meant to be single use, we want to ensure the device is inexpensive, making it a viable option for surgeons to use. To be considered a low cost, we compared other products currently on the market and decided upon the device costing about $500. Because this procedure will occur during a liposuction procedure, the decellularization and filtration process should occur within an hour. This will ensure the patient is not under anesthesia longer than they have to be and the process is efficient. Since the device must be a closed system to remain sterile, we cannot use centrifugation as an option to separate blood and oil from the adipose tissue. To centrifuge we would need to separate the lipoaspirate into multiple tubes that would be placed into the centrifuge to be spun. This would mean the device is no longer a closed system as there is no method currently that would allow us to transfer lipoaspirate into multiple containers in a sterile manner. Lastly, our device will need to be able to process up to a liter of lipoaspirate. Currently competitors using the decellularizing technique are not doing it on a large scale process which is what we are working towards.

3.1.3 Revised Client Statement

To provide patients requiring breast tissue reconstruction cheaper opportunities for and easier access to the best care by transforming the current standards in reconstructive surgery through the development of the first point-of-care scaffold device for tissue repair made of the patient's own adipose tissue for optimum biocompatibility and tissue regeneration.

3.2 Project Approach

The project will be approached by designing multiple concept iterations aimed at identifying the best design or solution to optimize decellularization protocols, the ideal materials, and the best controlling system. After the best design was identified, a prototype was developed to meet the needs of a fast acting, closed system, point-of-care decellularization device. We tested and validated the prototype with a surrogate that mimics the different components of liposuction tissue and eventually tested with human adipose tissue gathered by a plastic surgeon from the operating room. Through multiple design iterations and testing of the prototype, we hope to start the beginning of a device that can be classified as an FDA Class II medical device for rapid translation to patient care and market.

Chapter 4 Design Process

4.1 Needs Analysis

Based on our client statement, goals, and objectives/constraints for our project, we can assess the overall needs of the device. The main need of the device is to provide patients who have undergone a mastectomy or lumpectomy a cheaper and more biocompatible option for cell migration into a scaffold for tissue regeneration using their own tissue. The materials used for the device must allow the device to be cheaper and easier to access than what is currently on the market, so it can be produced at a cheaper rate, also lowering the cost of treatment and increasing accessibility for patients. Additionally, we wanted to ensure that this device is fast and efficient. Physicians will use this device during a liposuction procedure and must complete the entire process without adding a long wait time as the tissue is prepared to be reinjected into the body. Using the patient's own tissue will allow the decellularized adipose tissue to be patient-specific

as their own tissue is what is being added back into the body. To provide the patient with maximum sterility, the device will be a closed system and be made for one-time use. Lastly, the tissue will be reinjected into the body to be as minimally invasive as possible. This will allow the patient not to have to deal with additional scarring that methods like fat grafting would leave.

4.2 Systems and Subsystems

The two primary systems of this device are to decellularize adipose tissue for use in breast tissue reconstruction and to create an injectable scaffold. To accomplish the decellularization of adipose tissue, there are two subsystems. The first subsystem uses mechanical forces to break up the tissue. The second subsystem filters blood, oil, tumescent liquid, and other contaminants from the extracellular matrix.

The second primary system we must create an injectable scaffold. The subsystem of this function is to retain the ECM on a filter in a compartment that has a port to remove the scaffold after filtration. Each subsystem will be used to accomplish the main systems of decellularization of adipose tissue and the creation of an injectable scaffold made of ECM removed from adipose tissue.

4.3 Functions and Sub-functions

The main product function of this device is adipose tissue decellularization. Some sub-functions under this category are lysing adipocytes and removing oil, blood, and contaminants from the extracellular matrix using biocompatible methods. Another function is to create an injectable scaffold/gel after decellularization with the sub-function to preserve the ECM's proteins, shape, and integrity to foster adipocyte cell growth, migration, and adhesion. The last function is to maintain a closed system for use in the operating room; the sub-functions

here are scaffold sterilization and easy transition to be used with liposuction methods such as tubings connected to the canister where lipoaspirate is held during the procedure.

4.4 Functional and Performance Specifications

Table 2: Functional and Performance Specifications

Functional Specifications	**Performance Specifications**
Adipose tissue decellularization	Using a max **5 Liters** Finishing decellularization in under **1 hour** Retains **less than 10%** of oil, blood, tumescent liquid, and other contaminants Retains **more than 90%** of ECM
Create injectable scaffold	Volume of scaffold dependent on injection site
Maintaining a closed process	Keep scaffold **100% sterile**

To be sure the functions are meeting their expectations, performance standards were set and identified based on the needs of our device and summarized in **Table 2**. The first functional specification is for adipose tissue decellularization. A performance specification is to use a maximum of 5 liters of lipoaspirate, as that is the maximum amount of fat that can be taken out of a patient during a single liposuction procedure [54]. To be sure the adipose tissue is decellularized, the performance specification of retaining less than 10% of oil, blood, tumescent liquid, and other contaminants but leaving more than 90% of the ECM must be met in the final product of the device. Since this device needs to be used all in one procedure, the device needs to perform quickly. The performance specification is for completion in under an hour so that the patient does not have to undergo multiple surgeries. The process should be completed in the

amount of time it would take to remove excess skin due to fat removal and close the collection site so that the scaffold can be used immediately after the closure for insertion into the breast. The following main functional specification is to create an injectable scaffold, the performance specification here is dependent on the injection site, and the amount of scaffold injected. For example, if the patient wants a complete breast replacement, the injectable scaffold has to be able to fill the area desired, this means the volume of ECM created must fill the desired area. Lastly, the device must maintain a closed system; the performance specification here is to ensure that the field and work area and the inside of the device are 100% sterile. No outside contaminant should be introduced to the system and therefore introduced into the patient. This is measured by contaminant testing and product testing before use on a patient.

4.5 Primary and Secondary Specifications

Our device's primary specifications are to decellularize the adipose tissue and separate the contaminants from the proteins within the extracellular matrix by removing oil, blood, tumescent liquid, and other contaminants with safe methods that are biocompatible with the body, for example the lack of chemicals, so it will not cause adverse reactions. Another primary specification is to preserve the shape and integrity of the extracellular matrix to create an injectable scaffold. These are the functions that our device cannot function without. The secondary specifications are to be easily used in the operating room, transportability like keeping the device relatively small and compact while keeping everything sterile.

4.6 Pairwise Comparison Chart

Table 3: Pairwise Comparison of Device Functions

Pairwise Comparison Chart

Objectives	Adipose tissue decellularization	Use FDA approved chemicals	Create injectable scaffold	Preserve proteins and integrity of the ECM	Maintaining a closed process	Easy Complexity (not too many passages, quick, manual device, cheap)	Totals	Rank
Adipose tissue decellularization	X	0	0	0	1/2	0	1/2	**5 (W = 2)**
Use FDA approved chemicals	1	X	1/2	1/2	1	1/2	3 1/2	**2 (W = 5)**
Create injectable scaffold	1	1/2	X	0	1	1	3 1/2	**2 (W = 5)**
Preserve proteins, and integrity of the ECM	1	1/2	1	X	1	1	4 1/2	**1 (W = 6)**
Maintaining a closed process	1/2	0	0	0	X	0	1/2	**5 (W = 2)**
Easy Complexity (not too many passages, quick, manual device, cheap)	1	1/2	0	0	1	X	2 1/2	**4 (W = 3)**

After determining the functions the device needs, they were compared to find the ranking of importance in a pairwise comparison chart. The most important function needed is preserving the ECM's proteins and integrity. Preserving the integrity of the ECM to create a usable scaffold for injection back into the patient will allow cells to adhere and migrate to the scaffold and rebuild adipose tissue in the desired location. After preserving integrity the two main focuses are creating an injectable scaffold and using FDA approved chemicals. The end goal of the device is to create an injectable scaffold that will allow for proper cell regeneration with no random cell death which is why it is more important than other categories. This device will not be using chemicals currently but if that is what is required to help get rid of excess oil on top of the mesh, it will be an important category to focus on as we need to ensure it will get quick FDA approval. We then made sure the device is not complex as many surgeons or nurses will be required to use

the method if the device is widely distributed, there is no time for complicated devices in the operating room. The last two focuses are maintaining a close process and adipose decellularization. These are the lowest priority due to the fact that currently we had no time to figure out how to make it a fully closed system and rather focused on filtration and just making a prototype that works to isolate the ECM. The group that works on this project after us should consider a closed system a first or second priority as this device is meant to be working with human tissue that should remain sterile at all times. Adipose tissue decellularization is a low priority as well due to the fact that since the device is patient specific, injecting back their own blood or oil should have no adverse effects.

4.7 Conceptual Designs

The team completed a brainstorming session to come up with design ideas that would satisfy our project specifications through rapid ideation and sketching. The brainstorming methods used were rapid ideation and sketching. The team thought about different solutions independently then met as a group and spent 15 minutes jotting every idea down, as well as any new ideas that popped up. Then, sketches were drawn to narrow down five conceptual designs.

Our first design idea, as shown below in **Figure 19**, was to lyse the cells through sonication. Sonication is a method that uses pulsed, high frequency sound waves to agitate and disrupt cell membranes. After sonication, the cells, blood, and oils would be filtered through a mesh filter.

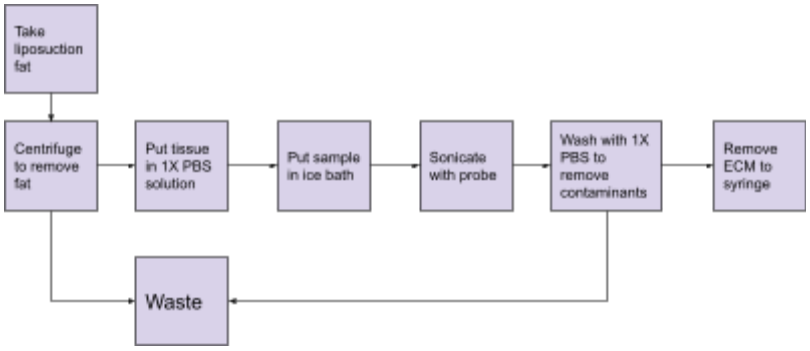

Figure 19: Process diagram of lysing cells through sonication. First the liposuctioned fat would be removed from the patient and centrifuged to remove the fat and oil from the tissue, the fat and oil would be removed and put into the waste container. Next the tissue sample would be placed in a solution with 1X DPBS and placed in an ice bath to alleviate the effects of heat from sonication. The tissue sample would be sonicated and the contaminants would be removed by washing the tissue with 1X DPBS and the ECM could be extracted into a syringe to be inserted into the patient.

Our second design, shown below in **Figure 20**, was blending the adipose tissue in a laboratory blender with a buffer, using the force of the blades to destroy cell membranes. Then, separating the ECM out using a centrifuge.

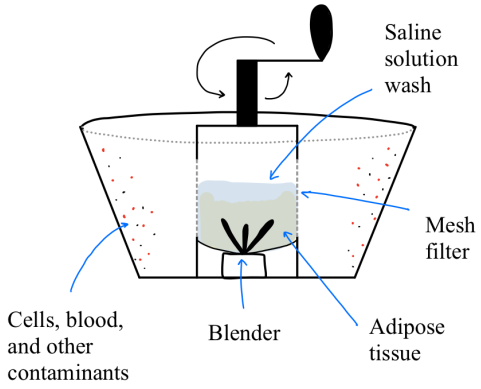

Figure 20: Diagram of combining the blending technique and centrifuge technique. The adipose tissue liposuctioned from the patient and blended to break up the tissue with a saline solution wash to help filter out contaminants. The mesh filter would be closed during the blending and once the blending is completed the mesh filter could be opened and the machine would centrifuge to push the cells, blood, fat, and other contaminants out to be collected in the waste bin outside. The leftover ECM can be collected into a syringe for insertion to the patient.

Our third idea, shown in **Figure 21** below, was washing the cells in a detergent solution, using biocompatible chemicals such as triton. Most detergent solutions are hydrophobic and can effectively break down cell membranes by disrupting interactions at the surface. Then rinsing out the chemicals and other contaminants with a saline solution, leaving only the ECM.

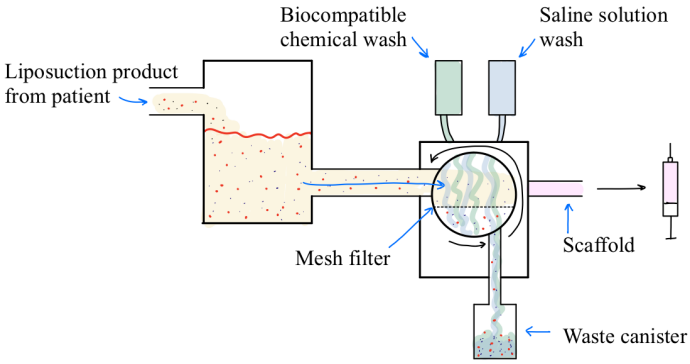

Figure 21: Diagram of "washing machine" device. This machine will take the liposuctioned fat from the patient through a vacuum into the washing chamber. In the washing chamber the fat will be centrifuged at a high speed with the mesh filter opened to remove the fat which will flow into the waste container. Once the fat is removed, the mesh filter is closed, the tissue can be washed with a biocompatible wash, and then tumbled to incorporate the wash into the tissue to remove an optimal amount of cells from the tissue. The wash will be removed by opening the mesh filter, allowing the wash to drain, closing the filter again, rinsing with a saline solution, tumbling, and opening the filter again to let everything drain into the waste container. The leftover ECM will be vacuumed out into a syringe for insertion to the patient.

Our fourth idea was using osmotic shock to break down the cells. The cells would be immersed in a hypotonic solution which would weaken them by increasing internal pressure as water rapidly enters the membranes. Then, sudden dilution would cause the pressure to rapidly decrease which would lead to the cells to burst and lyse. When diluting the tissue, the solution would leave through a mesh filter, so that the fat from the cells, blood, and fluid are also filtered out, leaving only the ECM. A process flow diagram of our system is shown in **Figure 22** below.

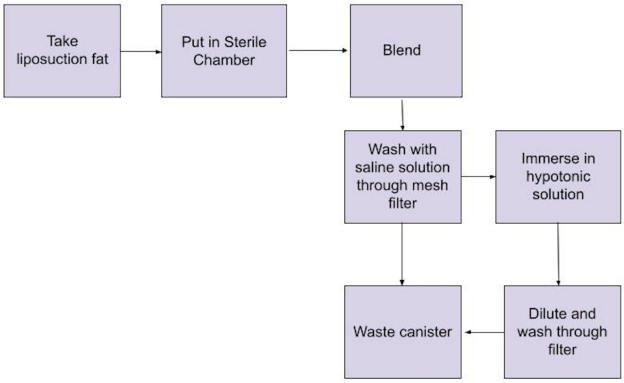

Figure 22: Process flow diagram of mesh filter and high concentration potassium chloride buffer solution to osmotically shock the cells. The liposuctioned fat tissue will be taken from the patient and put into a sterile chamber. Within the vacuum chamber, the sample will be blended with blades to cut the fat down and washed with saline solution through a mesh filter to remove fat and oil. Then the sample will be immersed in a hypotonic solution for a time. The hypotonic solution would then be diluted and washed out through another smaller mesh filter, leaving only the ECM. The leftover ECM can be taken and put into a syringe for insertion to the patient.

Our fifth design idea, shown in **Figure 23** below, was to quickly freeze the adipose tissue in liquid nitrogen. Freezing would cause the cells to swell and the membranes to burst as ice crystals formed. Then we would thaw the tissue and rinse out the dead cells using a saline solution leaving only the ECM. All of our designs would be in a closed system, ideally a vacuum chamber, to maintain sterility, an important project specification we identified.

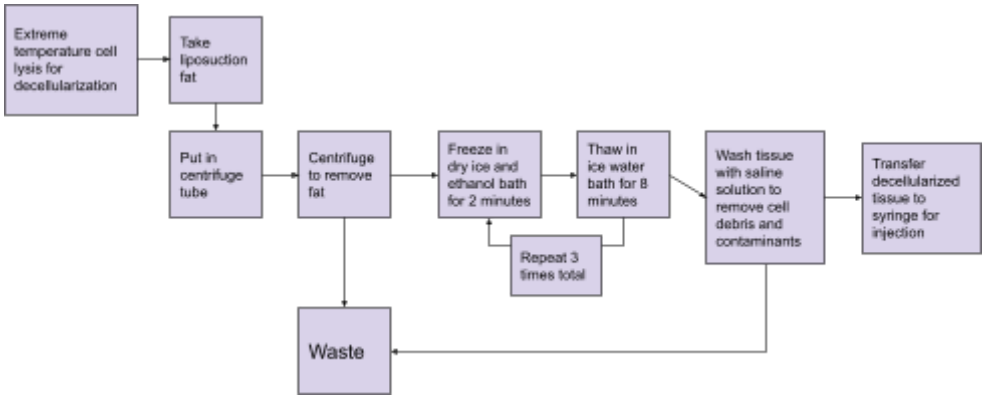

Figure 23: Process flow diagram for thermal lysing of cells for decellularization. The liposuctioned fat will be taken from the patient and centrifuged to remove fat and oil from the tissue, the fat and oil will be added to the waste container. The samples will then go through a freezing and thawing process where they will be frozen in a dry ice and ethanol bath for two minutes and thawed in an ice water bath for eight minutes and repeated a total of three times. The tissue will be washed with a saline solution to remove

cells and other contaminants and will flow into the waste container. The leftover ECM will be placed into a syringe for insertion to the patient.

4.7.1 Redesigned Conceptual Designs

After we met with our advisors and sponsors, our ideas were refined and taken back to the drawing board. New solutions included the combination of ideas 2 and 3. A blender was added to the washer machine method, shown below in **Figure 24**, to break up the lipoaspirate in order to remove the oil and blood. The biocompatible chemical solutions will still be used in the original washer machine idea to aid decellularization while filtering out the waste.

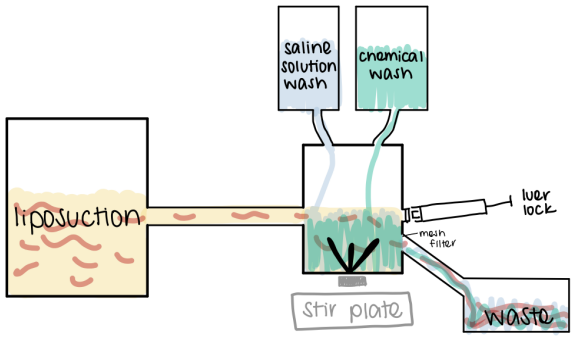

Figure 24: Combination of design 2 and 3. The liposuction fat will be moved into a separate container containing a blender with 2 ports at the top for saline and chemical wash solutions. Additionally, there would be a waste container collecting the oil and blood waste being filtered out from the fat. With a luer lock on the side collecting the ECM.

Based on feedback of the combined idea and the constraint of not using chemical washes, we went back to the drawing board to come up with a different solution that did not contain a chemical wash. The new idea, shown in **Figure 25** below, had a battery powered blender at the top of the device that would come down and blend the fat while having a mesh filter and waste bin at the bottom collecting the liquid waste.

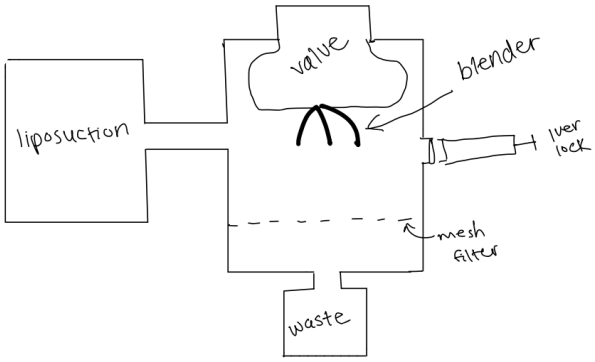

Figure 25: Battery power blender. The liposuction fat will be moved into the blender device and the power button will be turned on for the blender. As the blender is cutting up the fat, the oil particles and blood will be filtered out through a mesh filter at the bottom of the device flowing into a waste bin. Once the entire fat is blended and no more oil and blood is in the fat, the ECM will be removed through the use of a luer lock.

Based on the feedback of idea seven and the addition of the constraint of hopefully not having any powered parts, we decided to take a new approach completely. We came up with a device, shown in **Figure 26**, that will use a grinder method and multiple mesh filters to ensure only the ECM would be the end product. A CAD drawing of the grinders used is in **Figure 27**.

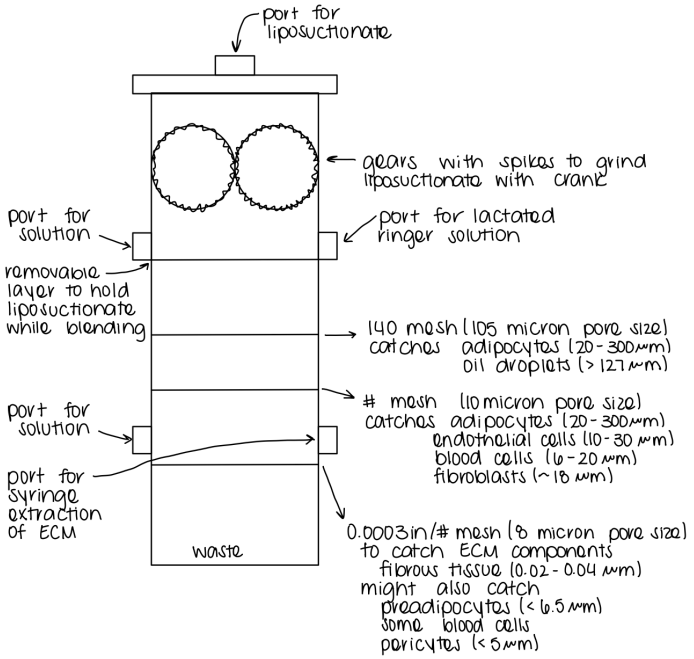

Figure 26: Grinder tower design. This design would have the liposuction tissue come in at the top through a port and with the use of a manual grinder, the tissue will be grinded up as it came into the

device. From here a removable layer will be used to hold the fat until grinding is complete before being washed with a lactated ringer solution and moving down the tower. The lactated wash will help move the tissue down the filters as each filter is designed to catch different components. The last filter will catch the ECM and underneath there will be a waste layer to collect any solution that made it to the end. A port will be used to remove the ECM from the device.

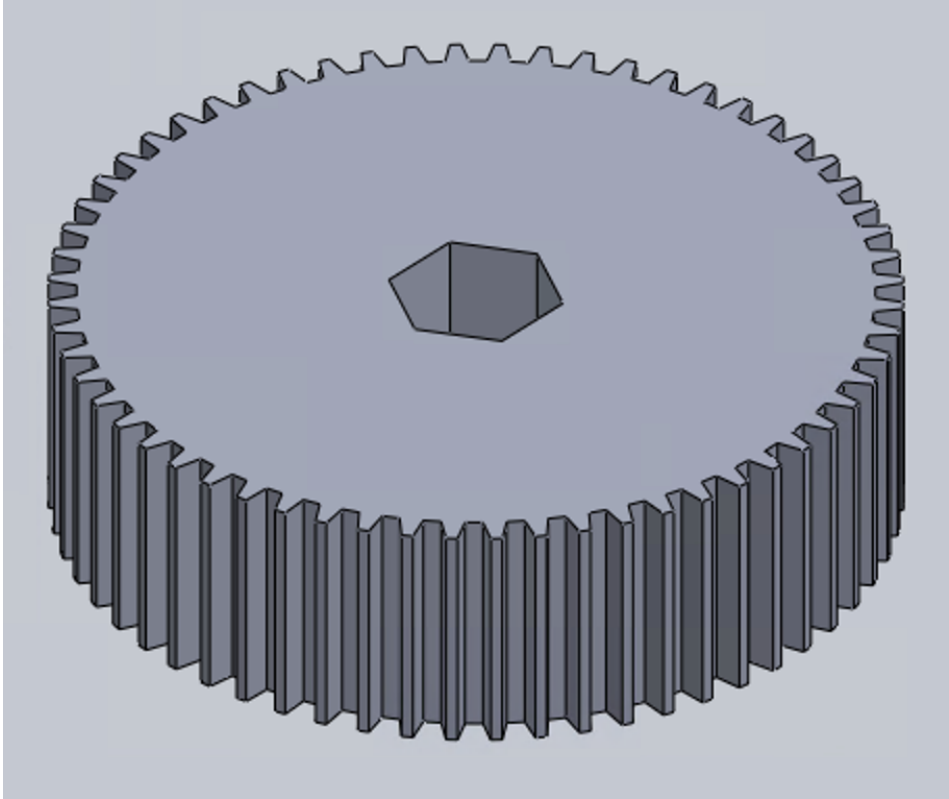

Figure 27: CAD design of grinder for grinder tower design. This was 3D printed in plastic twice to be turned using a crank that went in the middle. This idea was soon discarded as we determined mechanical grinding was not strong enough to lyse cells.

After further brainstorming and feedback, we drew out a tangential flow design, seen in **figure 28** below, that combined the blender idea with a continuous pump to allow for continuous filtration that would minimize clogging.

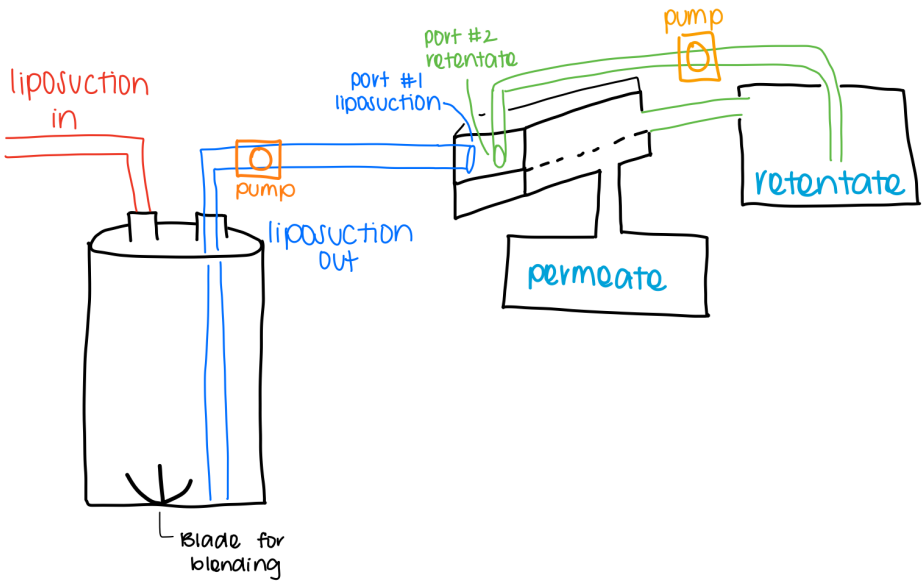

Figure 28: Tangential Flow design. In this design, the liposuction is vacuum pumped into the blender and the tissue is blended up, lysing the cells. Then the blended tissue is pumped into the second container, where the ECM is collected on the mesh, and the material is continuously pumped through the second container to minimize clogging and get optimal ECM.

Based on further feedback about the devices, one that uses no chemicals, is simpler than the tangential flow idea, and provides stronger lysing of cells than grinding, we came up with a single compartment blender idea, shown in **figure 29** below.

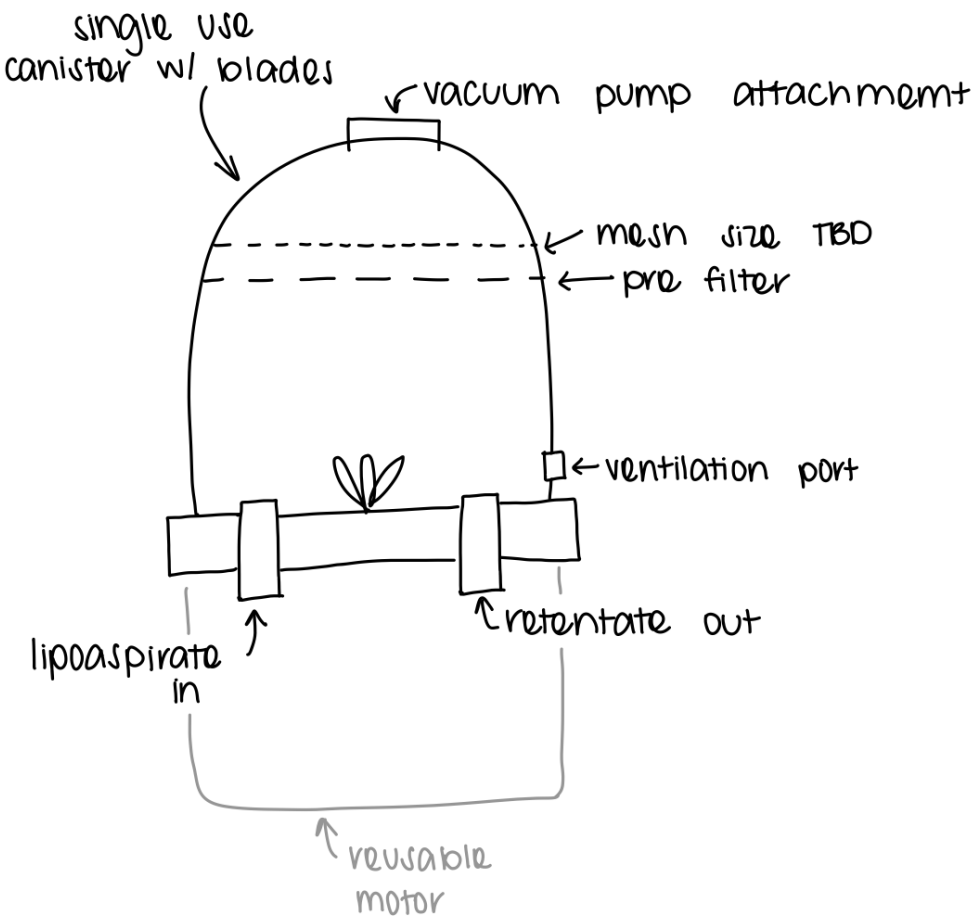

Figure 29: Single compartment design, similar to two compartment design except the mesh is in the same container as the blender. The lipoaspirate would be vacuum pumped into the container and the material would be blended for a given time to lyse the cells. Then, the container would be flipped, so that the blended lipoaspirate could be filtered through the mesh filters, where the ECM would collect on top of the second mesh.

4.8 Concept Map

The overall concept of our device was to remove the cells, oil, blood, tumescent liquids, and any other contaminants found to obtain the ECM. Based on our brainstorming session and project specifications, we created a concept map showing how we hope to accomplish our overall goal shown in **Figure 30** below.

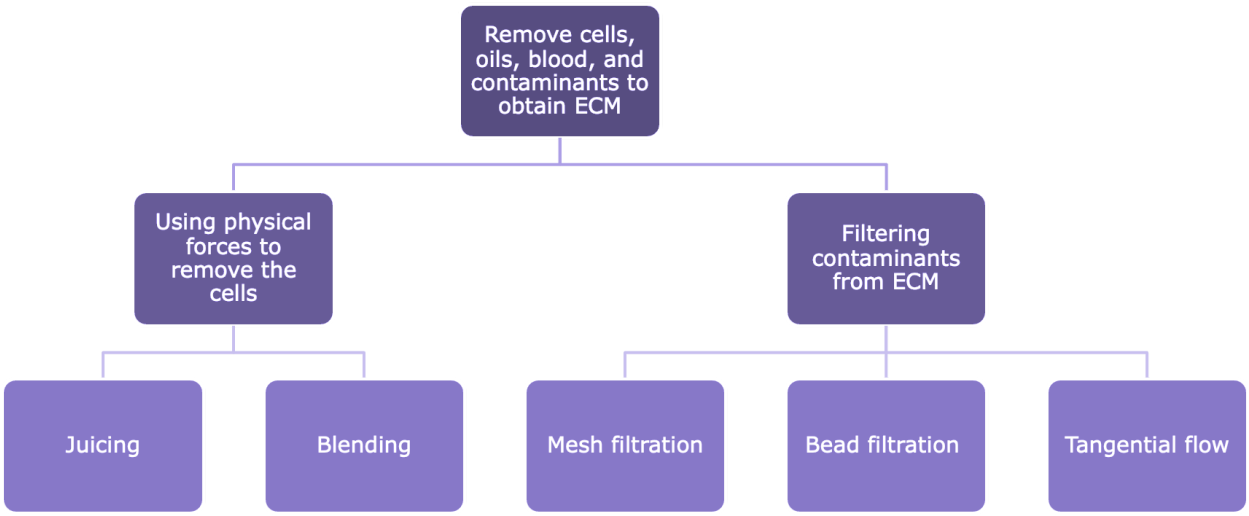

Figure 30: Concept Map of ideas on how to complete the functions listed in the second layer.

The overall function of the device will be done using physical force to break up the cells, and using vacuum and mesh filtration to filter out the contaminants found in the liposuctioned fat. This will ensure the cells are lysed leaving behind ECM only creating an acellular ECM scaffold [55]. Physical forces include blending the tissue to separate all the components, and juicing the tissues to retain a drier ECM material through centrifugal force. And finally, to ensure the ECM is completely rid of any contaminants, filtration occurred to guarantee every cell, blood or oil is removed. Mesh filtration and tangential flow would work similarly. For mesh filtration the blended lipoaspirate would be suctioned over the filters and the top filter would filter out large chunks of fat that were not blended, and the second filter would filter out blood, oil, and tumescent fluid leaving the ECM on the filter that can be suctioned out a side port for reinjection. The tangential flow system would also use a continuous flow to push blood, oil, and tumescent fluid through the filter while leaving the ECM on top for collection. Bead filtration would simultaneously blend/break up the lipoaspirate and then the ECM would hopefully fall through to the bottom since it is a smaller size compared to blood, oil, and tumescent fluid.

4.9 Preliminary Design

Based on the feedback from our advisors and the need to hit every point of our criteria, three new designs were made that fit in the criteria and can be considered for preliminary data testing and the final design could be 3D printed for prototyping. This section will go over our engineering design process through multiple iterations of designs.

4.9.1 CAD Design for Juicer

Our first design came from the mechanisms of a juicer. As a juicer typically works, the vegetable or fruit is inserted into the device and blended up while separating the fibers and liquid into 2 different chambers. For this idea, the goal was to build a juicer in a way that it can be considered a closed system that can blend up the fat – decellularizing it – and then separating the ECM from the oil, blood, and liquid wastes. To ensure the fat is able to be fully decellularized, the idea - while not shown in the **Figure 31** below - is to have it be blended up by a blender prior to it being juiced. The juicer will work with a hand crank to keep simplicity for FDA approval.

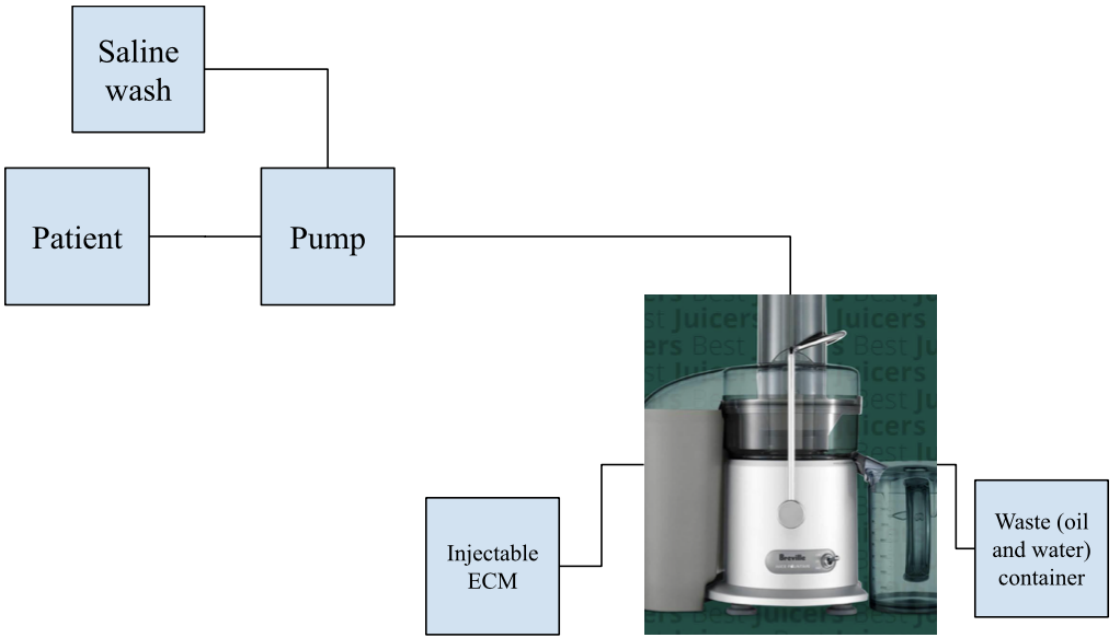

Figure 31: This is the process flow diagram for the potential juicer idea. The hope was that the lipoaspirate from the patient and saline would be suctioned through the top of the juicer which would then blend the material and separate the drier ECM fibers to the left side, with the waste falling to the bottom of the canister.

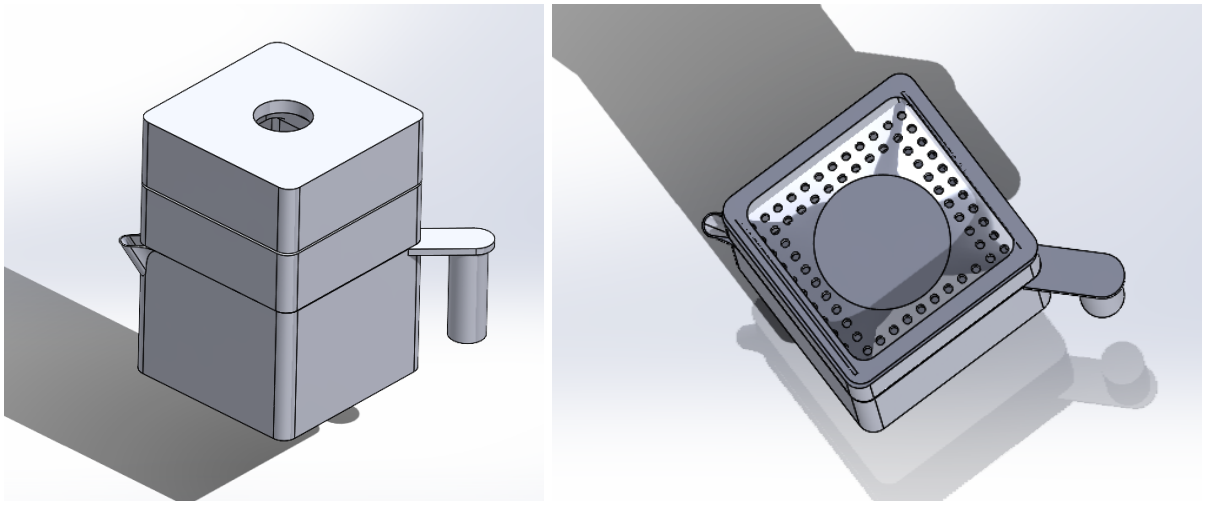

Figure 32: CAD for juicer idea. The liposuction fat will enter the device at the top and get blended prior to moving down onto the juicer blades where the liquid waste and tissue will be separated into two separate compartments resulting in an injectable scaffold.

4.9.2 CAD design for Blender

Our second design, shown in **Figure 33**, would be similar to our blender design stated above in conceptual design. This design would blend the liposuction fat in a blender, but instead of being a one compartment system, the fat would move to a separate container where it would be filtered leaving only the injectable ECM scaffold. Again, the idea is to have the final product work with a hand crank for simplicity.

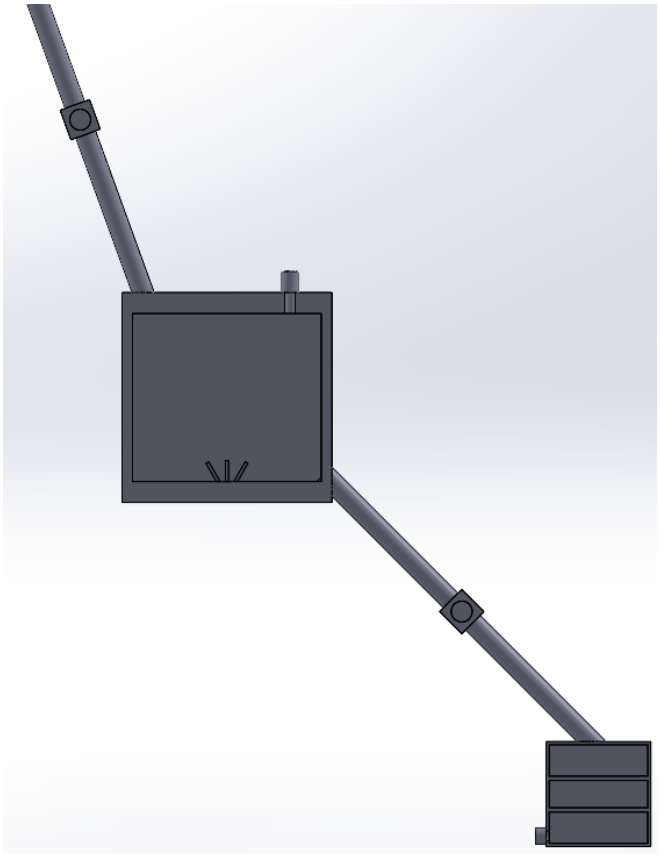

Figure 33: CAD for blender idea. During the liposuction procedure the pump used to suck the lipoaspirate will also suck it into that chamber that houses blades through the left port. The blades will act like a blender. There will be another port on the right that is intended for a saline solution to be injected into the chamber to wash as the lipoaspirate is blending. The blenders will be controlled by a magnetic rotor making them spin without involving electrical components. The port on the bottom right will lead to a separate chamber of two levels of differently sized meshes that will decant the solution leaving only the desired ECM at the bottom. Using a luer lock syringe we can extract the ECM for reinjection back into the patient.

4.9.3 Design for Tangential Flow

This design, suggested by professor Page, includes a blender to break up and decellularize lipoaspirate collected from the patient and tangential flow to significantly reduce the volume and leave behind only the ECM retentate, shown in **Figure 34** below. As previously mentioned in Chapter 2.8.1, tangential flow passes the fluid parallel to the filter while performing a continuous filtration with the permeate being put in a waste container and the retentate being recycled back into the feed.

This design would take the lipoaspirate from the patient and collect it through a port into a canister designed by the team with a blender blade inside, using the same mechanics and pump as in any liposuction procedure. The blades would blend the lipoaspirate as it is being collected by the patient for continuous flow through the tangential flow filter. The blades would not hit the collection tube but would create a vortex to make sure all the lipoaspirate is blended evenly. Continuous blending will not affect the solution created by blending, as proven by the procedure from Zhang et al. [53]. After blending, a pump would bring the solution through a tube to the mesh filter for tangential flow filtration. As it moves across the filter the permeate or waste would go through the filter and collect in the waste container. After the filter the retentate or the ECM would be collected in the retentate collection container. It would need multiple passes through the filter to be sure the volume is reduced and the waste is removed so it will continue to go through the filter as it is collected with another pump and brought back through the filter until adequate volume reduction is achieved. There would be a luer lock system port, not shown in the drawn figure, at the retentate collection container that can be used to collect the ECM once filtration is complete.

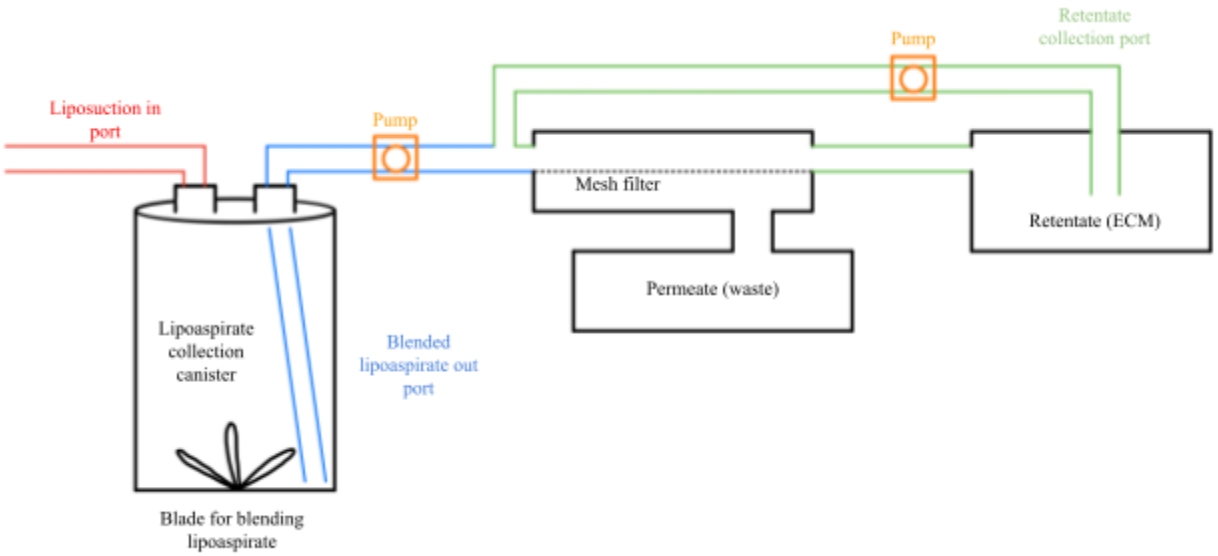

Figure 34: Preliminary drawing for tangential flow concept. Tangential flow will be used to filter out the waste, oil and water, from the ECM for a large volume reduction. The end goal retentate is the ECM with little waste included. The retentate will go through the tangential flow filter with multiple passes.

4.9.4 Pugh Analysis

Table 4: Pugh Analysis of Brainstorming Ideas

Criteria	Weight	Gold Standard (ACF)	Juicing	Blending	Tangential Flow
Adipose tissue decellularization	**2**	0	-1(2) = -2	0(2) = 0	0(2) = 0
Use FDA approved chemicals	**5**	0	+1(5) = 5	0(5) = 0	0(5) = 0
Create injectable scaffold	**5**	0	-1(5) = -5	0(5) = 0	-1(5) = -5
Preserve proteins, and integrity of the ECM	**6**	0	+1(6) = 6	0(6) = 0	0(6) = 0
Maintaining a closed process	**2**	0	0(2) = 0	+1(2) = 2	+1(2) = 2
Easy Complexity (not too many passages, quick, manual device, cheap)	**3**	0	0(3) = 0	+1(3) = 3	-1(3) = -3
	Total	0	4	5	-6

The pairwise comparison chart rankings in **Table 3** were used as the weights of the pugh analysis chart in **Table 4**. The pugh analysis chart narrowed down the decision-making process of finding our leading concept. The gold standard we compared all of our ideas to was ACF. These scaffolds are currently the best option in the market since they are biocompatible, and maintain the integrity of the extracellular matrix well enough to show positive results such as improved collagen synthesis and dermal thickness. Therefore, after comparing all three ideas from the brainstorming, it was decided that the juicing and blending ideas will be prototyped and preliminary testing would occur.

4.10 Testing and Analysis

There are many testing methods we can do to ensure our device is built properly and functions properly. One of the testing methods we intended to use was an absorbance based DNA quantification, using commercially available kits [41]. The kits do their job by digesting a piece of the tissue sample and isolating it and purifying the DNA. A spectrophotometer can then be used to measure the amount of DNA content per mg of dry tissue. The tissue can be deemed decellularized if there is a DNA content of below 50 ng per mg of tissue [41]. Another method we planned to use was staining the sample with Hoechst 33345 to use optical microscopy to confirm the lack of visible nuclei [41]. Another staining that can be performed is with Dil or its derivatives, to stain the outer membrane without affecting cell viability [42]. Although not affecting cell viability is not important to us, it will allow us to calculate how many intact and alive cells are left, which will allow us to determine if the scaffold has been decellularized or not.

Another main goal we had for our device is to maintain a sterile field for the entire duration of decellularization. A test we can use to check if the device maintains sterility is a Direct Transfer Sterility Test [43]. In this test, a small volume of the sample is taken and

incubated in a growth medium. After the incubation period is up we can test for turbid areas. If there are turbid areas it indicates that there was microbial growth which is something we do not want to happen under any circumstance.

We also wanted to stain with masson's trichrome which would allow us to differentiate between the cells and collagen and other connective tissue fibers. Since we wanted to isolate ECM from adipose cells this test would allow us to quantify how much ECM we were able to isolate and what percentage of fat remains.

4.10.1 Validation Testing Methods

Validation testing methods are intended to ensure the right product is being built. The product we wanted to make is a device that can decellularize adipose tissue to make an injectable scaffold. Ideally, we would perform a histology staining of the tissue after blending and filtering to ensure that only the ECM remains, with very little cells and water content. The tests would be run with human lipoaspirate in a hospital and we would test for the time it takes the procedure to run and whether the device can take human lipoaspirate and adequately decellularize the tissue and filter out everything but the ECM.

4.10.2 Verification Testing Methods

To verify that the device is being built right, the device needs to be tested based on the specifications it was designed for. The device needs to be able to filter extracellular matrix from lipoaspirate after decellularizing and disrupting the cell walls of adipocytes. The solution will contain oil, blood, tumescent lipoaspirate liquids, and fibrous extracellular matrix. In order to prove the device is able to filter, testing with mesh will need to be done to separate the components. After the components are separated the components would be weighed and

compared to their original weight to show how much of each was removed from the original mixture. Other verification methods that can be used could be image analysis to quantify the size of oil droplets in the fibers removed. To test that this device is able to achieve those goals, the device needs to be tested by surgeons in the OR.

4.11 Feasibility Study

Our team ran preliminary testing of porcine fat and carrots. Boiled carrots were chosen when we ran out of pig fat because it is most similar to lipoaspirate in the aspect that there were fibers and cell walls that could be broken for decellularization. With these samples, we tested the efficacy of juicing and blending for decellularization. For the control using the pig fat, we cut it up into smaller pieces to try and replicate lipoaspirate. Next, using the same cut up pig fat, we took a sample and blended it in a small spice blender. It was difficult to use liposuctioned pig fat because it did not behave at all similarly to human lipoaspirate as the pig fat was very stringy and hard, did not blend well, and had more of a clumpy consistency versus a homogenous, liquid consistency of human lipoaspirate. Additionally, at this time we did not have a juicer so we did not test the juicer on the pig fat. For the control using boiled carrots, we mashed the carrots using a mortar and pestle as the control, to create a similar consistency to the cut up pig fat. We then juiced a sample of the mashed carrots in the juicer and blended another sample in a nutribullet blender for three minutes, according to prior literature. To analyze our pig fat and boiled carrot samples, we performed histology staining. We first cryosectioned our samples and then stained them using the H&E technique (hematoxylin and eosin).

When we started testing filtration methods we chose vacuum filtration to focus on because that method worked quickly and effectively. Since we are not allowed to use centrifuges in the operating room we needed a method that would aid in quick filtration rather than allowing

the material to filter using gravity. The vacuum accelerates the material that is able to go through the mesh at a given time and is commonly used in the OR. Before the experiments were started we looked at different mesh sizes and saw if each mesh size would filter out different amounts of fibers. Under vacuum filtration it was noted that almost all of the tested mesh sizes retained similar quantities of fibers on top except for the 20 mesh. Due to this we decided that we would use the smallest mesh size, 200, in our final prototype.

After deciding what mesh sizes to use, experimentation continued. Before putting the mixture through the mesh filter we allowed the blended mixture to decant. Other devices doing this in the market allow lipoaspirate to decant to get rid of excess water. We usually allowed mixtures to decant for 5-10 minutes. The mixture would separate into three distinct layers DPBS on the bottom, then a combination of fibers and DPBS in the middle, and frothy oil on top. During experiments we would either suction out the oil layer on top before pouring it over the filter or we would suction out the two bottom layers of DPBS and fibers to leave the oil in the container as we wanted to get rid of as much oil as possible. Decanting allowed us to remove much more oil than if the entire mixture was just poured over the mesh.

4.12 Preliminary Data of Mechanical Disruption Testing

The carrots were first boiled for 10 minutes and mashed with a mortar and pestle to simulate the broken up texture of lipoaspirate. Then, about 4 mL of DPBS solution was added to each sample. Images of the blended, juiced and control carrots are shown below in **Figures 35, 36,** and **37**, respectively. The blended carrot had a more liquid consistency, whereas the juiced carrot was more fibrous and had small chunks in it. The juiced carrot did not change much because a juicer is meant to be used on large solid fruits and vegetables but the carrots put in were small chunks not able to be pushed through the canister well.

In addition to carrot testing, pig fat was also imaged. **Figures 38** and **39** depict the control and blended pig fat, respectively. DPBS solution was added to the pig fat samples to prepare for histology staining.

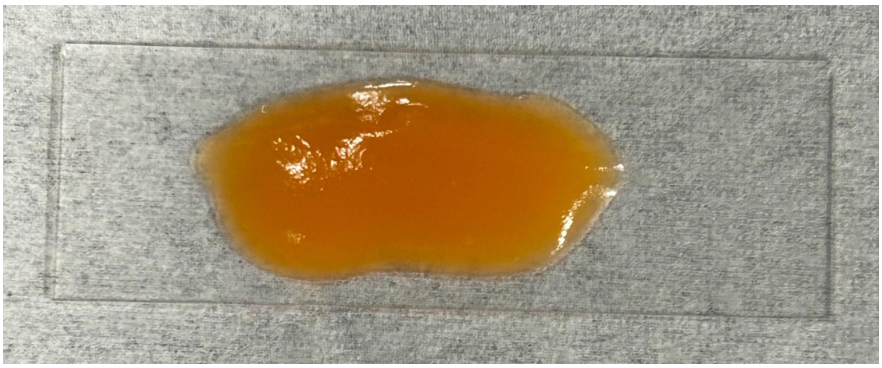

Figure 35: Blended carrot. Has the consistency of a puree, keeping most of the water within the cells, fibers seem to be broken up.

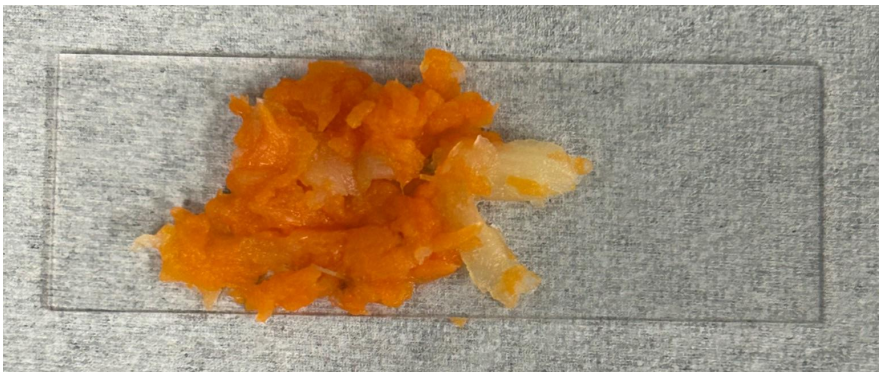

Figure 36: Juiced carrot. Still a little chunky and hydrated, however it did lose some of the water when put through the juicer. Has a lot of fibers still intact.

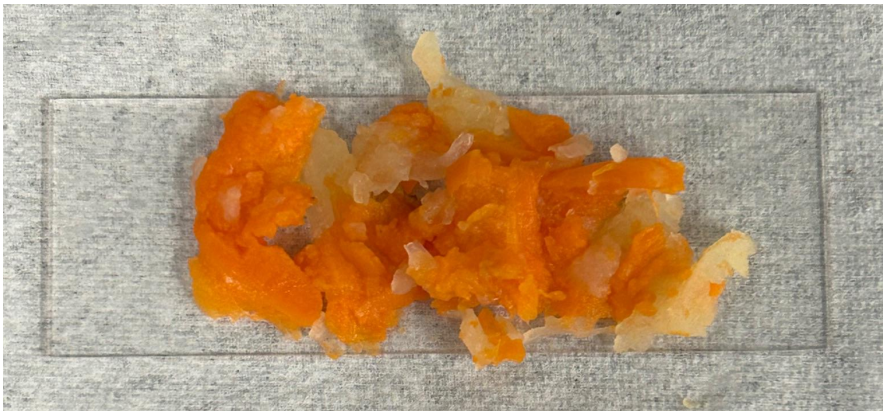

Figure 37: Mashed carrot used to replicate lipoaspirate. Has a similar texture to lipoaspirate, it has fibers, water within the cells so when broken it should release the liquid and leave behind the fibers and ECM.

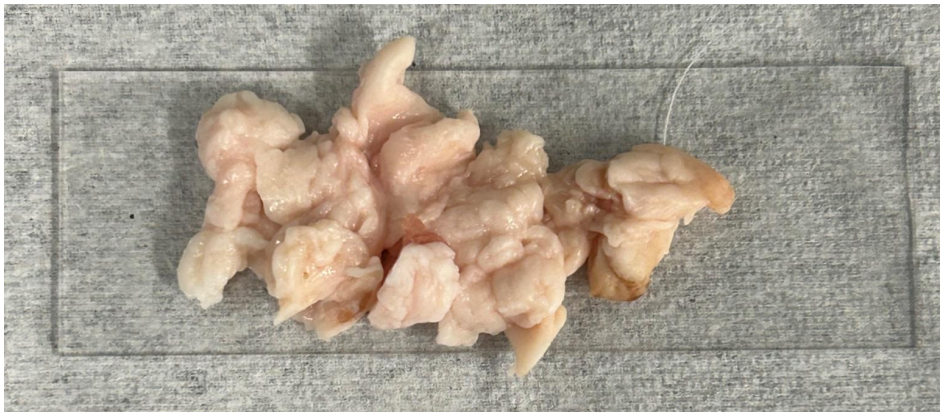

Figure 38: Cut up fat used to replicate lipoaspirate. The consistency of the fat was chunky and stringy, however it did not replicate lipoaspirate very well.

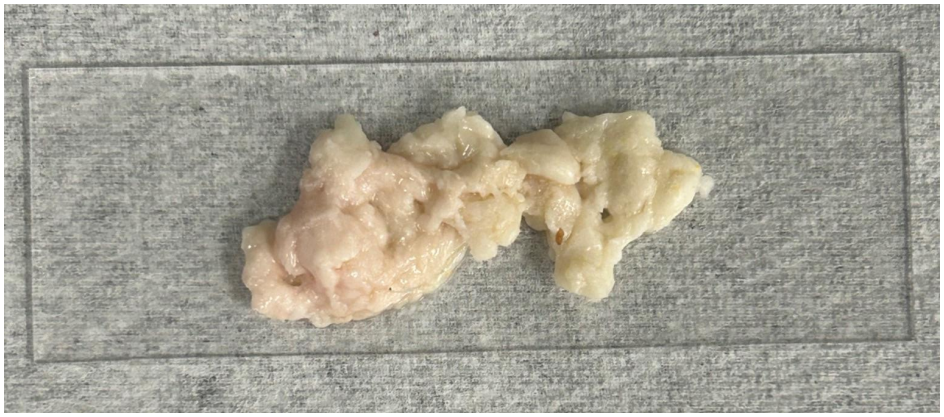

Figure 39: Blended fat. The consistency is still stringy but not as chunky.

4.12.1 Results from Histology Staining

After blending or juicing, the pig and carrot samples were taken to the lab to be stained and imaged. We used the H&E method to stain our samples and used the ZEN microscope to image them. It is important to note that the pig fat we were given had been frozen and thawed, so the control may have had cell damage from that. Additionally, the spice blender we used to blend the pig fat was not strong enough to achieve the desired consistency we wanted, and there were still big chunks of fat after blending. Additionally, since the tissue was not fresh, most of our samples had deteriorated. The blended pig fat, though only used with a small spice blender with low power, still showed the breaking of cell walls compared to the control, as shown in **Figures**

40 and 41. The juiced carrot was very similar to the control, as shown in **Figure 42 and 43**, whereas the blended carrot showed broken cell walls, as shown in **Figure 44**.

Cells in the pig fat images are stained yellow. The blended pig fat did a better job of rupturing the cells than the control, which was our desired goal. In images of the carrot samples, the nuclei are stained purple whereas the cytoplasmic components are stained pink [55]. The juicer did a better job of removing water, however the blender did a better job at rupturing the cells.

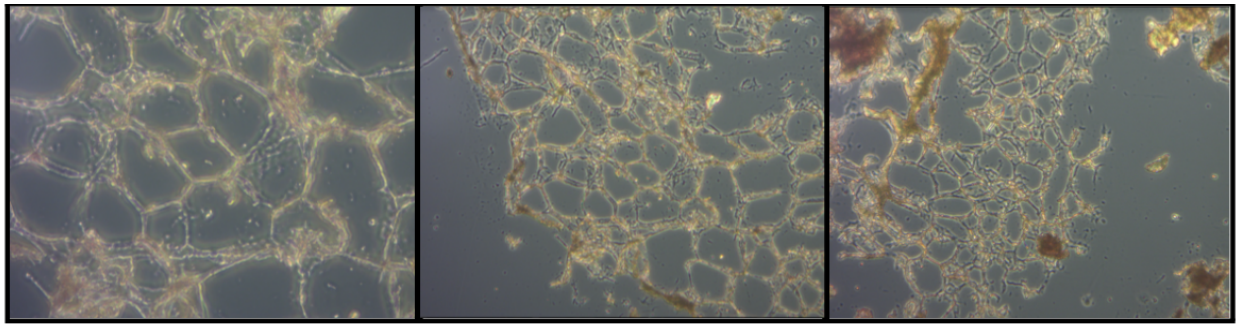

Figure 40: Histology stained images of control pig fat. (20X objective). Some damage to cells, however most cell walls are still intact.

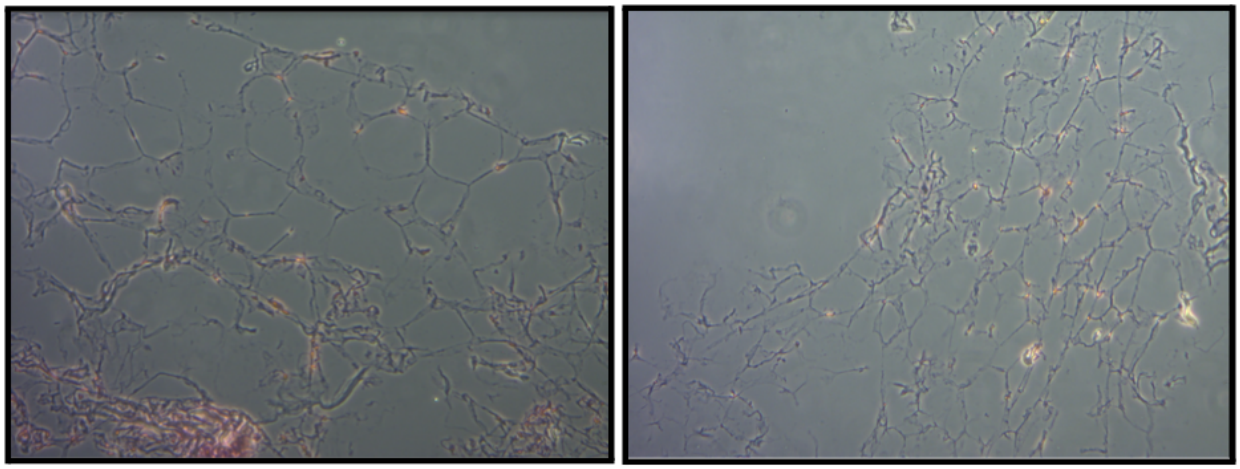

Figure 41: Histology stained images of blended pig fat. (20X objective). There is cell damage as most of the cell walls are ruptured. Can also see some fibers floating around.

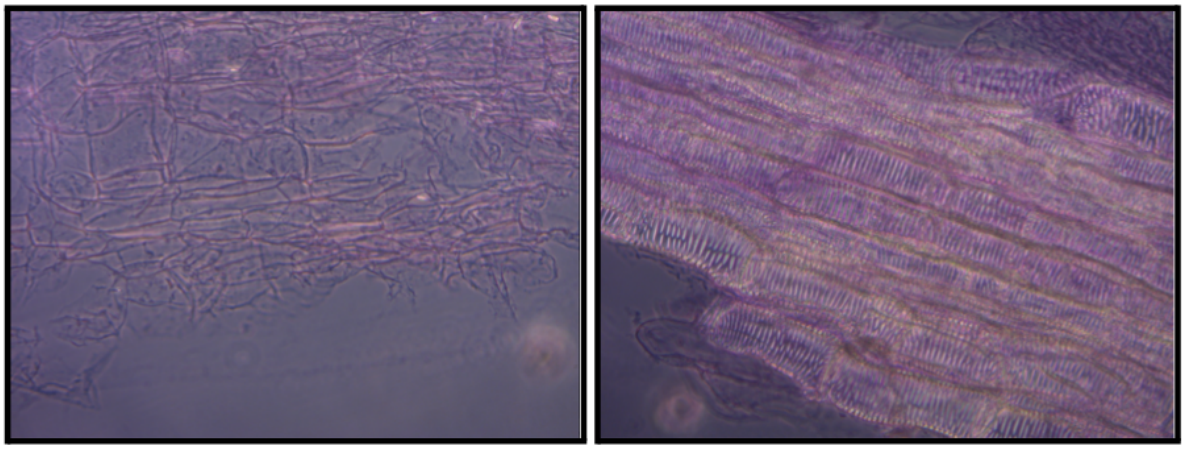

Figure 42: Histology stained images of control carrot (20X objective). Most cell walls look to be intact. Very fibrous. Image on the right is a cross section from the top of the cells.

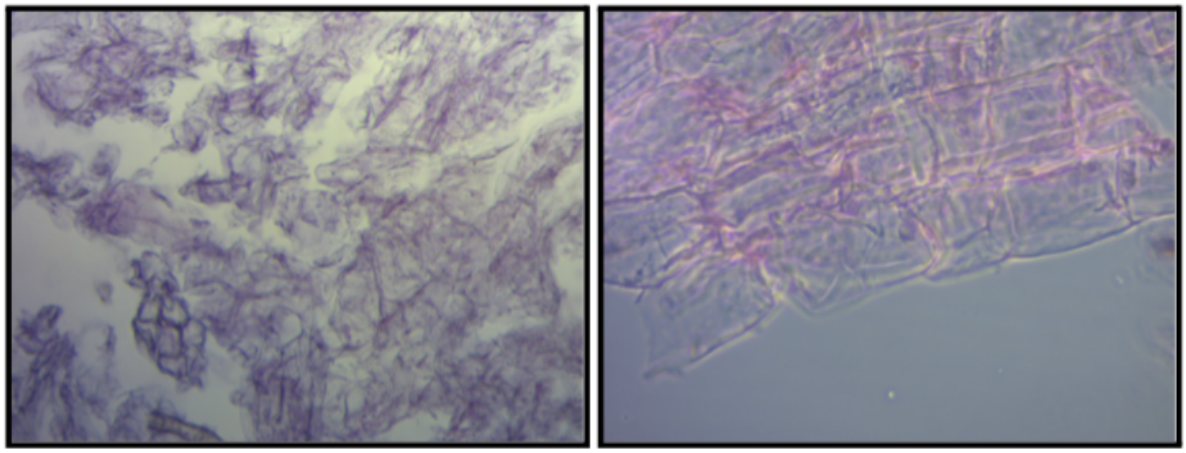

Figure 43: Histology stained images of juiced carrot (10X and 20X) objective. Some cell damage. Looks very fibrous.

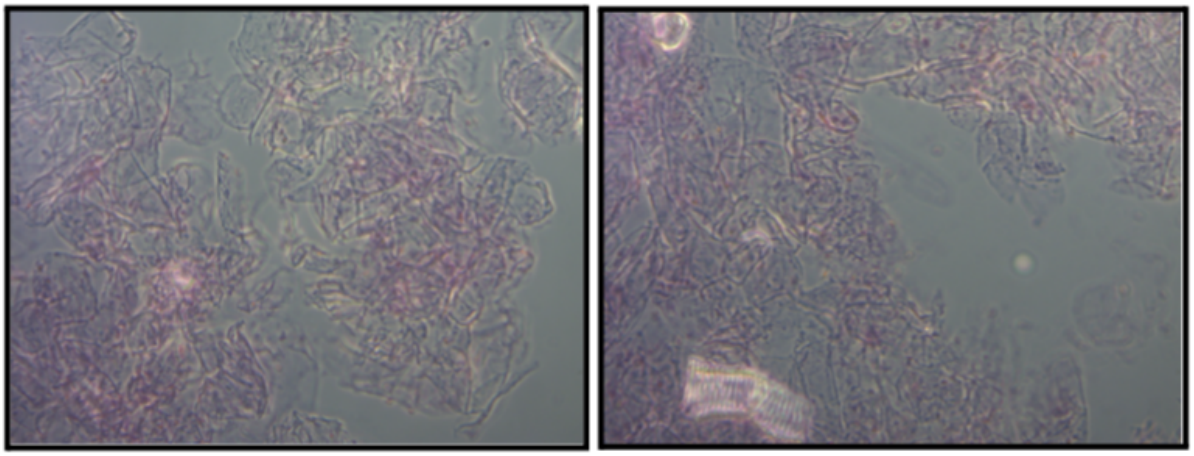

Figure 44: Histology stained images of blended carrots. Lots of cell damage and ECM floating around with cell debris.

4.13 Final Design

The final design is a two compartment device. The first compartment takes in human lipoaspirate through a port that attaches to the liposuction device canister to blend the tissue and allow it to decant. After decantation, the ECM and water content is transferred to the second compartment through tubing using a vacuum pump where it will be filtered through two layers of mesh. The first mesh filter, size 20 US mesh, acted as a pre-filter that would catch any large remaining waste. The second mesh filter, size 200 US mesh, would catch the ECM, while the oil, blood, tumescent liquid, and other contaminants filter through to the waste container. The ECM can be collected with a syringe through a luer lock system. The blender container holds 32 oz of liquid and is made of plastic and the filter/waste container also holds 32 oz and was 3D printed using resin, however, if manufactured, it would also be made of plastic. The ideal tubing used would be an outer diameter of ⅝ in and inner diameter of ⅜, which is the standard tubing used in OR. The blade in the blender would be a 3 piece blender blade as it is effective at breaking down material. The connectors used had an outer diameter of ⅝ inches on both ends.

4.13.1 Process flow diagram

Figure 45 below depicts what the final device looks like with dimensions and setup. The steps of the procedure is to:

1. Connect liposuction tube to lipoaspirate in port
2. Allow lipoaspirate to be suctioned into the blender container.
3. Let the lipoaspirate blend for 60 seconds
4. Open the vent and turn on vacuum pump
5. Allow the blended lipoaspirate to vacuum through funnel filters and waste container
6. Let the vacuum run for 5 mins to let all aqueous content to be pulled through mesh
7. Connect syringe to luer lock and suction out ECM

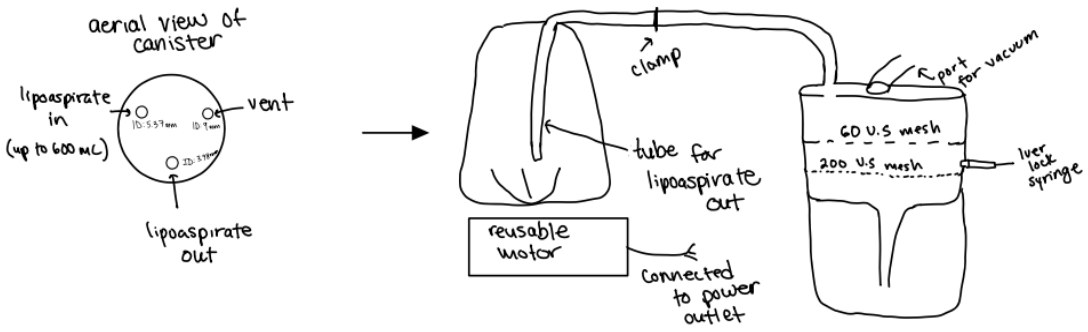

Figure 45: Diagram of final design with all components

4.13.2 CAD designs

The second compartment of the final prototype was designed in SolidWorks, as shown in **Figures 46-50,** and 3D printed with resin, as shown in **Figure 51**.

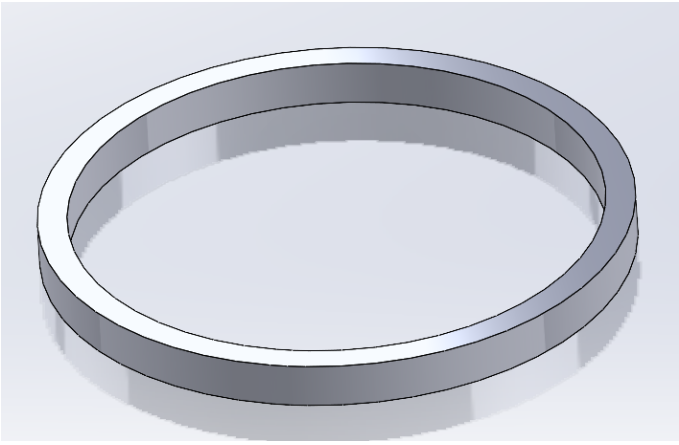

Figure 46: The ring to secure mesh in place, this component will be glued on top of both the 20 and 200 mesh to keep them in place

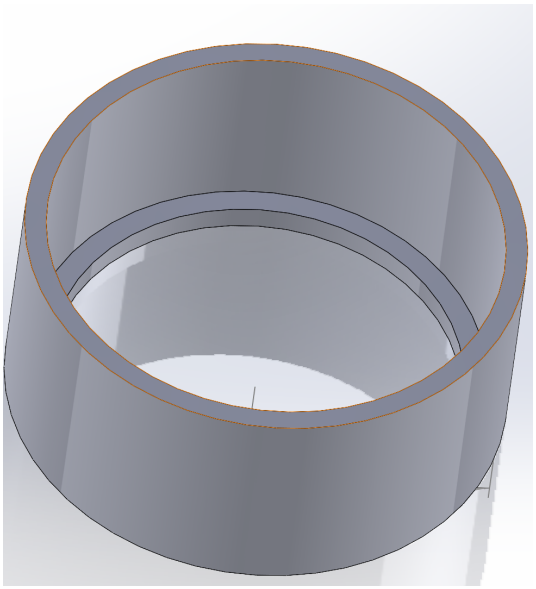

Figure 47: Top compartment to hold 20 mesh. The mesh will rest on the ridge and the water and ECM will fall through into the bottom compartment.

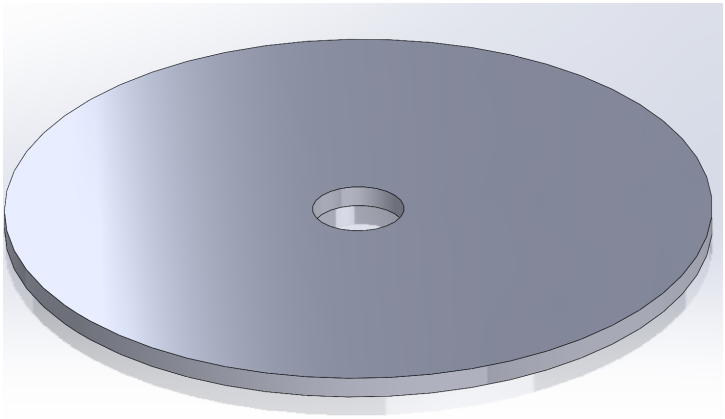

Figure 48: Cover for closed system. This is glued on top of the top compartment. The hole in the middle is for the tube that connects to the lipoaspirate out tube from the first container (blender).

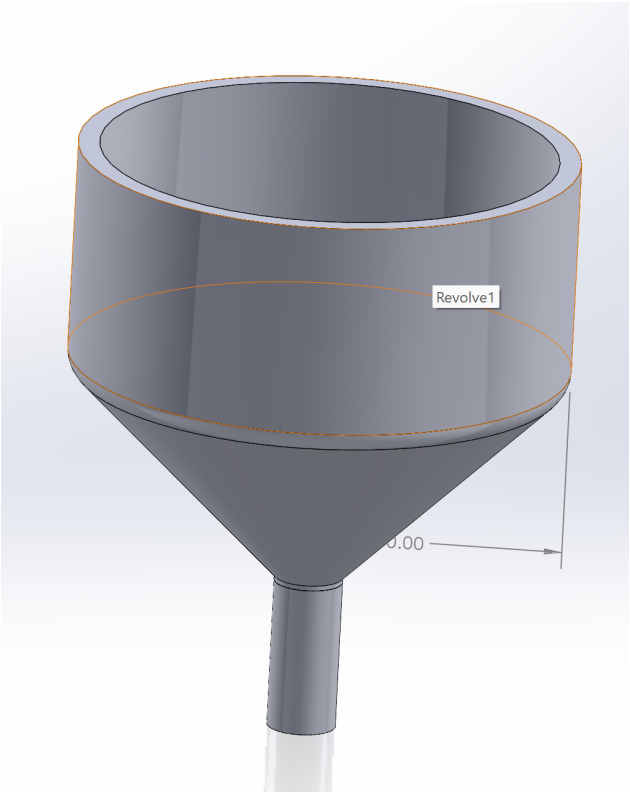

Figure 49: Funnel compartment that will hold 200 mesh and collect ECM on the 200 mesh which is glued to where the funnel starts.

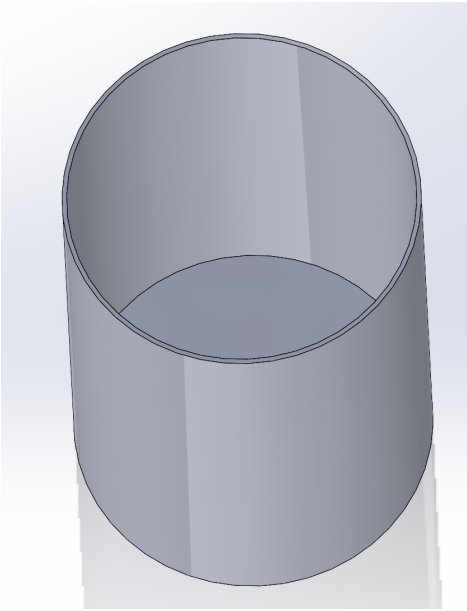

Figure 50: Waste container where the water content will filter into, the funnel will sit on top of this compartment.

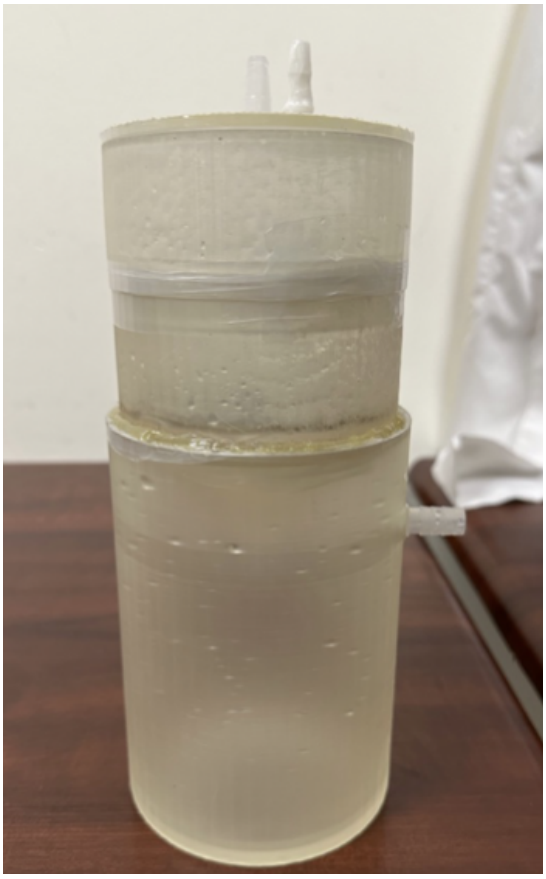

Figure 51: Final design of mesh filtration compartment 3D printed in resin.

Chapter 5: Design Verification

Based on preliminary testing, the blended pig fat tissue shown in **Figure 39** showed a majority of broken cells and cell walls when compared to the control pig fat. However, due to the natural stringy but solid texture of pig fat, it was decided this would not be the best option to use to mimic liposuctioned tissue. Carrots were tested during preliminary testing and showed to have fibers that could be used to mimic the natural fibrous components of human tissue. It was decided that carrots would be used to continue testing different methods to meet the intended device specifications.

5.1 Testing of Mesh Sizes

The test performed involved multiple different mesh filter sizes and filtration methods to see which filters would collect the fibers and allow water and oil to filter through.

5.1.1 Gravity Filtration

The first test was blending boiled carrots with DPBS and an oil component to test gravity filtration. We used 67.5g of both bacon grease and carrots, and 200mL of DPBS to create a mixture that contained the components of lipoaspirate. We then poured 34mL of the blended mixture over 4 different mesh sizes; 40, 60, 120, and 200. We allowed the material to filter through the mesh with gravity to quantify the amount of fibers retained, as shown in **Figure 52**. With gravity filtration, the mixture was run at room temperature which caused the grease to solidify instead of staying liquid which clogged the filters and caused the filters to retain DPBS and oils.

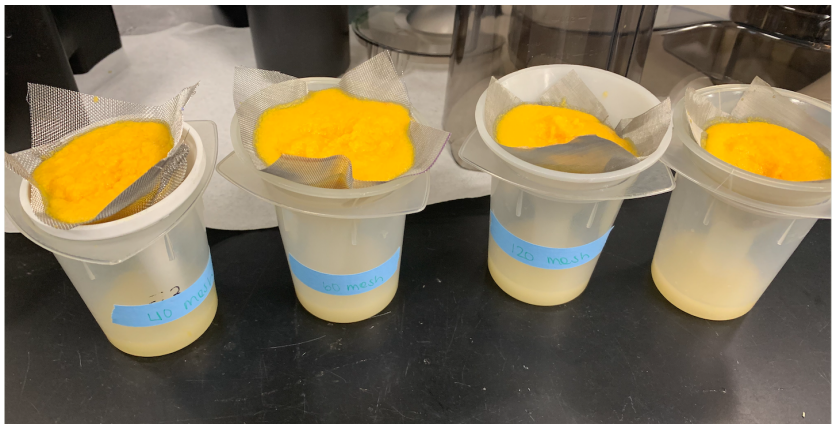

Figure 52: Mixture being poured over the meshes to test gravity filtration and which mesh would be the best filter.

Figure 53: Mesh testing for gravity filtration results from top to button: the 40 mesh, 60 mesh, 120 mesh, and 200 mesh.

The results are shown in **Figure 53** above. The 40 mesh filtered out only some solids but the liquid released was cloudy. The 60 mesh filtered out some solids but the liquid remained cloudy. The 120 mesh filtered out more solids and the liquid was slightly less cloudy. The 200 mesh filtered out the most solids and the liquid was clearest after going through that filter. We concluded that the 200 mesh size was best to keep all of the fibers on top while filtering water and oil into the waste chamber underneath.

5.2 Mimicking Lipoaspirate Material in a Carrot Lipoaspirate Surrogate

Since we were not allowed to test with human lipoaspirate we made our own mixture based off of the common lipoaspirate ratio. As previously mentioned, the correct lipoaspirate ratio is 10% extracellular matrix, 5% oil, and 85% aqueous solution. However, during testing the extracellular matrix ratio used was 5% and the oil ratio used was 10%. To replace the 5% of the extracellular matrix we used the fibers from juiced carrots. To replace the 10% oil we used melted lard initially, however, it would solidify at room temperature, so we switched to canola oil as the substitute for oil from human adipocytes. For the 85% aqueous solution we replaced it with DPBS. Everything was warmed to 98℉ to mimic human body temperature conditions.

We then tested the different mesh sizes again, this time with 20 US and 150 US mesh using a vacuum pump assisted filter. The results for this can be seen in **Figure 54**. We began with an initial 30 g of carrot fiber in the surrogate lipoaspirate mixture and used a vacuum pump to pull DPBS and oil through the filter while fibers stayed on top of the mesh. As seen from table 5, except for the 20 US mesh, all meshes collected similar amounts of fibers as shown by the similar weights. We concluded that using the vacuum trap, all of the mesh sizes except for 20 acted similarly. The 200 mesh was cut a little too small for the flask and collapsed a little during filtration, allowing some of the mixture to bypass the mesh and go under it during filtration. We decided to go with the 200 US mesh and the 20 US mesh as the pre-filter for our final design.

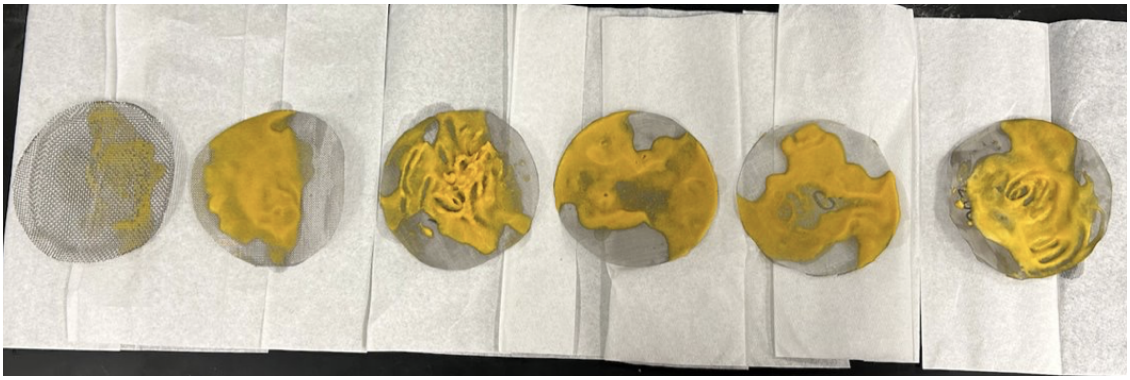

Figure 54: From left to right: 20 Mesh, 40 Mesh, 60 Mesh, 120 Mesh, 150 Mesh, and 200 Mesh with the carrot lipoaspirate surrogate made of lard filtered through results.

Table 5: Data from Testing Lipoaspirate Mixture
This table shows the data from the test with carrot surrogate lipoaspirate mixture. The goal was to measure fiber retained after vacuum filtration and quantify how much liquid was removed to see if both oil and water were filtered out. Smaller mesh sizes acted relatively the same under vacuum filtration.

Mesh Size	Mesh Weight Before (g)	Mesh Weight After (g)	Liquid Weight Removed (g)	Carrot Fiber Weight Removed (g)
20	9.45	10.03	29.01	0.58
40	3.91	8.94	27.43	23.52
60	3.77	10.00	26.11	22.34
120	2.22	8.39	26.58	24.36
150	1.40	7.69	26.77	25.37
200	2.02	7.31	21.49	19.47

5.3 Separation of Fibers from Carrot Lipoaspirate Surrogate

As previously mentioned the goal of our device was to completely isolate the ECM from the human lipoaspirate. Due to constraints of university rules we were not able to test our device with real human lipoaspirate during mesh trials and prototyping. To test the separation of “ECM fibers” from “lipoaspirate,” we used the liposuction surrogate discussed in **Chapter 5.2**.

5.3.1 Protocol for Separation

Throughout testing a common procedure was established to streamline testing and ensure data was comparable. Once the device was completed this procedure was applied to the final prototype.

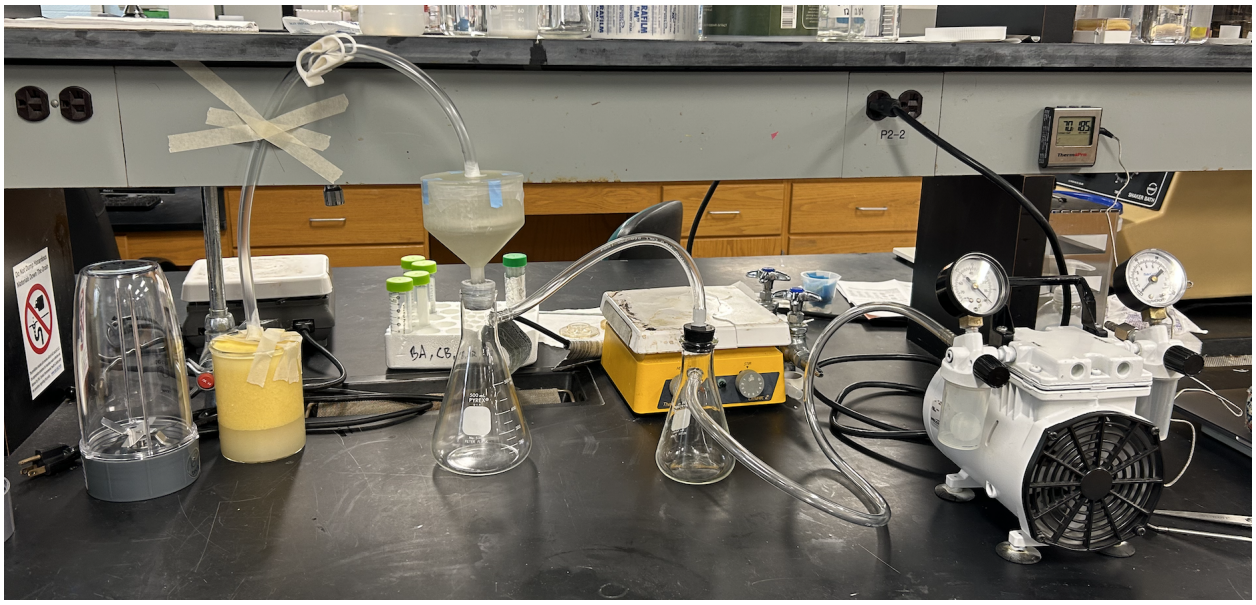

Figure 55: The complete set up of the final experiment that we ran. The beaker on the left has a tube attached inside the blender that was placed to suction the water and fibers after decanting.

1. Setup the process according to **Figure 55**
    a. Add a clamp to the tube connecting the "blender" canister to the filter canister
    b. Setup a beaker with the previously made tube and clamp, with the tube resting in the middle of the beaker
    c. Attach the tubing to the top of the funnel with the mesh filter inside
        i. Before attaching, weigh the funnel with the mesh filter inside and record
    d. With a rubber stopper connect the funnel to an erlenmeyer with a side arm
    e. Through the side arm connect another tube to another erlenmeyer flask with a side arm to create a vacuum trap

f. Connect the side arm to the vacuum with another time

2. Warm the components to 98F
    a. 30 g of juice carrots to mimic 5% ECM
    b. 60 g of canola oil to mimic 10% oil
    c. 500 g of DPBS to mimic 85% aqueous solution
3. Blend together components for lipoaspirate substitute mixture for 60 seconds
4. Move to a large beaker with tubing resting in the middle to decant for 10 minutes (oil, fibers, and water should separate into their respective layers)
5. Once decanted, turn on the vacuum and allow the two bottom layers (DPBS and fibers) to go through the tubing and into the funnel
6. Close the clamp when all the fibers have been vacuumed into the funnel
7. Allow the vacuum to run for 5 minutes to be sure all liquids have been filtered out of the fiber
8. Weigh the filter with solid material on top

5.3.2 Separation Results

We then tested the lipoaspirate surrogate using vacuum filtration to find out how the surrogate would filter through the device. The lipoaspirate ratio used consisted of 5% ECM which we substituted with juiced carrot fibers, 10% oil which was substituted with lard, and 85% of the mixture was DPBS which represented other fluids used during liposuction. In total the mixture was run with vacuum filtration with 35mL of carrot lipoaspirate surrogate for each mesh test, this was made up of 29.75mL DPBS, 3.5mL lard, and 1.75ml of carrot fibers. Every component was warmed to 98℉ and blended for sixty seconds. It was blended for 60 seconds as that was the amount of time that yielded the maximum amount of ECM and further blending did

not increase the ECM yield [36]. To then see if the layers would separate we let the blended mixture decant for ten minutes and the results can be seen in **Figure 56**.

Figure 56: Three layers can be pictured in this image after allowing it to decant for 10 minutes. From bottom to top the layers are DPBS, Fibers and DPBS, and oil.

One issue that arose after the decant period, was that fat would coagulate on the top of the fibers rather than get filtered through with DPBS when pouring it over the mesh. One solution tested was having an additional beaker of warm DPBS to pour on top of the fibers during filtration to keep the lard from stiffening.

In the next round of testing, to avoid large amounts of fat being poured through the mesh and clogging it we suctioned/scooped out the top layer of fat after allowing the blended mixture to decant for ten minutes. During the experiment we used the 120 mesh, and used 590mL of carrot surrogate volume and put it through the filter. After doing so we also poured an extra 150mL of warmed DPBS to hopefully keep the lard in a liquid state and filter it through the mesh. After the experiment we took a small sample of the filtered out fibers and placed it under a microscope to see if the device was successful in fully filtering out oil and DPBS. From the results in **Table 6** we saw a 76.67% yield. This could be due to the fact that during the suctioning

of the top fat layer the tube was placed too far down the container and started sucking up fibers in the fiber layer. Once the sample was under the microscope we were able to see that there was still fat in the fibers despite the warm DPBS rinse so we decided to switch to experimenting using canola oil as it is a liquid at room temperature, which is more similar to the oil that is released from adipocytes in lipoaspirate.

Table 6: Data Obtained After Oil Removal
The results from the second experiment with a 76.67% yield of fibers retained after filtration.

	Weight Fibers added (g)	Weight Oil added (g)	Weight DPBS added (g)	Weight Filter before	Weight Filter after	Weight solid remove
Decant run	30	60	500	55.47	78.47	23

Figure 57: The oil froth that was suctioned out after the decanting period. Oil can be seen as well as fibers and a large amount of DPBS.

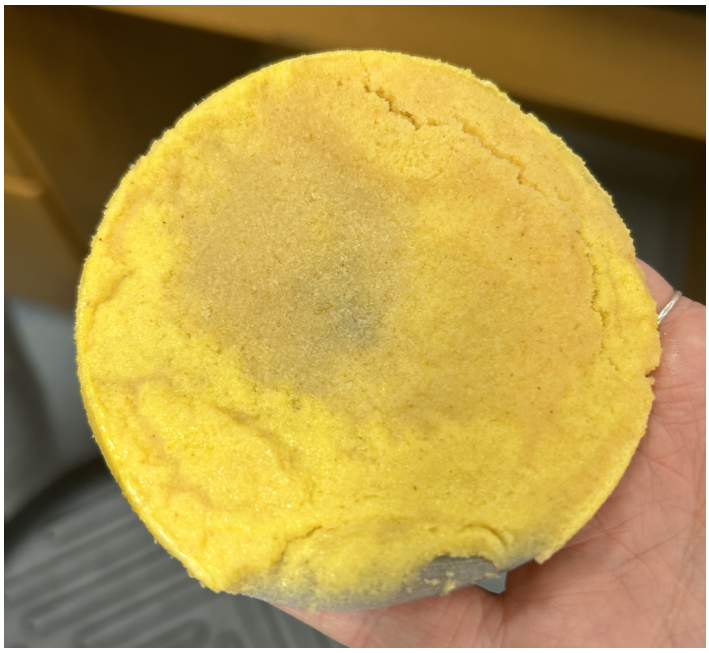

Figure 58: After suctioning out as much oil as possible and running the rest of the mixture through the set up most of the solids stayed on top and were mostly dry of any DPBS. However some oil was still trapped on top as well as some areas were still wet with DPBS.

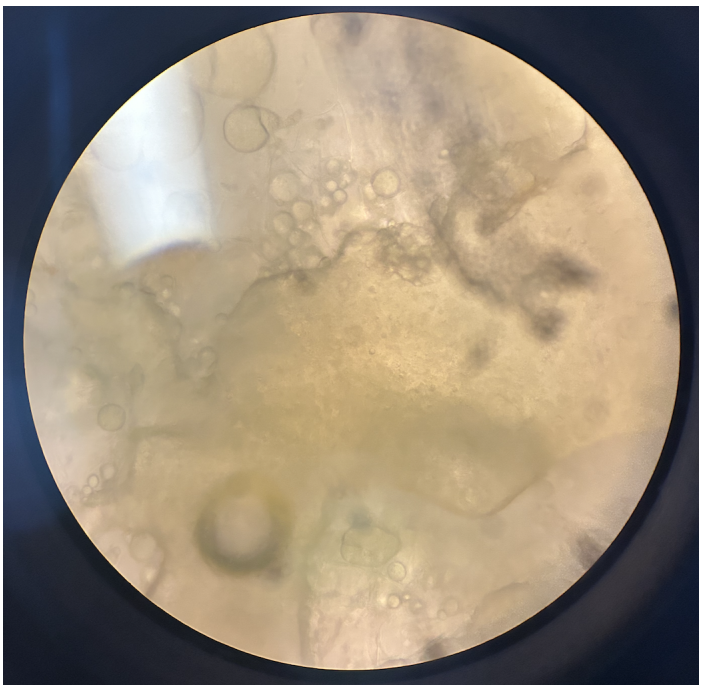

Figure 59: A picture of the solid mixture after filtration under 40X magnification. Here the oil droplets can better be discerned from the air bubbles

The next test was done using canola oil to more adequately mimic the oil that ruptures from adipocytes without the same hardening effect as lard. The 120 mesh was chosen and 590mL of mixture was filtered through the same vacuum filter apparatus. This time the mixture decanted for 20 minutes and the top layer of oil was removed, similar to the last experiment. A representative image of the oil removed can be seen in **Figure 57**. Using canola oil gave slightly better results as seen in **Table 7**. We also allowed the vacuum filtration to go 10 minutes longer than in previous experiments with the hope that it would continue to remove excess DPBS. We saw an 80.7% yield this time compared to the previous experiment's yield of 76.67%. **Figures 58 and 59** show the solids removed from this testing method.

Table 7: Data Obtained from Starting Suction at Bottom of Canister
This table shows the results for the third round of experimentation. In this experiment rather than suctioning out the oil layer at the top, we placed the tubing at the bottom of the decanted mixture and sucked out as much DPBS and fibers as we could. This was to test if this was a better method that got rid of more oil.

Decant run (min)	Weight Fibers added (g)	Weight Oil added (g)	Weight DPBS added (g)	Weight Filter before (g)	Weight Filter after	Weight solid remove
15	30	60.19	500	2.24	79.88	24.21

One of the last experiments we tried was to suction the water and fiber layer from the bottom of the canister rather than suction out the top oil layer. We clamped the tube being placed into the canister so that it would not take up any oil from the mixture. After the experiment the filter weight was found to be 122.35 grams compared to the initial weight of 55.34 grams. This gave a percent yield of 223.4% which suggests that there was a lot of DPBS retained and the

vacuum needed to be run for longer to continue to dry out the fibers. A representative image of the process results can be seen below in **Figure 60**.

Figure 60: After suctioning from the bottom it was possible to remove most of the DPBS and a large majority of fibers leaving only a small amount of DPBS and the frothy oil layer. After filtration the fibers the mesh kept on top looked like this.

This experiment of suctioning the water and fibers from the bottom of the blending canister was performed in triplicate. The results of each trial can be shown below in **Table 8**. Samples from the filtered out waste liquid and retained filtered fibers were taken and observed under a microscope at 10X magnification. As shown by the representative images in **Figure 60**, the waste liquid showed minimal amounts of fibers and a lot of DPBS and oil, while the filtered fibers showed a large amount of fibers. From the samples taken, 3 representative images of the components of each sample were taken and analyzed in ImageJ, seen below in **Figures 61 and 62**, to visually quantify the amounts of water, oil, and fibers; the average results of this visual quantification can be seen in **Table 9**.

Table 8: This table summarizes three trials from our experiment of suctioning the water and fibers from the bottom of the blender.

	Initial Weight Fibers (g)	Fiber Weight Collected (g)	Percent Yield
Trial 1	30	25	84%
Trial 2	30	23	76%
Trial 3	30	24	80%

Table 9: This table represents the average of all the collected areas of fibers, oil, and DPBS for filtered waste and fibers collected.

	Avg. Total Area (µm)	Avg. Area Oil (µm)	Avg. Area Fibers (µm)	Avg. Area DPBS (µm)
Filtered Waste	4,915,200	889,096 18%	629,855 12%	3,484,560 70%
Fibers Collected	4,915,200	480,662 9%	4,644,065 94%	355,145 7%

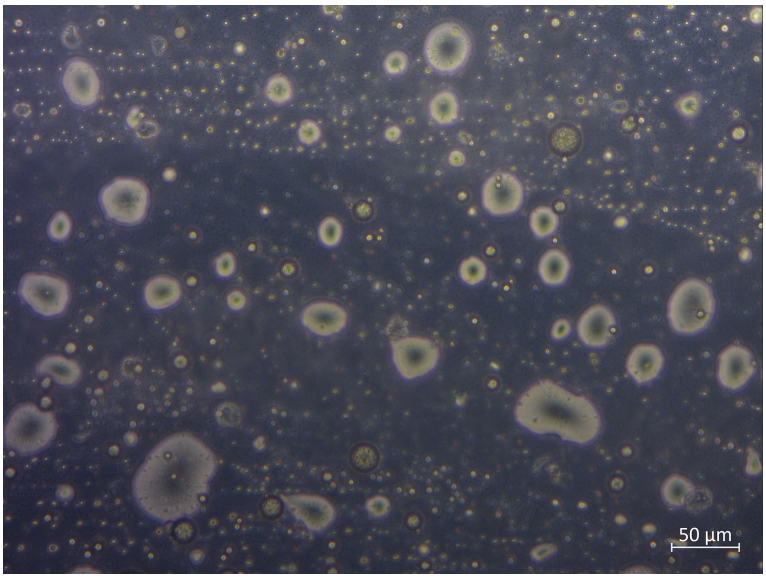

Figure 61: ImageJ of Liquid Waste under 10X. Scale bars are shown so that we were able to visually quantify the percentage of ECM to oil and DPBS. This was to verify if our device worked to filter and retain mostly fibers.

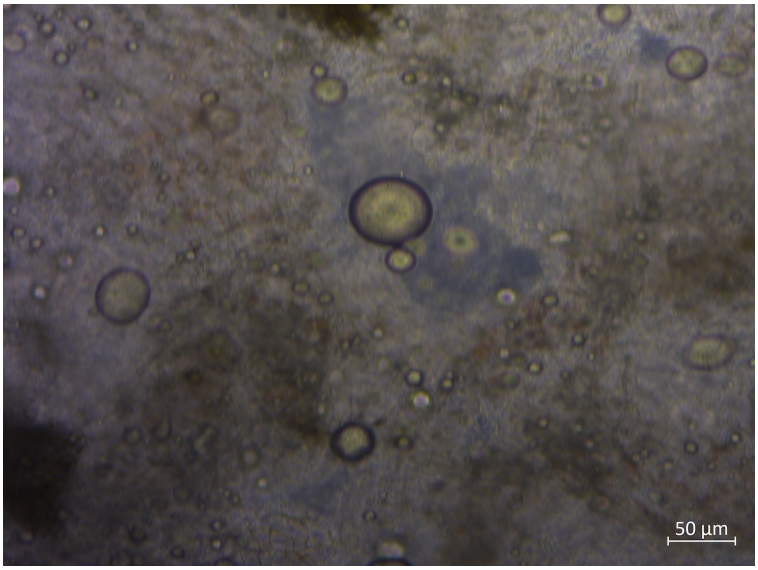

Figure 62: ImageJ of Solid retentate collected under 10x. Scale bars are shown so that we were able to visually quantify the percentage of ECM to oil and DPBS. This was to verify if our device worked to filter out greater than 90% of the contaminants.

Chapter 6: Final Design Validation

After testing different designs and techniques, a final device was built and tested. To ensure this device worked for our intended user, the device must be tested in an operating room with the intended client. Proper histology staining of the human lipoaspirate throughout each stage of the device would also need to be performed to ensure the device works for the surgeons' desired needs and performs how it is expected to. However, due to limitations, histology could not be performed on the trial run with human lipoaspirate.

6.1 Human Lipoaspirate Testing

The final device was used to perform a single run with human lipoaspirate at University of Massachusetts Medical School by Dr. Giorgio Giatsidis' team of medical professionals, Dr. Hiroshi Fukimaki and Nicholas Jarostchuk. This allowed us to see how the intended user used the device, how the device works with human tissue, if the device decellularizes tissue and allows it to be reinserted into the body, and user feedback from the client.

6.1.1 Human Lipoaspirate Procedure

The set up and protocol for this testing was similar to how the carrot lipoaspirate was tested. The lipoaspirate tissue was suctioned into the device's blender through a vacuum port attachment on the blender container. Once the lipoaspirate was in, the vacuum was turned off and the tissue was blended for 60 seconds. Then, the lipoaspirate was left to decant for 25 minutes and the vacuum was turned on to suction the blended tissue into a second container, the filtration compartment. The filtration compartment contained the pre filter and filter levels with a waste container at the bottom to collect the tumescent fluid filtered through. The ECM was then collected via a syringe through the luer lock on the container.

6.1.2 Human Lipoaspirate Results

The procedure written above was conducted at University of Massachusetts Medical School by a team of medical professionals. It was expected after the human lipoaspirate was blended and left sitting for a few minutes, the lipoaspirate would decant however, this was not the case for the tested tissue. As seen in **Figure 63** below, the lipoaspirate did not decant and thus the procedure had to change slightly.

Figure 63: Post Blending and Decanting Stage of Human Lipoaspirate Testing

Since the blended tissue did not decant into the layers we had originally observed with the carrots, the blended lipoaspirate had to be suctioned into the filter component instead of collecting only the fibers and tumescent fluid and blood as planned. Once the collected blended lipoaspirate tissue was filtered, the filtering compartment was opened to view what was collected in both the pre-filter and main filter for presumptive ECM collected levels. **Figure 64** below shows the larger pieces of tissue that were not fully blended meaning the pre-filter layer worked to separate these pieces from the ECM collection. **Figure 65** shows the collection of the presumed ECM and the total amount collected from this mesh layer.

Figure 64: Pre-Filter Collection Level

Figure 65: Presumed ECM removal and Total Collection Weight

There was a total volume of 572g of lipoaspirate as shown in **Table 10** however, only 322g was filtered through the device as the tubing did not fully reach the bottom of the canister to reach all of the blended lipoaspirate, as we assumed the lipoaspirate would decant similarly to the carrot lipoaspirate surrogate. The weight of the material that collected on top of the second mesh was 16 grams. We therefore multiplied the total filtered amount by 10%, about the percentage the ECM makes up in lipoaspirate as said in Zhang et al., and calculated a total yield of 49.69% as shown in the calculation below:

$$\frac{16g}{(322g*0.10)} \times 100\% = 49.69\%$$

Table 10: Human Lipoaspirate Tissue amount used and calculation

Lipoaspirate Tissue	**Amount (g)**
Total used for blending	572
Total filtered amount	322
Collected ECM	16

6.1.3 Human Lipoaspirate Histology

Due to limitations with primary human tissue, only one test could be performed and histology at WPI could not be performed to evaluate product composition. We hoped to analyze the presumed ECM obtained with histology methods such as Masson's trichrome to differentiate between cells and collagen and other connective tissue fibers. If there were small amounts of oil left, less than 15%, the prototype would have accomplished the goal of removing contaminants. We also would have analyzed the volume of waste to determine if there was proper filtration between the presumed ECM, blood and fat. To do histology staining, we would take the extracted presumed ECM from the device and take cryosections of the material and use the previous stains to verify our results. We would expect to see that a majority of the sample turns

blue when using Masson's trichrome, since the stain turns collagen blue. Since our objective was to maintain the integrity of the ECM components and isolate them, we would hope that about 50% of the sample shows blue. We also would have stained with Hoechst 33345, we would use optical microscopy to confirm the lack of visible nuclei. It would be okay if there were some nuclei remaining as we do not need complete decellularization but there would hopefully only be 10% or less of visible nuclei to prove that the mechanical methods can decellularize large amounts of tissue.

If we had the time we also could have used the absorbance based DNA quantification using a kit as talked about in **Chapter 4.10**. Since our goal was 80% or more of cellular death, we would hope that the data shows there is less than 50 ng per mg of tissue which would mean that the material can be deemed decellularized. We would also do the direct transfer sterility test. We would take a small volume of the isolated presumed ECM and incubate it in a growth medium to see if there are turbid areas. If there were no turbid areas our device would have completely kept the lipoaspirate sterile and could be called a completely closed system.

6.2 Design Objective Evaluation

The design objectives from **Chapter 3.1.1** will be referred back to to evaluate the design objectives were met. For a brief summary, the objectives discussed previously, were to test ways to break down fat obtained by liposuction in a biocompatible way, to test ways to remove water and oil to retrieve the ECM, have a device easy to use in the OR with a process that takes less than an hour, and lastly, to create a closed system device for sterile us in the OR. Through testing with Dr. Giatsidis's team, we were able to test our device and evaluate if it met the objectives previously stated. The device seemed to blend and break down lipoaspirate material as the product that was blended was able to be filtered through our device. Though, since histology was

not able to be performed, the complete breakdown of fat obtained by liposuction could not be quantified. After the filtration step, the contents of each layer of mesh were looked at visually, the pre-filter did show large chunks were removed and the main filter did retain about 50% of what is believed to be ECM, though the contents of what was retained could not be validated because histology could not be performed. The device was able to work in less than an hour, it took 60 seconds to blend the lipoaspirate material and was left to decant for about 25 minutes, but the solution was not decanting so in the future that step would be skipped speeding up the process. Based on feedback from Dr. Hiroshi Fujimaki and medical student Nicholas Jarostchuk, the device was easy to use, as they were the ones who performed the testing with human lipoaspirate. The device was created to be used as a closed system, however, after testing the device was taken apart to observe what was removed from each filter component.

Chapter 7: Discussion

The preliminary testing performed with a carrot lipoaspirate surrogate showed great results even when visually quantified, as it showed high levels of ECM retentate with low levels of oil and DPBS. However, testing with human lipoaspirate could only be performed once and the results were not able to be histologically quantified so the team was not able to accurately assess the final product obtained by the device. However, several of the previously mentioned requirements and specifications from **Chapter 4.4** were met. The device maintained a closed process that was easy to use in under one hour. The decellularization process of the device did not use chemicals. After filtration, the volume of lipoaspirate was reduced, indicating that the final filtrate volume was significantly reduced and presumptive ECM was retained. Visually, the filters worked as they were predicted to, as the prefilter removed large chunks of lipoaspirate and the filter retained what is presumed to be ECM. Since histology could not be performed, the

decellularization of adipose tissue, composition of the final product, and preservation of integrity of ECM could not be verified. As previously mentioned, the histology that would have been performed would be a Masson's trichrome and a hoechst 33345 stain. If the device worked as intended, the histology would show at least 50% of the sample would be stained blue with Masson's trichrome to indicate ECM content in the final product. Based on theoretical calculations, it was estimated that presumptive ECM yield was about 50% ECM, based on values from prior literature, so histology of the retentate gained from testing would show 50% of the sample was collagen to prove that the device would isolate ECM, just not efficiently enough. The hoechst stain would show us if we properly decellularized the lipoaspirate, as nuclei get stained. If we mechanically killed all the cells, there should be less than 10% of nuclei stained in the sample.

7.1 Standards

To ensure the device will be able to go under the FDA approval process in the future, the team needed to ensure all safety and effectiveness of the device met the current standards for medical devices.

The first standard our device needed to follow was ISO/TS 21560:2020, general requirements of tissue-engineered medical products. The requirements for material, manufacturing, quality control, and unintentional biological effects caused by the scaffold created by this device can be found in this ISO standard [56]. This allowed the team to better understand what requirements are needed in order for the device to be manufactured and to ensure no unintentional effects occur once the device is used in the operating room.

The second standard our device needed to follow was ISO 13022:2012, medical products containing viable human cells - application of risk management and requirements for processing

practices [57]. This ISO standard covers the necessary guidelines for manufacturing handling and managing risks correlated to viable cellular components obtained by human origin.

The third standard the device needed to follow was ASTM F3354-19, standard guide for evaluating extracellular matrix decellularization processes [58]. This standard displays the criteria needed for decellularization and ECM integrity acceptance for selected attributes.

The fourth standard the device needed to follow was ISO 10993-1:2018, biological evaluation of medical devices [59]. This standard specifies the general principles and evaluation of medical devices to ensure the safety of patient's once the device is on the market.

The fifth standard the device needed to follow was ISO 80369-7:2021, small-bore connectors for liquids and gases in healthcare applications [60]. This standard specifies the design and functional performance specifications of small-bore connectors for intravascular or hypodermic connections. This standard ensures the luer lock used prevents any leaks and is able to be easily used to move the collected ECM to a syringe for reinjection.

The last standard the device needed to follow was ISO 13485:2016, medical devices - quality management systems [61]. The standard explains the manufacturing and management specifications needed to ensure the medical device meets customer and applicable regulatory requirements.

7.2 Ethical Impacts of Point of Care Device for Tissue Reconstruction

7.2.1 Economic Impact

When considering the patient, the price of a double breast reconstructive surgery with implants is about $10,000. This type of surgery is quite expensive and inaccessible for many patients. With the device, we hoped to lessen the cost by combining liposuction surgery with

breast reconstruction surgery. The device will be similar to the price of fat grafting and the Revolve system which are around $5,000 and $9,300, with the hope to keep the cost in between this frame.

However, when considering what the device would do for the economy, the development of this product could cause an increase in the demand for tissue engineering products and tissue reconstruction for cosmetics. This technology would advance the field, make tissue reconstruction more available to patients, and create new markets and opportunities for companies in the medical and biomedical fields. With new opportunities in the medical and biomedical industry, new jobs will be created to develop, manufacture, and control the quality of the device. If the device continues to be manufactured and used, it can reduce the cost of healthcare procedures and impact the patients costs and the healthcare systems costs.

7.2.2 Environmental Impact

The production and disposal of a device for adipose tissue-derived scaffolds could have environmental impacts, depending on the manufacturing processes and materials used. These impacts would need to be carefully considered and mitigated to ensure that the benefits of the technology outweigh the environmental costs. When comparing the point-of-care scaffold to Revolve, the device has reusable aspects and can be made of recycled materials. The blender motor is reusable, which keeps the consumables of the device low. Since the consumable blending canister and filter will most likely be made of plastic, another way to keep the environmental impact low is to use sustainable and recycled materials. The consumable blending canister could be made of recycled plastics or bioplastics or sterilized and recycled by the manufacturing company. The blender blades could also be made of recycled metals and could be sterilized and recycled again by the manufacturing company.

Our device currently uses a reusable motor while the rest is disposable. This allows the device to be sterile for every user however, this can impact manufacturing as the disposable parts will need to be constantly made for doctors to have constant supply of them. While this can become very costly, the majority of the materials used for our device is hard resin and these parts can be made from a cheaper alternative. Additional to this, when manufactured, we would want the device to be built at a larger scale that is able to handle the large quantity of lipoaspirate that is removed during liposuction. This would also play a role in the cost of manufacturing our device.

7.2.3 Societal Impact

Some societal impacts in the scope of the medical field that can be caused by the creation of this device are developments in tissue regeneration and advancements in medical research. Adipose tissue scaffolds, in the scope of this device, will be used for soft tissue reiteration and reconstruction. It will provide a natural environment for cells to grow and develop into, leading to more effective and efficient tissue reconstruction. This development will advance medical research and lead to new insights into tissue development and disease progression for patient specific models.

As for the societal impacts in the scope of society and the average person, some examples could be easier access to reconstructive surgery and an increased demand for safer cosmetic procedures such as use of the tissue scaffold in other areas of the body. Our device would be affordable and easy to use for reconstructive surgery not only in the breast tissue but in other areas of the body. The device could become widely available which could cause an increased demand in cosmetic procedures for reconstruction or enhancement to the breast and other areas of the body.

7.2.4 Ethical Impact

Adipose tissue-derived scaffolds may raise ethical concerns if the adipose tissue used to create the scaffolds comes from living donors. However, in this case, there would be no ethical concerns as the donor is the patient receiving the treatment.

Another large ethical impact that would happen is animal testing. Over the years animal testing has become a controversial subject. Before it can be FDA approved there would need to be trials to validate that the reinjected scaffold does actually promote cell proliferation. This could be an unknown amount of trials and many could deem it unethical to use animals to test a procedure mostly used for cosmetics. When it comes to ethics about doing mostly cosmetic procedures on the human body there is no major known controversy.

Like any other medical treatment there could be side effects. Due to the fact that we have not done any animal testing with this technology, we have not found potential adverse side effects to this treatment. Since it is a patient's own tissue there should be no concern about biological rejection after the treatment. However it is always possible that the scaffold does not actively promote cell proliferation and rebuild the soft tissue like it is supposed to. The same risks are associated with other methods such as fat grafting, where the reinjected cells have a possibility to die off and create uneven regrowth. The procedure is safe as the decellularization happens completely outside of the patient and involves no toxic chemicals since our device uses just mechanical disruption.

Chapter 8: Recommendations

The limitation of time constraints and resources available took a toll on the progress of this project in device development prototyping and result validation. If there had been more time for testing and histology staining with human lipoaspirate, modifications could have been made

to enhance the ECM retention to get as close to the specification of retaining 90% of ECM. Unfortunately, obtaining human lipoaspirate and testing with professionals who are allowed to handle human tissue was challenging and could only happen once towards the end of the year. Since the tissue was human, the team could not do histology staining at WPI. Since histology could not be performed, the components of the filtered product could not be quantified, and the verification of an injectable scaffold could also not be quantified. Based on this limitation, it would be beneficial for future teams to perform histology testing to quantify every step of the process. The lipoaspirate tissue should be tested with histology after liposuction but before blending, after blending but before filtration, after the prefilter, after the filter, and after the waste liquid. Visual and histological observations should show the amount of decellularization of adipose tissue, the composition of the final product, and the preservation of ECM integrity could be proven.

Looking back at the functional and performance specifications previously mentioned in **Chapter 4.4**, the device can be looked at analytically to see which specifications were met and where the device was limited and could not meet the specifications. For example, the prototype device could not handle a maximum of 5 liters; it could only hold about 0.7 liters of lipoaspirate, as the blending canister used for prototyping had a maximum fill of 32oz. In future iterations, the blending canister should be designed to hold 5 liters of lipoaspirate material that can be blended and filtered through the device in under an hour. In addition to holding a larger volume, the prototype was designed with an internal tube to collect the fibers and tumescent fluid after decantation. Since decantation did not occur in the blended lipoaspirate, this tubing is no longer needed, and the port could be put closer to the bottom to collect all the blended lipoaspirate so nothing is left behind and the total amount of blended lipoaspirate can be pulled through to the

filtering step. The device was created to be a closed system, but the methods used to confirm sterility were not performed. The device was not sterile before testing as it had been used for the carrot lipoaspirate surrogate testing, so it was assumed not to be sterile after testing. However, when the device is completed, it would be a sterile closed process as the blending and filtration canister would be single-use and disposable. In addition, the final device should be a one-compartment system for ease of use in the OR. An example of a one-compartment system can be seen in **Figure 29**. If the device can blend and filter in the same compartment, then the motor blender would be reused, and the blender and filter compartment could be completely disposable or sterilized and reused.

Not all methods of separation previously mentioned were tested. In the future, the team would like to see how tangential flow filtration can filter the components of human lipoaspirate, so a design iteration with tangential flow filtration would be an exciting development for the future as the filtration could be continuous and used at the same time as the liposuction is being performed. With our initial testing and prototype device, we hope to give another team significant intellectual property to advance their device iteration.

8.1 Future Recommended Testing

The team compiled a list of recommended testing protocols for the future to be done with human lipoaspirate to further verify and validate the proposed prototype. They are described below.

8.1.1 H&E Staining

Hematoxylin and Eosin (H&E) staining is used to stain cell nuclei in tissue. Visual inspection of the structure of adipocytes will give insight to how much cell lysis and damage occurred with the processing technique of the device, i.e. blending.

There needs to be 5 test groups: unprocessed lipoaspirate, blended lipoaspirate, blended tissue collected on the pre-filter, blended tissue collected on the mesh filter, and blended tissue in the waste container. The team hypothesizes that there would be a higher degree of cell lysis in the blended lipoaspirate than the unprocessed lipoaspirate, visualized through the presence of more cell nuclei. The team also hypothesizes that there will be less cell nuclei seen on the blended tissue collected on the filter because the cell nuclei would be removed through mesh and vacuum filtration into the waste container.

Protocol on Frozen Sections from WPI Histology:

1. Fix frozen section slide in FS fixative (10-30 seconds each)
2. Rinse in distilled water till clear (5-10 dips)
3. Stain in Hematoxylin (1 minute)
4. Rinse in distilled water (5-10 dips)
5. Differentiate in acid rinse (2 dips)
6. Rinse in distilled water (4-6 dips)
7. Dip in alkaline rinse (8-10 dips)
8. Rinse in distilled water (5-10 dips)
9. Counterstain with Eosin (4-5 dips)
10. Two Rinses in 95% ethanol (5-7 dips each)
11. Three washes in 100% ethanol (5-10 dips)

12. Three washes in Xylene (5-10 dips / 1 minute each)
13. Coverslip the slides using synthetic mounting medium
14. Observe the stained tissue sections under a brightfield microscope and analyze the tissue structures and cell morphology using suitable image analysis software. Hematoxylin stains nuclei blue to purple, while Eosin stains cytoplasm and other tissue components pink to red.

8.1.2 Bradford Assay

The Bradfordd assay will be useful in determining protein concentration in the ECM scaffold. The principle of this assay is that the binding of protein molecules to Coomassie dye under acidic conditions results in a color change from brown to blue. Knowing the protein content left in the ECM is helpful in determining whether the ECM is viable for reinjection.

There needs to be 5 groups. These groups are the control (unprocessed lipoaspirate), blended lipoaspirate, blended tissue collected on the pre-filter, blended tissue collected on the mesh filter, and blended tissue in the waste container. The team hypothesizes that the highest concentration of protein would be from the final product in the mesh filter as the final product we are hoping for is an ECM scaffold.

<u>Standard Protocol from BME Biomaterials Lab [62]:</u>

1. Dilute the protein sample in a suitable buffer, such as phosphate-buffered saline (PBS), to a known concentration. A good starting concentration is 1 mg/mL.
2. Prepare the Bradford reagent by mixing the Coomassie Brilliant Blue dye with a suitable acidic solution, such as phosphoric acid or sulfuric acid, in a ratio of 1:5 or 1:6, respectively.

3. Add an appropriate volume of the Bradford reagent to the protein sample, usually in a ratio of 1:5 or 1:6.
4. Mix the solution thoroughly and incubate it at room temperature for 5-10 minutes to allow the dye to bind to the protein.
5. Measure the absorbance of the solution at a wavelength of 595 nm using a spectrophotometer. The Bradford dye changes its color from brown to blue when it binds to the protein, and the intensity of the blue color is proportional to the amount of protein in the sample.
6. Prepare a standard curve by measuring the absorbance of known concentrations of a protein standard, such as BSA, using the same protocol as for the protein sample. Plot the absorbance values against the known concentrations of the protein standard to generate a standard curve.
7. Use the standard curve to calculate the protein concentration of the sample based on its absorbance value.

8.1.3 Hoechst Staining

Hoechst stains for any free DNA, the nuclei, in the scaffold. Though DNA in the final scaffold is from the same patient, free DNA can still have inflammatory effects on the body, so it will be useful in quantifying the amount of DNA in the final scaffold.

There needs to be 5 groups. These groups are the control (unprocessed lipoaspirate), blended lipoaspirate, blended tissue collected on the pre-filter, blended tissue collected on the mesh filter, and blended tissue in the waste container. The team hypothesizes there will be the least amount of nuclei in the sample collected on the pre-filter as most of the cell waste would be filtered out into the waste container.

Standard Protocol:

1. Prepare the sample of cells by washing them with phosphate-buffered saline (PBS) and fixing them with a suitable fixative, such as 4% paraformaldehyde or methanol.
2. Prepare the Hoechst staining solution by diluting the Hoechst dye, such as Hoechst 33258 or Hoechst 33342, in PBS or Hank's balanced salt solution (HBSS) [63].
3. Add an appropriate volume of the Hoechst staining solution to the fixed cells, enough to cover the cells completely.
4. Incubate the cells with the Hoechst staining solution at room temperature or at 37°C for 10-30 minutes [64].
5. After the staining is complete, wash the cells with PBS or HBSS to remove any excess dye.
6. Mount the cells on a glass slide using a mounting medium, such as glycerol or mounting media containing DAPI, and cover with a coverslip.
7. Observe the stained cells under a fluorescence microscope using a UV filter to visualize the Hoechst-stained nuclei. Analyze the nuclei to measure various parameters, such as nuclear size and shape.

8.1.4 Cell Migration Study

This in-vitro study would help determine how quickly cells repopulate the scaffold. This can then be compared to the current gold standard of fat grafting using Coleman fat. This will help determine how effective the device is in promoting cell proliferation and migration in comparison to current methods. There would be three test groups, one using ECM gathered from centrifugation, one using fat gathered from fat grafting/Coleman fat, and the ECM from our

scaffold. The team hypothesizes that the scaffold from our device would promote the most cell migration.

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