ENGINEERED TUMOR MODELS

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Submitted by:

Bishoy Abdelmalek Cecilia Berniac Brian Brooks Michael Reilly

Advisor: Raymond Page, BME Department

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Authorship

Section	Primary Author(s)
Abstract	Cecilia
Chapter 1	Michael
Chapter 2	Brian
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Abstract

1 in 8 women in the U.S will develop invasive breast cancer [1]. In 2021 alone, it is estimated that over 280,000 new cases of invasive breast cancer are expected [1]. One of the forms of metastatic breast cancer that accounts for about 10-15% of all breast cancers is triple-negative metastatic breast cancer (mTNBC) [32]. mTNBC is considered to be the most aggressive form of breast cancer due to its quick spread, the difficulty of treatment, and the likelihood of recurrence [32]. There has been much research dedicated to studying this disease and many attempts have been made in harvesting and culturing cells from biopsies of solid tumors to build cell lines that can predict the response of cancer cells to therapies. Despite the billions of dollars invested yearly in this cause, a method of accurately mimicking the actual tumor microenvironment does not exist.

Our project is focused on designing and testing an in vitro engineered tissue strategy that would enable the study of the response to therapy that is more representative of the tumor microenvironment in vivo. Applications are not limited to but focused on mTNCB, as we hope that our design is applicable across multiple cancers and contributes to personalized medicine efforts. Our design must include improved cell viability maintenance, allow for cell-cell interaction, and be representative of the tumor microenvironment (TME).

1.0 Introduction

The goal of our project is to recreate the tumor cell microenvironment in vitro to test and analyze the isolated environment rather than both the tumor cells and the surrounding tissue to give us a better idea of what kind of response is expected. This will allow for the creation of personalized treatments as well as guided cancer therapeutics for each patient because the models should allow for the study of the progression of the tumor in vitro and will give researchers a better idea of how to approach each patient's case as time passes.

Our project focuses on the testing of breast cancer, one of the most common cancers found in women in the US, which normally begins with the overgrowth of epithelial cells lining ducts and/or lobules. Breast cancer most often occurs due to the presence of estrogen or progesterone receptors or unregulated HER2 protein production, but about 10-20% of cases are triple-negative, meaning that the cancer was not fueled by any of the classic causes listed above making it especially difficult to target. In the estrogen and progesterone cases, the breast cancer cells with the corresponding receptors (PR-positive, ER-positive, or hormone-positive) will be stimulated to grow and divide causing the tumors to form over time. In the cases with unregulated HER2 protein production, the HER2 gene is mutated and becomes unregulated in production so the growth factor causes continuous uncontrolled tumor growth.

Breast cancer can appear in multiple ways, some more common than others. It may appear as a breast lump, nipple abnormality, pain in the breast, or even skin abnormalities on the breast itself appears more common than the cases where there are axillary lumps that appear, breast ulceration where the tumor breaks through the skin, back pain, breast infection or inflammation, and even breast swelling.

The significance of this project is that it will create a solution to the inability to properly test therapies on different patients' tumor cells through the isolation of their tumor microenvironment in 3D. Currently, there are none that allow this specific type of testing, after almost $600 million has been spent to study breast cancer alone. This project will find an efficient and cheap way to extract, culture, and study tumor cells and their reactions to different therapies.

2.0 Literature Review

2.1 Background

Extensive research was done in preparation for the designing process in order to understand the current efforts toward creating an accurate tissue-engineered cancer tumor model representative of the patient's microenvironments. In addition, research on breast cancer, specifically, triple-negative metastatic breast cancer was done to understand the cell line we will be working with.

2.1.1 Physiology

The breast, medically known as the mammary gland, is possessed by both men and women. Females have more breast tissue than males, and the breasts contain the lobules which produce the milk, ducts that carry the milk to the nipple, and fatty tissue which protects these parts. These lobules can count anywhere from 15-20 lobes depending on the female, and the ducts are where breast cancer forms [3]. There are invasive and non-invasive forms. For instance, there is Invasive Duct Carcinoma (IDC) which starts in the ducts and accounts for approximately 80% of breast cancer cases. There is also Invasive Lobular Carcinoma (ILC) that starts in the lobes and accounts for approximately 10% of the cases. See **Figure 1** to see the different types of breast cancer. The non-invasive forms include ductal Carcinoma is the formation of abnormal cells within the ducts which could develop into breast cancer on their own. And lobular Carcinoma is the formation of abnormal cells within the lobules which increases the risk of the development of breast cancer yet does not pose as much a threat of developing into cancerous cells themselves. A major threat with breast cancer as with all cancer types is metastasis, where parts of the tumor break off and are transported by blood vessels to other locations in the body. The high metastatic tendencies of triple-negative breast cancer are part of the high risks which are posed with this disease.

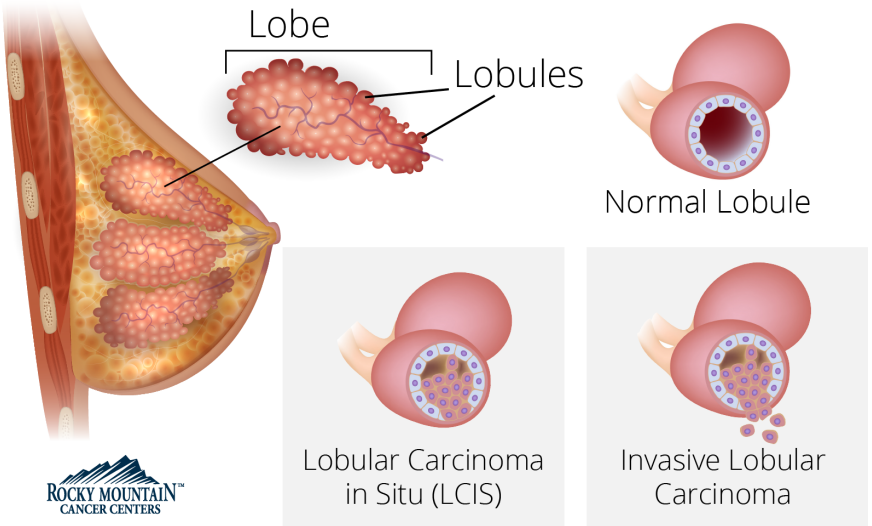

Figure 1: Breast Cancer Types [4]

2.1.2 Disease State

Triple-Negative breast cancer is the most difficult to treat as it is highly metastatic breast cancer that has several significant factors which can be attributed to the cause of most cases. The most common factors are hormones, estrogen, and progesterone. These two hormones have strong influences on cell growth, especially in the breasts of females. Cells with receptors for these hormones may be receiving signals from these hormones which relay to them to continue to grow and reproduce beyond which they are needed to. This causes the cells to develop into cancerous cells and possibly tumors. These cancers are categorized as estrogen-receptor-positive (ER+) or progesterone-receptor-positive (PR+) and about two out of every three cases of breast cancer can be categorized into one of these two states. These states of cancer allow for the potential for hormonal therapies to be used on the patient which would normally be ineffective if the patient's cancer was hormone-receptor-negative [5]. Another state in which breast cancer may be categorized as is based on the presence of the HER2 gene and protein. The HER2 protein is responsible for controlling the replication of healthy breast cells. In breast cancers categorized as HER2-positive, which account for 10-20% of breast cancer cases, there is a mutation in the gene which causes the overexpression of the protein. Since these proteins are receptors on the surface of the cell which promote the growth and replication of healthy cells, the excess of this protein causes uncontrolled cell growth. Similar to hormone-receptor-positive breast cancers, there are treatments that are able to specifically target and work against the HER2 gene and protein [6]. However, there are also cases where none of these factors are a cause, and these are categorized as triple-negative breast cancer (TNBC). This state is named after the case testing negative for both hormone receptors and excess HER2 proteins. This state of breast cancer is far more difficult to treat as the more straightforward and less damaging treatments usually target one of these three factors, so it limits the treatment possibilities greatly. These cases account for 10-20% of the remaining breast cancer cases, and as such, there is a great need for novel techniques for treatment, as the current methods usually include a combination of surgery, radiation therapy, and chemotherapy. All these treatments are far more risky and less direct methods of treating breast cancer, so more efficient and less risky methods are a strong focus of cancer research [7].

2.1.3 Clinical presentation

There are many signs which may lead clinicians to test for breast cancer, but the following is a list of some common and less common symptoms. Most common symptoms include a breast lump, nipple abnormalities, breast pain, and breast skin abnormalities. Less common signs comprise an axillary lump, breast ulceration, back pain, breast infection or inflammation, and breast swelling [8].

2.1.4 Clinical Outcome

Many people with metastatic triple-negative breast cancer are at high risk and may not survive. Based on data from the American Cancer Society, the 5-year relative survival rate depends on the stage of cancer, with localized standing at a 91% survival rate, regional at 65%, and distant at 12% [2]. Combined, cancer appears to have a 77% 5-year relative survival rate, but the rate is also dependent on the age and condition of the patient as well as other factors.

2.1.5 Economic impact

According to the budget summary data from the National Cancer Institute (NCI), over $5.9 billion was budgeted for the NCI in 2018 [9]. Of a similar budget in the 2019 fiscal year, over $4.1billion was allocated to research accounting for almost 70% of the budget. Of that percentage, over $1.3 billion was used for treatment research [10]. The project will impact the research sector, hopefully reducing the cost for testing novel treatments in development by providing a standard model to test on which will give more accurate data on the potential effects of the treatment. This would cut down costs for different levels of testing and also reduce the risk of some treatments as side effects may be able to be identified earlier in development. The hope is that our model will aid in preclinical trials for treatments and drugs for cancer and allow for more information to be gathered in less testing to accelerate the process of developing novel cancer treatment methods.

2.1.6 Current Treatment Methods

Currently, there is a distinct lack of different effective therapies for triple-negative breast cancer (TNBC). The most common treatment tracks include a combination of surgery, radiotherapy, and chemotherapy with some developments in targeted therapies. The first three of the aforementioned treatments all have their own risks and inefficiencies depending on the state of the cancer. The combination of these with the more aggressive course of the disease leads to higher mortality than the other forms of breast cancer. The combination of surgery and radiotherapy still poses a viable option for patients depending on the state of their cancer and the physical condition of the patient [11]. Conservative breast surgery (CBS) followed by radiotherapy to remove the tumor and retain as much of the breast as possible is viable and may be reasonable but still poses risks with exposing the patient to radiation. The aim of this course is to remove the tumor and then expose the surrounding tissue to radiation to eradicate any lingering cancerous cells to reduce the change of locoregional recurrence in the patient. For more severe cases, the breast may not be able to be conserved, so a mastectomy followed by radiation therapy may be advised.

The other common course involves chemotherapy. Chemotherapy involves the exposure of the patient to powerful drugs capable of killing the rapidly developing cancer cells in the body. Chemotherapy still has a poor prognosis for TNBC as there is a shorter interval where the patient is cancer-free when chemotherapy is used prior to or following primary treatment with TNBC and due to the more aggressive metastatic course of the disease [11]. With the disease developing faster and having an increased risk of developing metastatically, chemotherapy is also not universally effective for all cases of TNBC. Additionally, it still poses risks, both short-term and long-term.

Finally, some forms of target therapy have studies that support that they may be effective when treating breast cancer. One of such is mTOR inhibitors which have shown to be effective against other cancer types, and TNBC shows a high mTOR activation during its course [11]. The potential for the use of target therapies is still a possibility for development in the future.

As such, the lack of a highly effective form of treatment for patients displays the future for improvement in cancer treatment.

2.2 Prior Arts

Prior to the brainstorming and designing process, great efforts by the team were made to understand the current technologies being used or researched with the goal of creating a tissue-engineered cancer model. This section delves into prior arts with the objective being to have a solid understanding of 2D and 3D models to influence our design process.

2.2.1 Comparing 2D and 3D in vitro Models

A current focus of development for cancer treatment research has been 3D models for testing the treatments. The use of 2D models, while more simple and easy to replicate, does not accurately replicate the interior conditions and complexity of the human body and human tumors. Nevertheless, there has not been one singular method for a 3D model which replicates all the conditions which researchers seek in the trials. A course of experiments in *Stock et al.* shows a lack of evidence supporting a singular 3D model which they tested to display all or most characteristics of a real tumor [12]. However, the studies were able to find more specific applications in which certain 3D models would be useful.

In conclusion, there are advantages to using 2D and 3D cell culture. For instance, the 2D cell culture has a lower cost than the 3D cell culture, is better established since first being used in the 1900s, and is the easier model out of the two to observe and analyze [13]. Meanwhile, 3D cell culture has been found to be more physiologically relevant and allows for more complex systems and cell-cell interactions. Finally, 3D cell cultures provide better simulations of conditions and are more realistic for tumor model research [13]. For these reasons, our team has decided to go with the 3D cell culture model with the goal of accurately mimicking the tumor microenvironment and observing healthy tissue and tumor interaction in the body. **Figure 2** depicts the differences between the two models.

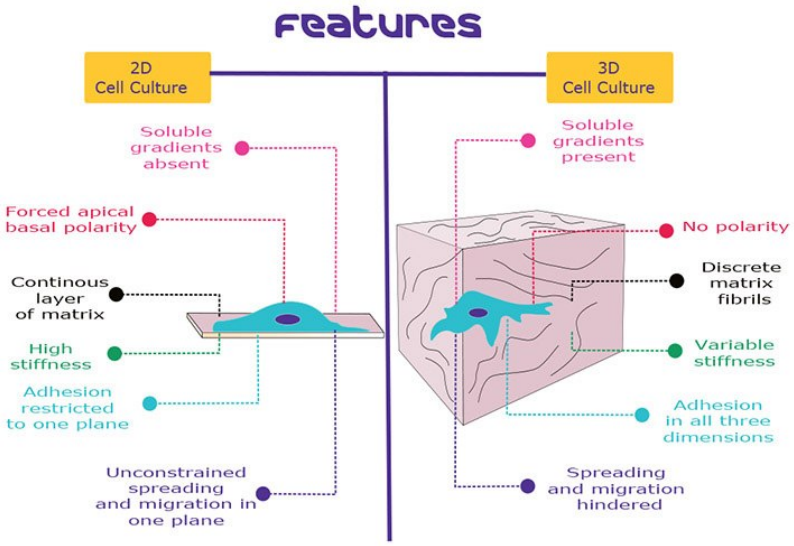

Figure 2: 2D Cell Culture vs 3D Cell Culture [14]

2.2.2 Spheroid Models

An example of a 3D cell culture model is the hanging drop method. This method creates spheroids by dropping cell suspensions on the underside of a petri dish lid and having the cells aggregate into spheroids while they hang using surface tension. The method is reliable and consistent as it does not require scaffolds to create the spheroids. The method allows for the creation of uniformly sized spheroids from a small number of cells. The method is useful to see cell-cell and cell-ECM interactions as well as when using patient-specific cells [15]. The method is limited by the size of the spheroids to be created, as the drops over 50 µL do not properly adhere to the dish and thus cannot form spheroids. Additionally, it is difficult to interact with the model after its creation with the risk of disturbing the model. **Figure 3** depicts a schematic demonstrating the principle of the hanging drop technique.

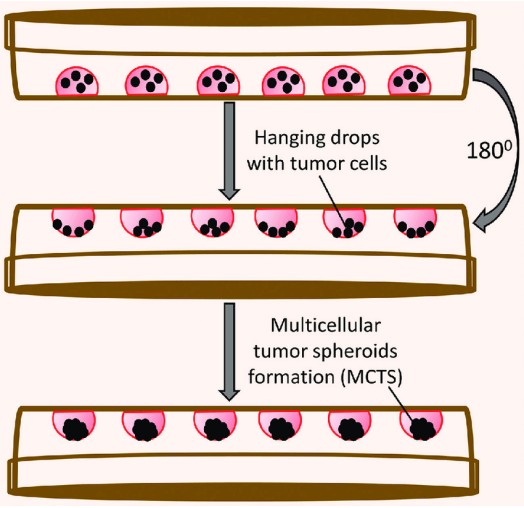

Figure 3: Hanging Drop Method Schematic [16]

2.2.3 Scaffold-Based Models

Unlike spheroids, the scaffold-based cell culture method, this method utilizes fibers and pores to act as an ECM structure where cells are able to grow and replicate. The foremost example is 3D bioprinting. These methods allow cells to grow in the microenvironment without the need to aggregate or form spheroids, but are limited by the bioinks available as well as the lack of vascularization to imitate metastasis [15]. **Figure 4** shows the schematic for scaffold-based tissue engineering.

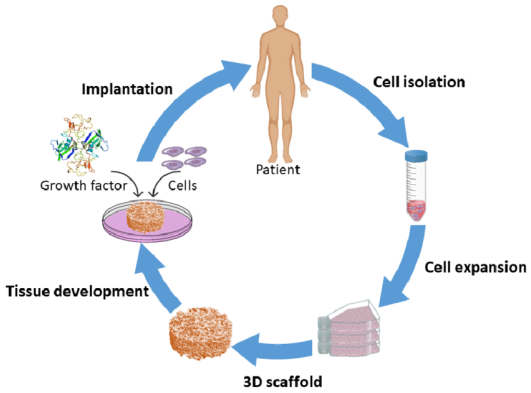

Figure 4: Scaffold-Based Tissue Engineering Schematic [17]

2.2.4 Suspension Culture

Another method of 3D cell culture and modeling is suspension culture which suspends cells in a container using agitation or an increase in media viscosity. The suspensions often include spheroids containing cells. Spinner flasks and bioreactors both make use of this culturing method. The agitation can promote cell-cell interactions, but can also modify cell physiology depending on the method of agitation. Bioreactors reduce the shear stress with agitation by rotating the container rather than a stir bar, but still do not allow for the control of the size of the spheroids created [18]. **Figure 5** shows the 3D cell culture techniques we have discussed so far.

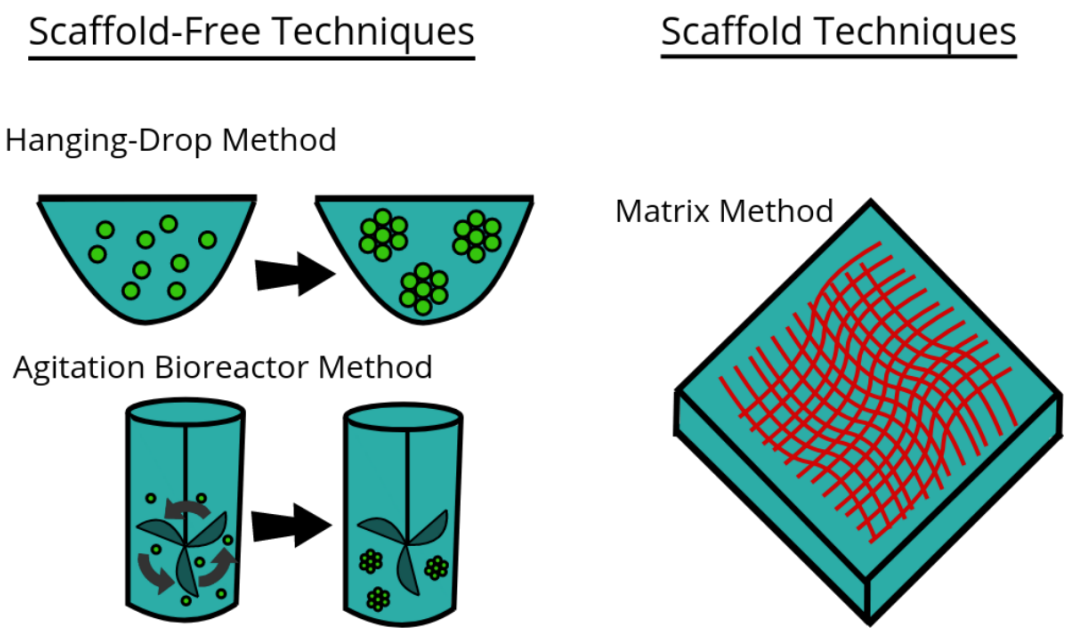

Figure 5: Scaffold-Free and Scaffold Cell Culture Techniques [19]

2.2.4.1 Bioreactor

Bioreactors are another method of suspending cells in a 3D culture to monitor the interactions and behavior of the cells when not cultured in a 2D environment. This method differs from previous methods as it utilizes agitation in the form of stirring or rotation to suspend

the aggregated cells in a solution. This also allows for the cells to be in their encapsulation within the bioreactor without anchoring to any surface. These aggregations are more manipulatable after creation than the hanging drop method but require a complex setup to recreate [18].

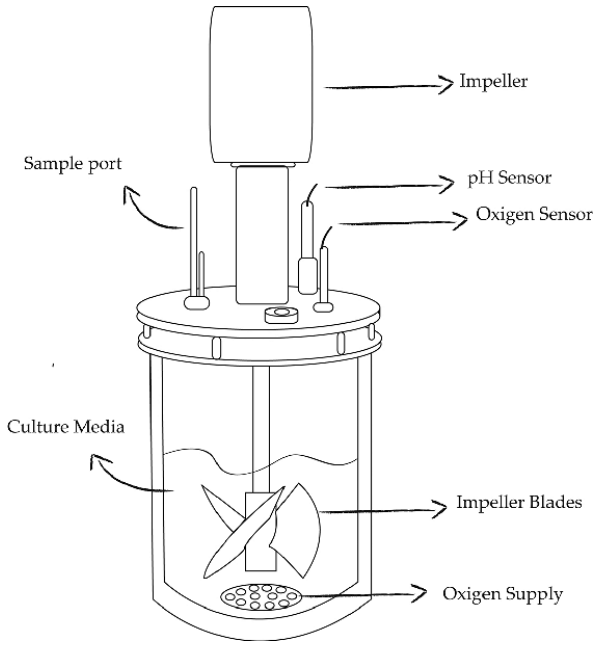

Figure 6: Bioreactor Schematic [27]

2.2.5 Microfluidic Devices

The final model our model looked at was microfluidic devices. Microfluidic devices have the advantages of being both highly scalable and reproducible as well as suitable for complex microenvironment and native physiology studies, see **Figure 7** for a graph of how different models compare. Microfluidic devices have already been developed to mimic the delivery of drugs to heterogeneous 3D tumor tissues in vitro. Due to the fact that cells possess heterogeneous microenvironments, many cancer cells fail to penetrate and treat all types of cells [20]. Most microfluidic devices are made of PDMS using soft lithography and share many of the same concepts as the hanging drop method by inserting and constraining the method into rectangular chambers on the device [20]. Advantages of microfluidic devices include the use of less volume of samples thus reducing the costs as well as their high throughput nature. Furthermore, this method allows for precise control of parameters and reduced experiment time thus increasing productivity. A disadvantage when working with microfluidic devices is that it is difficult to pattern the channels and takes much experience and patience. In addition, to our knowledge, there has been no other MQP team with similar goals to ours that have tested this strategy before which may be interesting.

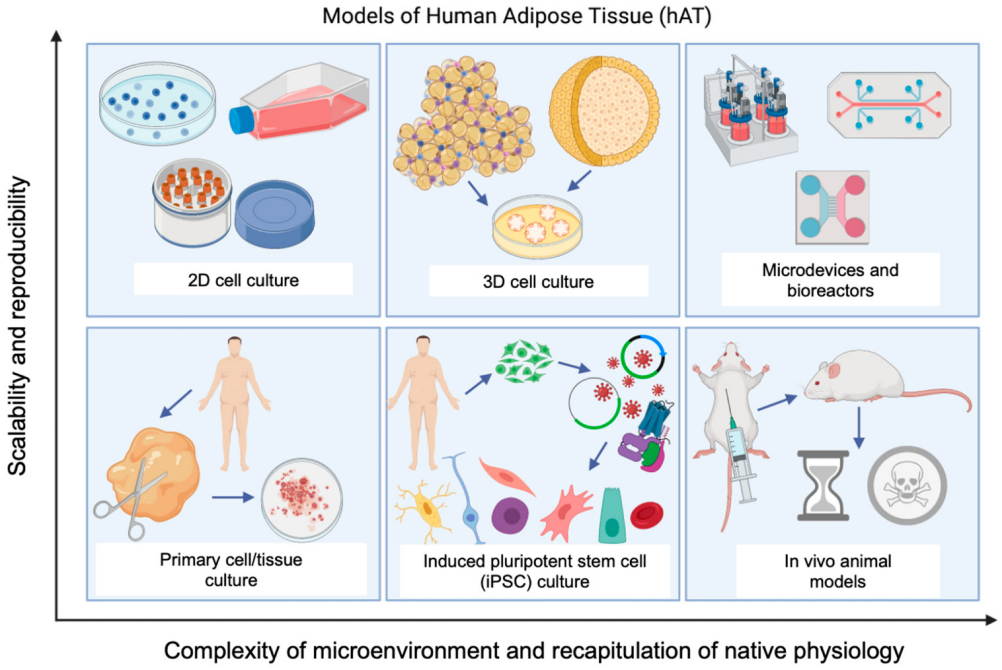

Figure 7: Comparing Tissue Engineering Methods [20]

An in vitro modeling of solid tumor interactions with perfused blood microfluidic device was made by Tae Joon Kwak and Esak Lee using human umbilical vein endothelial cells (HUVEC)-lined, perfusable, bioengineered blood vessel and tumor spheroids (MDA-MB-231 breast tumor cells and mesenchymal stem cells (MSCs) and their extracellular matrix (Matrigel) [25]. The results from their study found that their device enhanced the migration of tumor spheroids, promoted angiogenesis, facilitated vascular invasion, and preserved the structural integrity of HUVEC-lined microfluidic channels [25]. Their suggested application for their device is mainly drug screening. A concept schematic of the device is shown in **Figure 8.**

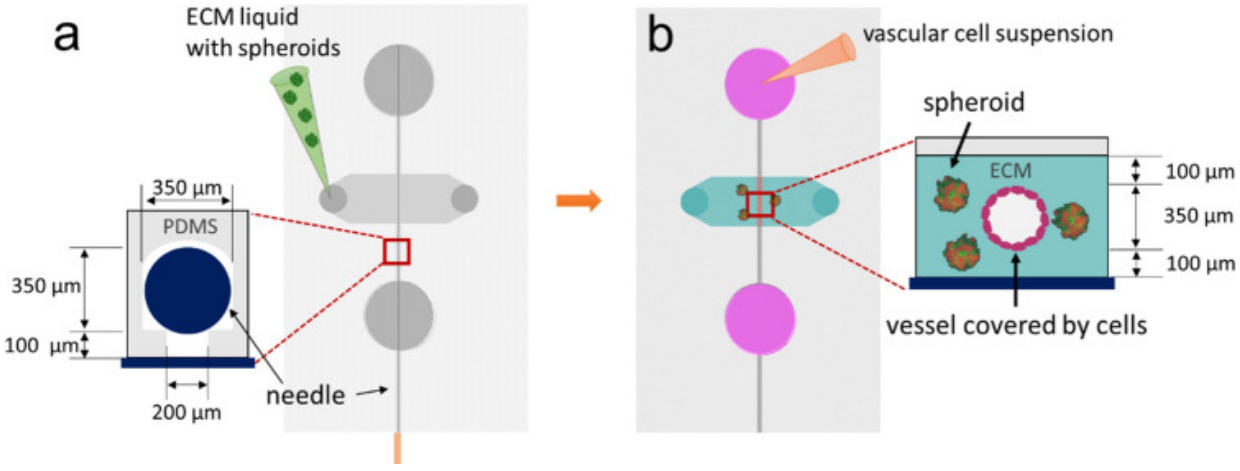

Figure 8: Schematic of Microfluidic Device [25]

Much of this study's objectives are similar to our project goals, and microfluidic devices have shown great promise in better mimicking a tumor environment. This particular study

studied the invasion of tumor spheroid mixtures in Matrigel, collagen I ECM, and fibrin ECM. Interestingly, they found that culturing tumor spheroids in fibrin ECM best preserved the integrity of the HUVEC channels and promoted angiogenesis and tumor cell migration [25]. A continuation of their study using their published methods could consist of testing different scaffolds as Matrigel is not the best scaffold to use in many cases as seen in **Figure 9.** Matrigel is a basement-membrane matrix extracted from Engelbreth-Holm-Swarm mouse sarcomas and has been used for many years for cell culture applications [26]. Despite its popularity, there are drawbacks to using Matrigel mainly due to its ill-defined and variable composition leading to uncertainty in cell culture experiments affecting reproducibility [26]. Another large disadvantage to using Matrigel is its difficulty in promoting intended cell behavior, which is very important to our project [26]. A closer look into different scaffolds such as polyacrylamide (PAM) and polyethylene glycol (PEG) could be further looked into in order to improve findings on the aforementioned device study.

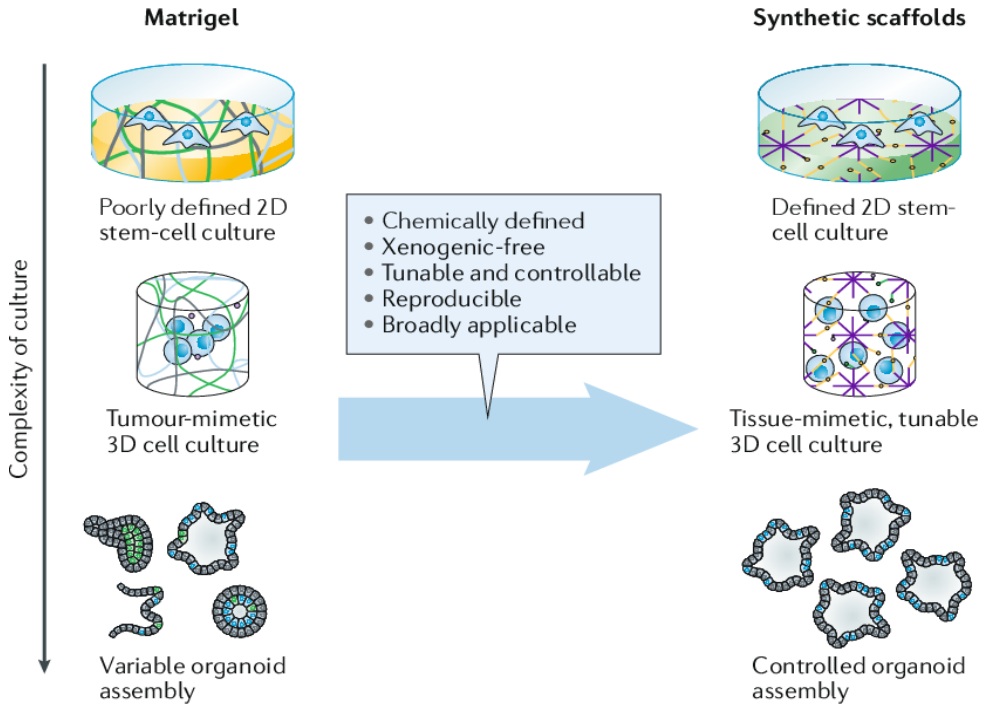

Figure 9: Synthetic Alternatives to Matrigel [26]

2.2.6 3D Bioprinter

3D Bioprinting is a technology that has been proven to have many benefits in the fields of tissue engineering and regenerative medicine. The use of CAD modeling software allows the ability to design and fabricate tissues in virtually any shape with very fine control of dimensions as well as near 100% reproducibility. For the purposes of our project, we would be using the Biobot Basic 3D Bioprinter shown in **Figure 10**. An added benefit to the Biobot basic is the use of a five-chamber extrusion head. Each of these five chambers can be loaded with varying cell suspension mixtures containing different types of cells. This would allow our team the ability to co-culture up to five of the many different types of cells that are often present in the tumor

microenvironment which could lead to even more accurate models of the tumor microenvironment.

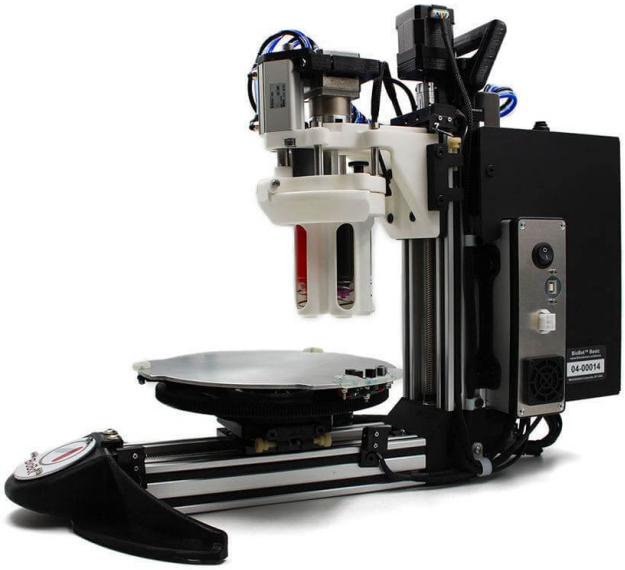

Figure 10: Biobot Basic

2.2.7 Tissue Rings

Tissue rings formed from molds is a method employed by some groups in order to attempt to create a model developed from the cells. The creation of the molds can differ in different situations from gel molds to 3D printed molds [22]. While research so far has not shown the cell's ability to interact as they would in the human body, these formations have the potential to allow for the mimicking of the microenvironment within the human body.

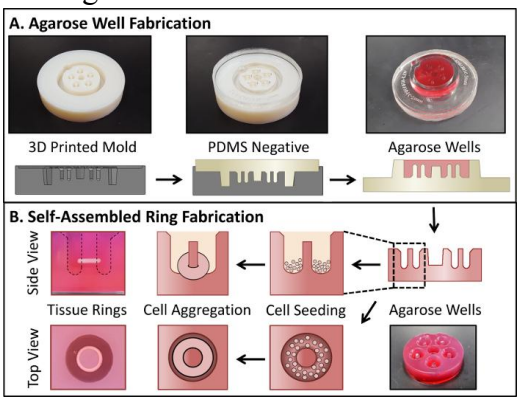

Figure 11: Process for the Formation of Molded Tissue Rings [21]

3.0 Project Strategy

The design process was focused on early in the projects' development in order to best reach our goal of creating a cancer tissue model that accurately mimics the body's microenvironment. This chapter outlines the client statement, objectives and constraints, and the final deliverables for the project.

3.1 Initial Client Statement

The initial client statement is as follows. There have been many attempts to harvest and culture cells from biopsies of solid tumors in order to establish cell lines that can be used to predict the response of cancer cells to therapies. While there has been some coordination between the response to drugs in terms of cell death, the tumor cells in vivo do not exist in isolation from the cells and tissue of the patient. Thus, the drug response is not measured in the context of the actual tumor microenvironment representative of the specific patient.

The goal of this project is to design an in vitro engineered tissue strategy that would enable the study of the response to therapy that is more representative of the tumor microenvironment in vivo. The applications for this project involve personalized medicine and guided cancer therapeutics.

3.2 Objectives and Constraints

After discussing with team members and advisors after reviewing the background and prior arts, a set of key requirements were found in order to direct our objectives for the project. The requirements are scalability, user-friendliness, cell viability maintenance, cell-cell interactions, and accurate representation of the tumor microenvironment. This set of requirements will help guide the research and designing process as well as help set performance and functional specifications to focus on. Finally, these requirements will aid in the final design choice.

The objective of this project is to create a model which incorporates elements which allow for better representation of some characteristics of the tumor microenvironment. Due to restrictions on time, space, as well as a budget of $1000; the team will seek to emphasize one factor in the tumor microenvironment in vivo as the focus of the model. Our design will seek to employ more simple and replicable tactics in order to achieve these objectives.

For our validation methods, our team will focus on meeting all the International Organization for Standardization (ISO) and Food and Drug Administration (FDA) standards. The main focus will be on their regulations on sterility, cell culture, and manufacturing. As our device will be considered a medical device, working according to the FDA and ISO standards will allow for the device to be more viable once it must be registered with the FDA for use.

3.3 Revised Client Statement

After researching and assessing the major methodologies related to the creation of an in vitro tumor model, our team was able to develop a better focus for our design. As tissue rings are both a cost-effective and underexplored topic, our team decided to utilize them for our model. Previously, there have been studies in which the ability of cells to aggregate in the ring formation were tested, and the ability for cells to aggregate under stress has been addressed in few. Studies have shown that ECM stiffness as well as shear stress, especially relating to tumor metastasis, can have significant effects on tumor formation [35, 36]. Our team, with the aid of Professor Raymond Page, seeks to test the effects of stress and irregular patterns on the aggregation of fibroblast and cancer cells in a ring mold. By providing differing angles in each mold, different stresses can be tested and potential differences in stiffness can be induced. These molds will be easy to reproduce and will allow for a better representation of the actual in vivo tumor microenvironment in vitro.

3.4 Project Approach

Our team approached the project in parts in order to best progress throughout the year. During the months from A term to B term, the team spent time doing background research, as well as familiarizing ourselves with the lab. During the later weeks of B term, our team spent time developing initial designs as well as growing a cell bank in order to perform tests after returning from winter break. Our team spent time in C term refining and testing our design while passaging the cell lines and preparing them to be seeded. Our team would use C term and D term to perform tests on our mold design and attempt to create tissue rings.

4.0 Design Process

This section of the report includes the process by which the team developed the tumor cell model to mimic the in vivo conditions of the tumor microenvironment. This part addresses the prior design information which the team took into consideration during the development of the model as well as the stages of the model and the protocols for its use.

4.1 Needs Analysis

A decision matrix was created using the above requirements as shown in Table 1**.** This allowed the team to compare the objectives in terms of importance to the project, where 1 meant the objective held greater significance. From the matrix, we found that it was most important that the device maintains cell viability followed by cell-cell interactions and an accurate representation of the tumor microenvironment. The two latter requirements are very important and central to the project's final objectives; however, in order for them to be accomplished, there must be cells for us to work with. For this reason, our design will primarily focus on maintaining cell viability in the early stages. Scalability and user-friendliness will be improved upon later on in the design steps as they are still features that are important.

Table 1: Decision Matrix for Final Design Requirements

OBJECTIVE	Scalable	User Friendly	Maintains Cell Viability	Allows for Cell-Cell Interactions	Accurately Represents Microevironment
Scalable	X	0	1	1	1
User Friendly	1	X	1	1	1
Maintains Cell Viability	0	0	X	0	0
Allows for Cell-Cell Interactions	0	0	1	X	0
Accurately Represents Microevironment	0	0	1	1	X
Total:	1	0	4	3	2

In addition to meeting these objectives, there were other factors which would affect the project as well. The first would be the cost of the model, as the model should aim to be low in monetary and resource cost. The biocompatibility of the model is also significant, as the model must both be of a material which is approved to be used in tandem with biological substances as well as one which is able to be sterilized prior to use.

4.2 Design Concepts

The thought process with which the team approached different methodologies for the project is laid out in the concept map pictured in **Figure 12**. The two different directions of the project would be to implement an artificial environment in which the cells would reside in or an environment consisting mostly of other cells to recreate the interior conditions. The different

methods of creating the environment were then categorized based on how they would be executed, with the artificial environment being achieved through a hydrogel ECM and the cell-grown environment being achieved through patient sample cells or cell lines. These execution methods were then used to determine the different strategies which could be used to achieve the project goal.

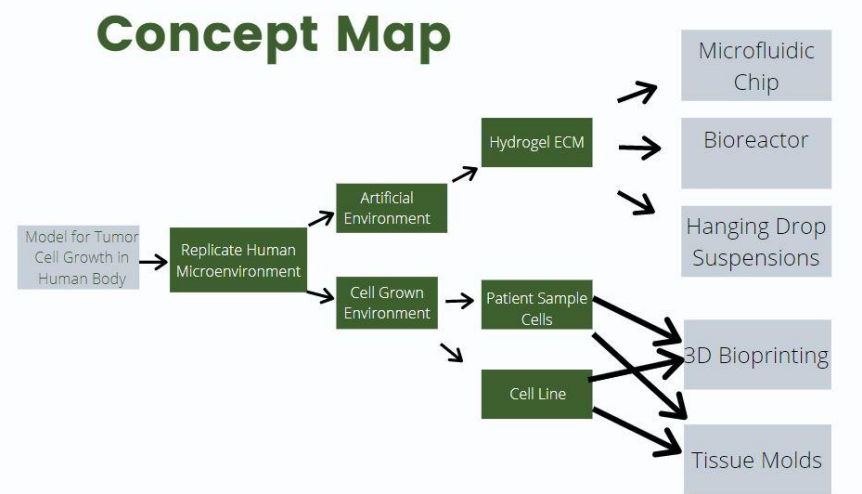

Figure 12: Concept Map for Different Project Possibilities

4.3 Preliminary Data

There have been multiple MQP projects with similar project goals which were reviewed. The client left it up to the team to either continue a past MQP project and improve it or come up with a new idea. The first MQP report we reviewed was “Engineered 3D Cancer Tumor Model” which was recently done in 2021 by Luis Corona, Alejandro Marzoratti, and Matthew Scott. Their focus was also on triple-negative breast cancer with their project goal being to create a 3D cell culture technique to create a tumor microenvironment that more accurately represents anatomical conditions than the standard tissue microstructures [21]. The main idea of their design was to use different molds that contain shapes on where the cancer cells can proliferate and cluster as shown in **Figure 13**.

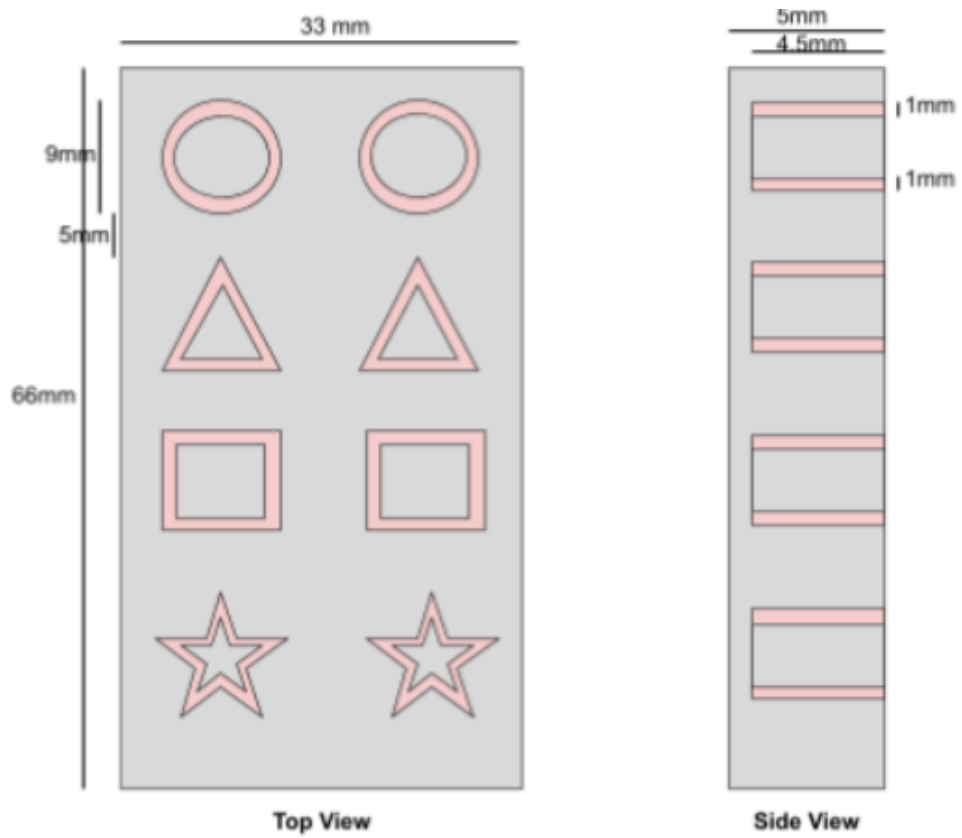

Figure 13: Top and Side View of Conceptual Mold [21]

The next MQP project we looked at, “Engineered Tumor Model” by Jacob Boles, Jessica Brewster, Kylie Smith, and Paige Waligora in 2020 where they also focused on metastatic breast cancer. The project shares similar goals to our project as well as the first MQP project we reviewed. Despite sharing similar goals the final designs were very different from the other MQP project, this team decided to do the hanging drop method to create a cluster of cells. The figure below shows their tissue ring model. The team included detailed recommendations that may be performed in the future that involve a BrdU assay [22].

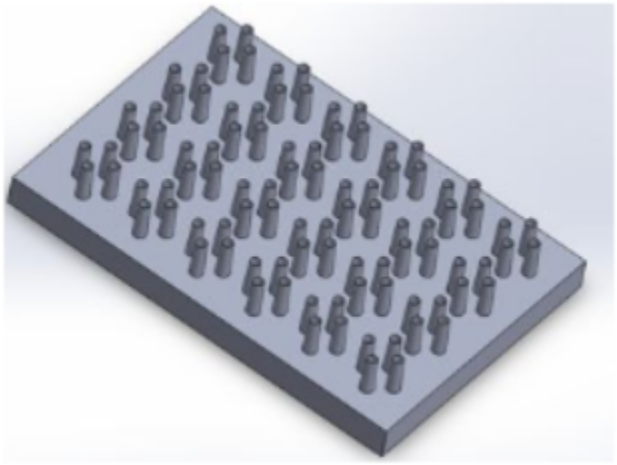
Figure 14: Tissue Ring Model [22]

Another MQP project with similar goals is the "3D Perfusable, Endothelialized Tumor Model" from 2018 by McKenzie Brunelle, Nicole Chittim, Ryan Conlan, Emily Newman, and Emma Sheils. The goal of this project was to design an in vitro testing system that more accurately mimics the tumor microenvironment as they lack a consistent endothelial monolayer and controllable fluid flow. The final design of their project consisted of a perfusion pump to recirculate media through tubing over a silk scaffold that incorporated an endothelial cell monolayer [23]. Their results were impressive where they successfully created a perfusion bioreactor system that resulted in a preliminary perfusable, humanized, endothelialized in vitro tumor model allowing for controlled fluid flow. Their design can be seen in **Figure 15** below.

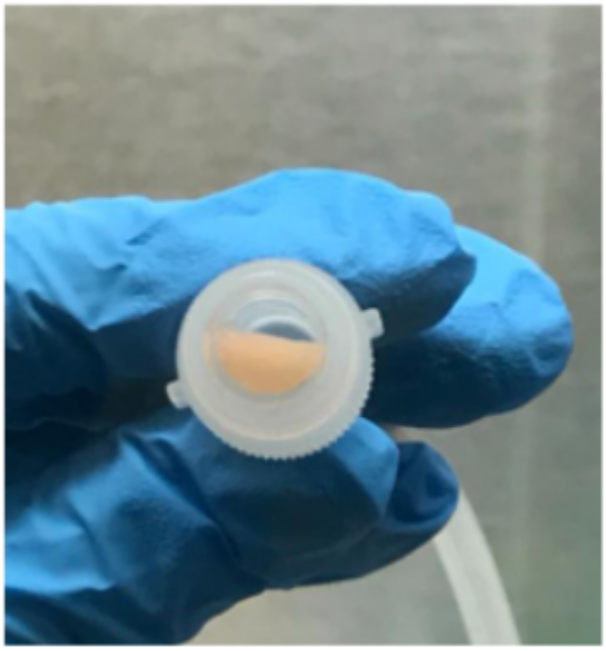

Figure 15: 10% silk scaffold cut to a half-moon with endothelial cells seeded on it. The scaffold is inserted into the 2 mm thick PDMS ring, which is then inserted into the Swinnex [23]

The final MQP project we looked at was "Engineered 3D Microtissues for Personalized Cancer Treatment" by Kylie Arnold, Madeline Blake, Marissa Gonzales, and Elena Raden in 2021. They share the same project goals as us and they developed a protocol to create an in vitro, 3D, ring tumor model for use in cancer therapy testing [24]. They decided to go with a Tissue Ring Model that was 3D printed for their agarose rings and posts. Their design can be seen in **Figure 16**. The hanging drop method was also used in their project as well as spheroid cell density tests. The preliminary results from their project showed great potential for using ring formations as in vitro cancer models.

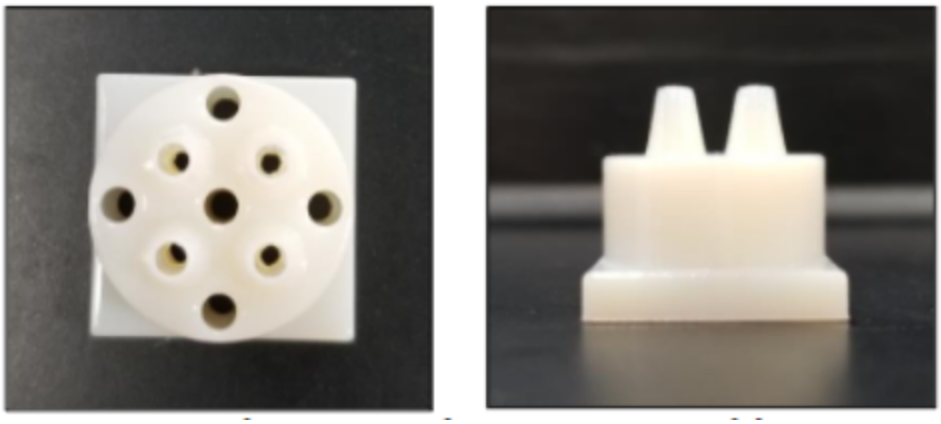

Figure 16: 3D Printed Component for Tissue Ring Model [24]

4.4 Conceptual Designs

This section will outline the potential and initial designs to create the tissue rings which will be later evaluated based upon the objectives each meets.

4.4.1 3D Bioprinting - Biobot Basic

The bioprinter would utilize preformulated bioink, which would contain the cells which would be seeded, and lays them in a 3D structure. This ink would allow us to create the ring structures which we seek to generate, and would give us control over the shape and size of the ring, making it sizable. However, the use of the Biobot is expensive in the limited access which others would have to the technology but also in the creation of the ink. Additionally, the ink contains other components than the cells and media for them to grow in which would not normally be present in the tumor environment. These components could alter the formation of the rings, and cause the cells to behave differently than they would in vivo.

4.4.2 Spheroid Model - Hanging Drop

This design relies on the ability of cells to aggregate on their own within a small spheroid. The cells would be cultured and seeded, then suspended in the droplets to form the spheroids through the previously detailed hanging drop method. These cells, once aggregated, could be tested upon. This method is not reliable, as the hanging drop method, while simple, does not allow for changes to media prior to cell aggregation, which can lead to cell death in areas of the spheroid with little nutrients.

4.4.3 Self-Aggregating Tissue Ring Mold

The tissue ring model has the team create a mold which the team would use to create a negative in agarose in which the cells would be seeded and allowed to aggregate in. The team

designed an insert dimensioned for individual wells in a 24-well cell culture plate as seen in **Figure 18**. The insert would have 4 prongs which would be suspended above the floor of each well by the extension across the top of the unit as depicted in **Figure 17**. By inserting the unit after filling the well partially with agarose, the team could create a mold in which the cells could be seeded. The mold was designed using CAD and was printed using PLA from an Ultimaker 3D printer. The mold includes four hollow prongs, each of a different shape: circle, triangle, square, hexagon, with different angles. This would be a reusable method which would not be heavily costly for the team and could be easily sterilized. While the unit was able to create wells in the agarose, it was not as effective as our team hoped. The structural piece of the print was difficult to remove, and potentially compromised the shape of the unit. Additionally, the grooves of the plastic it was printed with made it more difficult to sterilize prior to use and clean after use.

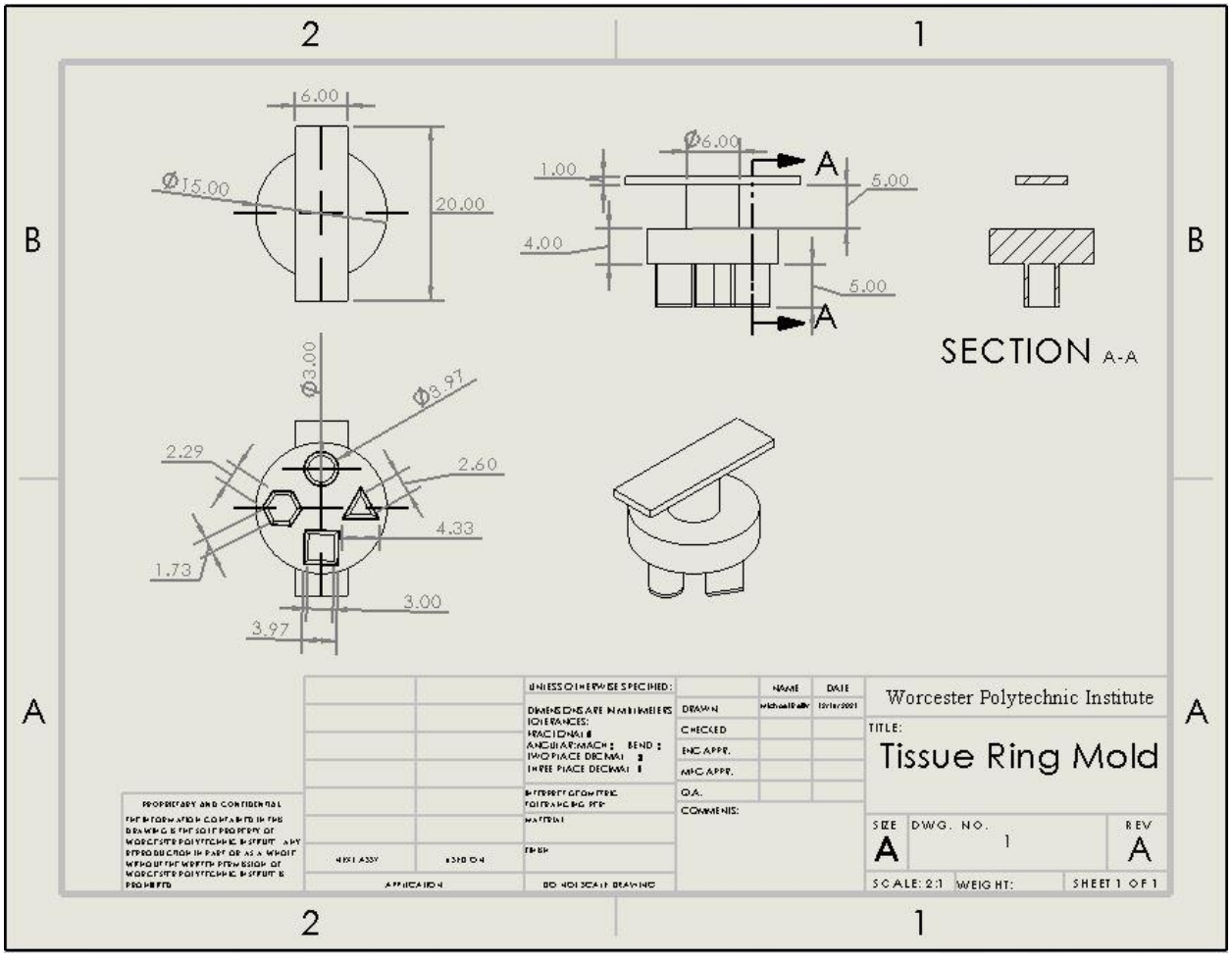

Figure 17: Solidworks part drawing for the well insert

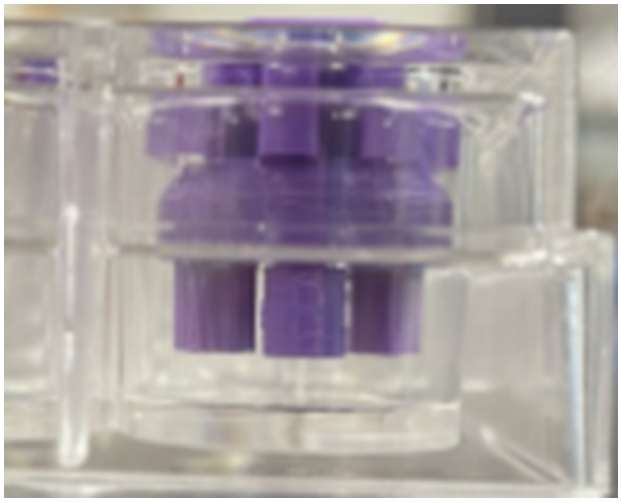

Figure 18: Initial print of unit inserted into well

4.5 Final Design

The design of the unit in order to create the ring mold negative includes four hollow posts of different shapes which allow for molds of different shapes to be created. The body of the unit measures with dimensions of 15x15x15mm and the extension across the top of the body has a length of 20mm. As seen in **Figure 19** the extension which will extend above the edges of each well to suspend the prongs into the wells, allow the agar to set around the prongs and create the mold negative. At the tip of each protrusion at the bottom of each shape is a chamfer which is set as a 2.5 degree angle to increase the ring integrity and longevity [34]. The design was printed using the Objet260 Connex printer with the biocompatible material MED610 with a glossy finish.

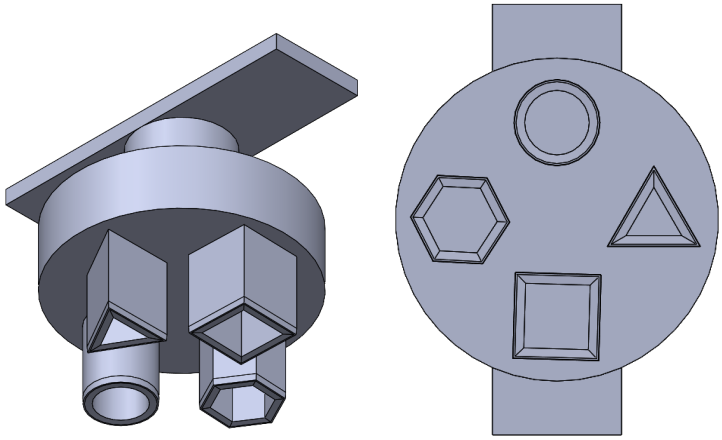

Figure 19: The CAD model for the insert to be placed in the well to create the agarose ring mold negative.

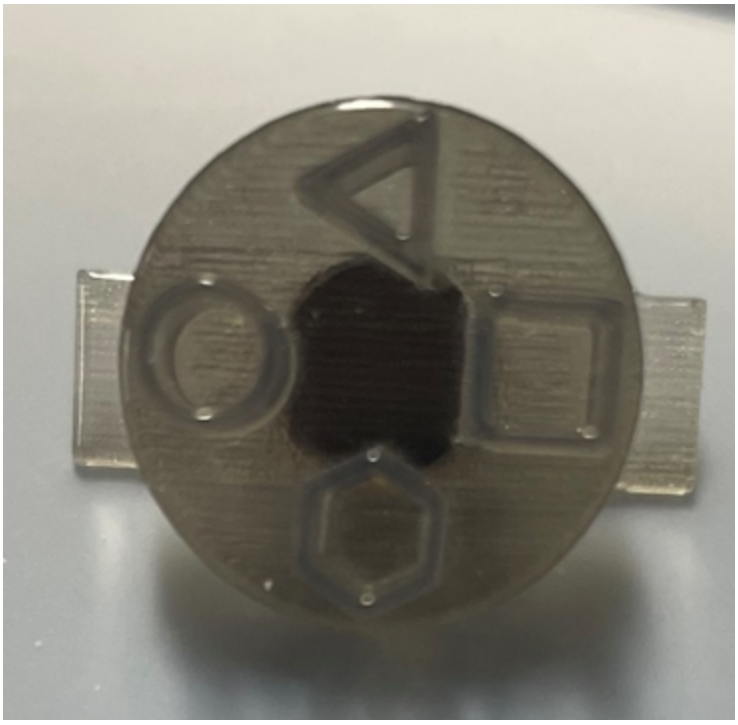

Figure 20: View of the bottom of the prongs of the well insert

4.6 Design Methodology

The final goal of the project is to co-culture human fibroblast and cancer cells in the model in order to create a ring which will display tumor microenvironment. For our human cells, our team decided to use HMF-52 fibroblasts and MDA-MB-231 tumor cells for our cancer cells. The MDA-MB-231 cells were fluorescently stained prior with green fluorescent protein, which would allow them to be differentiated from the fibroblasts under a fluorescent microscope. The cells were seeded in flasks and passaged to allow them to proliferate until they were confluent according to protocols outlined in **Appendix B** and **C**, using media created as detailed in **Appendix A**.

The team created 2% agarose and autoclaved it to sterilize it prior to creating the molds and seeding cells. By adding the agarose according to the protocol in **Appendix D**, to the wells and inserting the unit, the molds could be made for the tissue rings to form such as those depicted in **Figure 21**. We performed several trials with greater and lesser volumes of agarose, but the detailed amount allowed for the best mold formation from our insert.

Our team first needed to ensure the cells could aggregate in the wells formed within the agarose, so the fibroblast cells would first be seeded alone in each shape. For further trials, the cancer cells would be dispersed in a suspension with fibroblast cells and seeded. The amount of cancer cells in the suspension would be proportional to the fibroblasts at ratios of 1:2, 1:3, and 1:4 to better understand how well both cell types can aggregate at these densities.

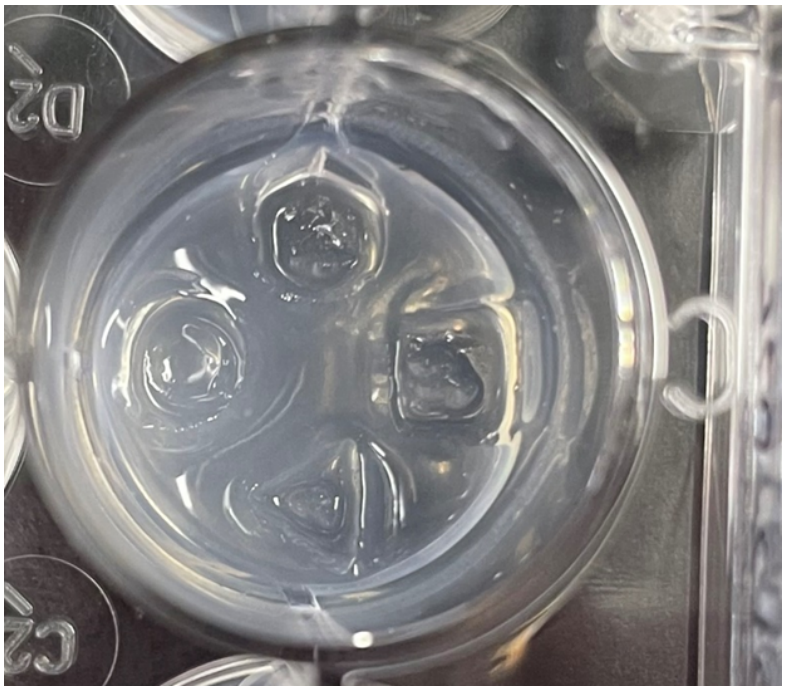

Figure 21: The four shapes left by the insert after inserting into agarose and allowing to set

5.0 Design Verification

This section addresses the results from the testing which was performed according to the protocol outlined in the previous section.

5.1 Expected Results

Using the well forming and seeding protocols described above, we expected to see the formation of self assembled tissue rings with varying mechanical and cellular properties based on the different experimental conditions. The tissue rings were expected to appear as those in **Figure 22** and be able to be extracted for mechanical and immunohistochemical testing. The ability for the CRL-2097 fibroblasts to secrete extracellular matrix was intended to be the driving force in the creation of these tissue rings but in the case of seeding only fibroblasts in the wells our team was unsure if there would be significant formation of the tissue rings as there would be no interaction between the fibroblasts and any other cell that would normally be found in the tumor microenvironment. With the introduction of tumor cells to the tissue rings we expected to see much more interaction between the cell lines and thus the formation of solid tissue rings. We chose to use a GFP labeled cancer cell line in order to more easily locate and analyze the cancer cells in the model. At 5% density of cancer cells our group expected to see some proliferation of the cancer cells into microclusters and also some migration of the cancer cells to various locations within the tissue. This migration would be possible because of the secreted extracellular matrix of the fibroblast cell line. In the 10% and 15% cancer cell density tials our team expected to see further development of cancer cell clusters and even more dispersion of the cancer cells throughout the tissue.

The expected results for each of the different ring mold shapes was expected to vary primarily with the difference in angle each of the shapes offers. Sharper, more acute angles such as those in the triangle and square shaped molds were expected to have larger quantities of cancer cells located at the angle itself and more proliferation of the cancer cells in this area. The expected cause of this was the tendency for breast cancer cells to prefer stiffer tissue which, once the rings had self-assembled, would be found at the more acute angles. This difference in stiffness in the tissue was anticipated to be the main catalyst for migration of the tumor cells which would be facilitated by the extracellular matrix secreted by the fibroblasts. In comparison, the tissue rings with more obtuse angles such as the hexagon and circle were expected to exhibit more even dispersion of the cancer cells and cancer cells microclusters due to the more evenly dispersed stiffness in the mold shapes. These stiffness values would be tested using mechanical testing and compared to the immunohistochemical testing results in order to see if a correlation existed between tissue stiffness and cancer cell migration and proliferation.

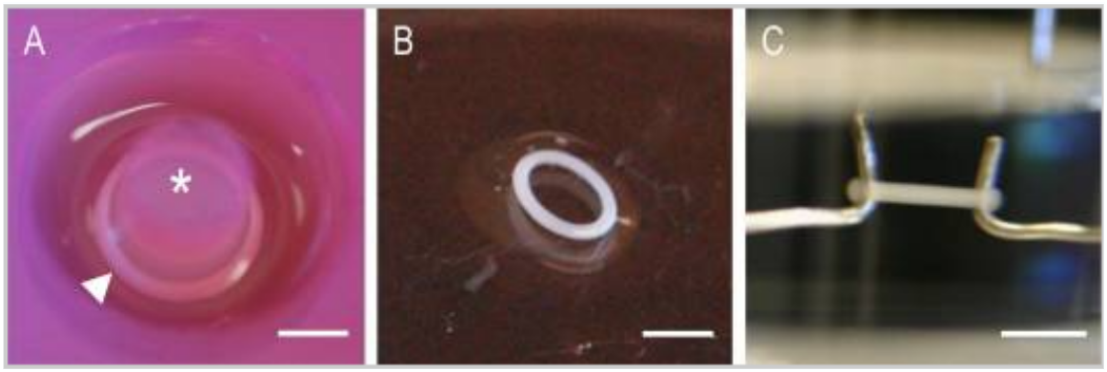

Figure 22: Self Assembled smooth muscle tissue rings. [30]

5.2 Results

At the conclusion of our testing period we were not able to successfully create a tissue ring mold that we could test. This was largely due to a large scale contamination issue in the lab space that did not allow any cell cultures or tissue structures to form. As can be seen in **Figure 23** the cells were successfully seeded into each of the wells but the hazy brownish structures are an unknown contaminant that found its way into the wells during each testing phase.

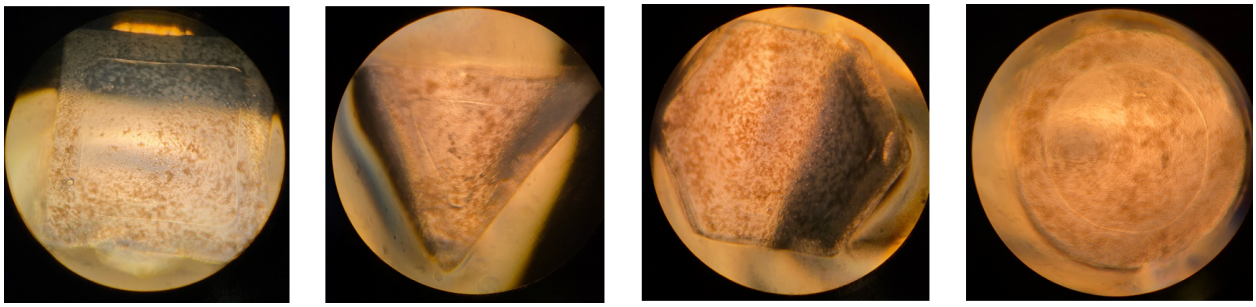

Figure 23: Images of contaminated tissue ring mold wells.

However, despite the unsuccessful creation of tissue rings our team was able to successfully design, develop, and fabricate an effective tool to be used in creating the ring molds in the future. This tool is easily manufacturable, scalable, cost effective, and can also be biocompatible because of its material. In addition to the creation of this tool, our team was able to successfully create working protocols for both the use of the tool to make agarose wells for cell seeding and for proper seeding of the cells into the wells.

6.0 Final Design and Considerations

The goal of this design is to provide a means for testing cancer treatments methods without a live subject. This would lead to reduced cost of care, allow for developments towards personalized medicine, and most importantly improve the quality of treatment for the patient. All of these components should be attractive to our customers and this design could be revolutionary in the oncology field as seen later on in the chapter, billions of dollars are invested in this cause. Developments towards personalized medicine leads to a great reduction in the cost of care for both the patients and hospitals in addition to impacting billions of patients around the world and millions of patients in the U.S to have improved quality of life.

6.1 Economics

This is the Business Model Canvas created for this design, see **Figure 24**. The following sections will delve into each segment in detail.

Figure 24: Business Model Canvas

6.1.1 Customer Segment

Our main customers for our design are the patients, pharmaceutical companies, oncologists, hospitals, insurance companies, and regulatory agencies such as the FDA. Although this customer will not directly work with our design, it will have a big impact on patients as our design would allow them to get better treatment due to the fact that therapies would be tested on tissue models specific to their biology. This allows for increased accuracy in the treatment and may lead to less of their money spent towards trying therapies that may not be the best for them. For this reason, pharmaceutical companies may want to use this design to test their therapies. Personalized medicine companies are becoming increasingly popular and our design would allow for high-throughput drug screening. Similarly, oncologists and researchers may want to use this to test the treatments used for patients or to test their research. In order for all of this to

happen there will have to be a great collaboration for hospitals and insurance companies to approve use of this design. To target this population we will emphasize that more accurate treatments save money and resources in the long run, billions of dollars are spent every year in the U.S alone on cancer treatments. Ideally, this number will go down as patients receive personalized treatment that has minimized the risk of rejection due to the design's autologous component. Finally, we will have to work with regulatory agencies such as the FDA to ensure that our design is safe and able to be used by the above customers.

6.1.2 Value Proposition

The goal of this design is to provide a means for testing cancer treatments methods without a live subject. This would lead to reduced cost of care, allow for developments towards personalized medicine, and most importantly improve the quality of treatment for the patient. All of these components should be attractive to our customers and this design could be revolutionary in the oncology field as seen later on in the chapter, billions of dollars are invested in this cause. Developments towards personalized medicine leads to a great reduction in the cost of care for both the patients and hospitals in addition to impacting billions of patients around the world and millions of patients in the U.S to have improved quality of life.

6.1.3 Channels

Once we have established what key components add value to our design we had to brainstorm on how our design will reach our customers. This was quite difficult to establish as our design would likely be an SOP that delineates how our design can be reproduced and they would follow the protocol in their own lab with their own resources. Direct sales would be the channel that allows our design to collect revenue. Exactly how this would be done would need to be further researched if brought to market.

6.1.4 Customer Relationship

A manner our team could build our relationship with our customers is by providing them clinical studies and access to personalized data. Clinical studies are an essential way to validate our findings and demonstrate that our design works. Moreover, access to personalized data would attract customers as more data allows for improvement in processes in activities such as research, marketing, and more. This data would be confidential and collected under patient consent.

6.1.5 Revenue Stream

Revenue would be collected through usage fees and data collection fees. The customer would make a one-time purchase to have access to the SOP as well as grant them access to personalized data. In addition to this, if the design/SOP is ever updated, the customer would have access to this update without any extra charge for a minimum of five years.

6.1.6 Key Resources

In order to accomplish our value proposition, channels, customer relationships, and revenue streams we will have to make good use of knowledge from oncologists and researchers, especially in the designing process. Funding will also be a key resource so that we are able to develop the idea and are able to market it.

6.1.7 Key Activities

Activities needed to accomplish value proposition, channels, customer relationships, and revenue streams are activities that focus on research and development, logistics, and marketing. As our design is simply an SOP, manufacturing, and transportation will not be of concern. Nevertheless, the logistics of how to keep track of usage will be established. Research and development will be the main priority at the start of the business and simmer down; however, will continue in order to improve the design. Marketing will be a large focus once a good design has been established and the patent has been awarded.

6.1.8 Key Partners

The key partners for this design are researchers, hospitals (specifically oncologists), pharmaceutical companies, and regulatory agencies. Collaboration with these entities is required for our design's success.

6.1.9 Cost Structure

The most important costs in our design are research and development, logistics, and marketing as previously discussed. At the beginning of development, money will go to research in order to make the best design possible with our resources. Once this has been accomplished, money will go into logistics and then marketing so the design is able to reach as many patients as possible for better treatment as well as be profitable.

6.2 Financial Projects Over Five Years

The following sections break down the financial projections of our design. This includes assumptions that this projection is based on as well as our reimbursement strategy.

6.2.1 Assumptions

Assumptions were based on research and logic. The first assumption made was that there are 1.8 million patients in the U.S and 1.8 billion cancer patients worldwide in 2020 [32]. Furthermore, to have a better of the market, we found that 150 billion dollars are spent on cancer care yearly [32]. We hope that our design will aid in reducing this number as it will help patients get more accurate and efficient treatment and greatly minimize failed treatments. Finally, we assumed that up to 10% of this population will use our design which accounts for 45,000 patients in the US and 45,000,000 patients worldwide. Although these numbers are ambitious, we believe that the benefits to the customers would be substantial and worthy. As for the selling price of our design, we have decided that nearly $3000 for the use of this method would fit as this is the average price of an SOP currently [33]. This includes access to the full SOP, personalized data,

and any updates for the next five years as well as technical support. However, it is important to note that the customer will have to pay for their own lab equipment, workers, and even their own bioprinter.

6.2.2 Reimbursement Strategy

Table 2 shows our costs incurred by the team. We expect more and more people to become interested in this technology, we are especially targeting pharmaceutical companies as they are the ones who will want their therapies tested. The manufacturing cost is currently only $1000 as this is our MQP project, but if this idea is patented we would likely need more funding to further develop. As this is just an SOP, there is no need for packaging, shipping, or distribution costs.

Table 2: Financial Projections for Five Years

	Year 1	Year 2	Year 3	Year 4	Year 5
Selling Price Per Method	$2,950	$2,950	$2,950	$2,950	$2,950
Number of Methods Sold (millions)	10	20	30	40	50
Manufacturing Cost	$0	$0	$0	$0	$0
Packaging	$0	$0	$0	$0	$0
Shipping	$0	$0	$0	$0	$0
Distribution	$0	$0	$0	$0	$0

Our design has the potential to be very profitable due to the benefits of using our design as well as the extremely low manufacturing costs as it is a method. If there are any “manufacturing costs” they would be very minimal and negligible when compared to potential net income. **Table 3** depicts the expected reimbursement for five years.

Table 3: Reimbursement Strategy

	Year 1	Year 2	Year 3	Year 4	Year 5
Total Manufacturing Cost	$0	$0	$0	$0	$0
Selling Price (million)	$29,500	$ 59,000	$ 88,500	$ 118,000	$ 147,500
Net Income (million)	$29,500	$ 59,000	$ 88,500	$ 118,000	$ 147,500

6.3 Environmental Impact

This project will have very little environmental impact if moved up to a larger scale. The low material cost and current manufacturing practice for creating the stamp mold generates very little waste and as each mold is small in size, the general cost per mold is relatively low when compared to other devices in the field. The reusability of the units allows them to be less wasteful with the most waste coming from the well plates and other equipment which would have to be disposed of due to sterility concerns.

6.4 Societal Influence

While it is unlikely that our product would cause any immediate shifts in society, there may be societal influence if the device were to reach a clinical testing stage. As a model which would allow for more accurate in vitro trials, the time for development of treatments for cancer could be decreased while still modeling them effectively. This could motivate companies or organizations to invest more resources in these treatments, as there would be less uncertainty with the development. This could then potentially extend beyond cancer treatment to study other disease courses with more advanced needs as well, which would aid the development of medicine.

6.5 Political Ramifications

As theorized in the previous section, this model may be able to change the landscape of medicine. This would have great influence in politics, due in part to the large amount of resources that countries invest in disease research. Changes to the large companies invested in disease research could change both politics and economics.

6.6 Ethical Concern

While the use of fibroblasts and cancer cells in the lab is common for disease research, the use of cell samples from people may lead to some ethical concerns. When applied to personalized medicine, there may be concerns about the restrictions which doctors and researchers have on the provided cells. In addition, it is important that if this model is used in a research setting that a well-diversified cell bank is used in order to collect important information on all races, genders, etc.

6.7 Health and Safety Issues

Our model would aim to aid with public health and to bolster health care. With the ability to more efficiently test new cancer treatments, cancer treatments should be able to develop more smoothly. The model produces some waste which must be disposed of as hazardous, but does not utilize any substances which would pose a great danger to handlers.

6.8 Manufacturability

The main aspect of manufacturing in our project is to do with the creation of the tool that creates the well shapes in the agarose gel. This device can be easily manufactured at low cost in a number of different ways. In our project we opted to use 3D-printing to create the tool which proved to be effective as we were able to generate multiple iterations of the tool using multiple different materials in a relatively short time frame. However, 3D-printing is not a feasible

manufacturing means for large scale production so if this manufacturing of this device were to be scaled up an alternative approach could be assembly line machining or the use of molds that would be filled with plastic and allowed to set into shape. The machining approach would also allow for the device to be made out of metal if this was deemed to be necessary.

6.9 Sustainability

This model utilizes minimal supplies to achieve its goal, and should aid in the sustainability concerns for medical devices. The reduction of resources which companies would normally have to invest in research would be a welcome change. Companies could create these models with supplies they likely have on hand, and would not need to reinvest in other technology in order to attempt to reduce their waste production.

7.0 Discussion

7.1 Discussion

Figure 25 is a chart of the functional specifications we shared in our concept evaluation section. As can be seen, we believe our model included all necessary components to observe the tissue microenvironment as it allowed for cells to be grown inside the molds. However, our biggest success was designing a model that is easily manufactured, scalable, high throughput, cost-efficient and easy to use. Nevertheless, there were some aspects of the model that need great improvement. For instance, we were not able to test the biocompatibility of our materials used due to the recurrent contamination. This contamination issue also did not allow us to determine whether the mold was truly sterilizable and non-disruptive to the cells.

Status	Achieved	Needs Improvement
Biocompatible		
Represents 3D in vivo interactions		
Includes necessary components for TME		
Easily Manufactured and scalable		
High throughput		
Cost-efficient and easy to use		
Sterilizable and non-disruptive to cells		

Figure 25: Functional Specifications Assessment

7.2 Limitations and Challenges

We believe our limitations and challenges go hand in hand. The biggest limitation and challenge was the constant contamination issues we had to deal with in the Spring Semester that came with the shared lab space, we believe it could have been the hood that was contaminated. Contamination kept us from getting our results later on in the year. **Figure 26** shows what healthy fibroblasts cells looked like at the beginning of the year, and what the cells looked like after Winter break. They are covered in a sort of haze. We truly believe this was out of our control as we took multiple steps to get ahead of this contamination. For instance, we routinely checked our cell media for contamination and used our own consumables. However, sadly even after just a night in the incubators everything was found to be contaminated. Our team did consider utilizing other lab space but this was highly risky and we did not want to extend this contamination to other labs. Another challenge we faced was keeping the agar sterile while heating in order to make the wells.

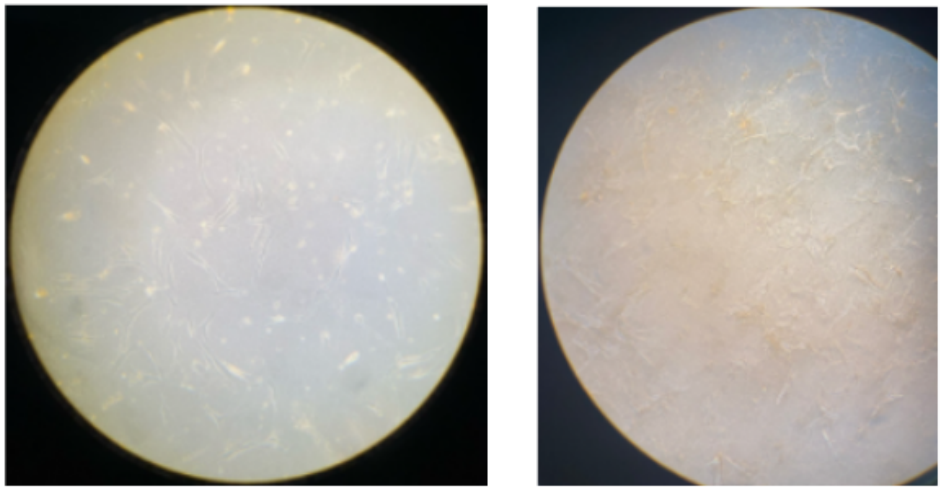

Figure 26: Healthy versus contaminated human fibroblasts

8.0 Conclusions and Recommendations

For future groups, we strongly encourage to start cell banking as soon as possible and create a large cell source that can be used during the testing phase. In addition, we suggest that groups have their own sets of consumables to ensure sterility and routinely test cell media for contamination. As for our model, we believe that the volume of agar in the well needs to be further investigated along with how long the mold needs to be in for. Sometimes if the mold was removed too early the shapes would break but if removed too late the mold would get stuck and tear the agar. We also think that it would have been interesting to explore the addition of collagen to the mold, this may provide further stability to the mold and perhaps enhance TME interactions.

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Appendix A: Media Preparation Protocol

Objective: Make cell culture media with 10% FBS and 0.5% pen-strep

Materials:

- Full media bottle
- FBS
- Pen-strep

Method:

1. Thaw 50 mL FBS (full tube) in warm water until completely thawed
2. Carefully pour 50 mL FBS into media bottle
    - If not able to pour without spilling then use pipettes
    - Rims of bottles should NEVER touch anything other than the cap
3. Add 2.5 mL of pen-strep to the media bottle with FBS
4. Gently mix the bottle without creating bubbles
5. Write with a sharpie on the bottle:
    - Opened: Date, Initials, 10% FBS + 0.5% PS
6. Carefully pour prepared media into 2-3 50 mL tubes for future use
    - Label tubes:
        - Fibroblasts, Date, Initials
7. Put everything in the fridge for future use
    - If using right away, put 50 mL tubes in incubator to warm

Appendix B: Media Change Protocol

Objective: changing media day after passaging or every 2 days

Materials:

- Prepared fibroblast cell culture media
- Aspirator

Methods: For 1 flask *

1. Put media in incubator to warm
2. Meanwhile:
    a. Check cells under microscope
3. Aspirate all media
    a. Do NOT touch bottom of flask with aspirator, only the side
4. Add 10 mL of media
5. Check cells under microscope
6. Return flask to incubator

Appendix C: Cell Passaging Protocol

Objective: Passaging cells

Materials:

- Prepared fibroblast cell culture media
- 15 mL conical tube
- 1 mL pipette
- Trypsin
- Aspirator
- DPBS (-)
- Big flask

Methods: For 1 flask *

1. Warm prepared media and trypsin in the incubator for around 5 minutes
2. Meanwhile, check cells under a microscope
3. Aspirate media from plate
4. Add 5 mL DPBS (-) to the plate and quickly and gently mix
5. Aspirate media from plate
6. Add 2 mL of trypsin and swish around quickly
7. Aspirate
8. Add 3 mL of trypsin and let sit for about 1 minute (under one minute though)
9. Add 9 mL of media to neutralize trypsin
    a. Rinse the flask with its own media with a 1 mL pipette
    b. Smack flask hard a few times to encourage cell lifting
10. Check cells under the microscope to make sure that nearly all the cells are lifted (floating)
11. Pipette contents of the flask into a 15 mL conical tube
12. Centrifuge for 5 minutes at 200 rcf
    a. Remember to balance!
13. Meanwhile waiting for centrifuge:
    a. Add media to the new flask (8 mL)
14. CAREFULLY aspirate media leaving cell pellet at bottom
    a. Leave a little bit of media if needed but do not touch the pellet
15. Add 8 mL of media to the 15 mL conical tube with cell pellet to resuspend cells
    a. Thoroughly mix by reverse pipetting with 1 mL pipette
16. At this point, if the flask was confluent there should be around 8 million cells
    a. Add 2 mL to the prepared flask with 8 mL of media
17. Check the cells under microscope and return to incubator

Appendix D: Cell Seeding Protocol

Objective: Create Tissue Rings

Materials:

- 24-Well Plate
- Model Mold
- 2% Agarose
- MDA Cells suspension
- Fibroblast cell suspension
- Media

Volumes:
Square = 26µl
Hexagon = 23µl
Circle = 20µl
Triangle = 19µl

Methods:

1. Create 2% agarose and autoclave it to sterilize the medium.
2. Add 0.7mL of agarose into 24-well plate
3. Set mold negative into the well and allow agarose to set for 10 minutes
4. Passage both the fibroblast and MDA cells and suspend them 1,00,00 cells/mL
5. Add cell suspension in 1mL increments to a tube to create mixture at proportions of 1:2, 1:3, 1:4 MDA to fibroblast cells
6. Seed the cell mixtures at the volumes corresponding to each shape listed above
7. Cover the wells and store in incubator for 48 hours
8. Check and image cells every 48 hours to change media and monitor tissue ring formation