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Design of a Bioreactor for Myocardial Patch Generation

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Design of a Bioreactor for Myocardial Patch Generation

A Major Qualifying Project Report:
Submitted to the Faculty
Of the
WORCESTER POLYTECHNIC INSTITUTE
In partial fulfillment of the requirements for the
Degree of Bachelor of Science
by

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April 30, 2009

Approved:

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Professor Marsha Rolle, Advisor

1. Bioreactor
2. Cardiac Patch
3. Cell-derived
Abstract
Heart disease is the leading cause of death in the United States, leaving the afflicted with lost or damaged cardiac tissue. Cardiac patches can be used to repair or replace this tissue. Current methods include using synthetic and extra cellular matrix patches. Completely cell-derived patches seek to improve on current methods, but the production processes are time-consuming and labor-intensive.

The goal of this project was to design a bioreactor to address these problems by generating multiple totally cell-derived patches quickly, consistently, and with minimal effort. A bioreactor was designed that allows the production of tissue patches without user intervention. The device contains a volume of media sufficient for extended culture periods to feed the cells. A controllable media distributor circulates media throughout the system that has the ability to generate up to 12 tissue patches per trial. This also allows multiple culture materials to be tested at once, depending on the needs of the user. This automated versatile device fits in a tissue culture incubator and is compatible with a variety of human cell types for clinical and experimental use.
Acknowledgments

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Cell Culture..................................................................................................................EG
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1 Introduction

Heart disease is the leading cause of death for both men and women in the United States. There are several diseases that can lead to loss or damage to myocardial tissue. Three of these diseases are myocardial infarction (MI), hypertrophic cardiomyopathy and congenital defects. These diseases are prevalent; there are 565,000 new cases of MI annually, one in 500 individuals suffers from hypertrophic cardiomyopathy, and 650,000 to 1,300,000 people in the United States are born with congenital cardiovascular defects. (American Heart Association, 2008)

Patches can be used to repair these defects and restore the natural shape of the heart. Synthetic patches such as Dacron and polytetrafluoroethylene (PTFE), or extracellular matrix (ECM) patches can be used. There are a number of disadvantages associated with each of these patch types. Synthetic patches are much stiffer than natural heart tissue; the modulus of elasticity is at least 4 orders of magnitude greater than natural myocardium. Because of this a Dacron patch would “tether” the heart muscle and reduce the mechanical function of the surrounding myocardium. This can reduce both diastolic and systolic function. (Kochupura, 2005) In pediatric patients, these patches are unable to grow with the patient, and additional surgeries are required. (Leor, 2005) ECM patches are comprised of decellularized tissue, often from non-human sources. Complications with these patches can include calcification and inflammatory response (Kofidis, 2002)

Patches that are totally cell-derived would solve many problems associated with current patches. These patches would have the same mechanical properties (ultimate tensile strength, compliance etc.) as the original tissue (Leor, 2005), could potentially be grown from the patient’s own cells, would be biocompatible, and would eliminate rejection.

Unfortunately, producing these cellular patches is challenging. The sheets can be cultured in a flask, but this method can take up to 30 days. (L'Heureux, 1998) Other culture methods have been researched that allow the patch to be easily removed from the culture surface. Unfortunately, the cell
sheets cultured with this method are only one cell thick and multiple sheets must be layered on top of each other to create a sufficiently thick patch. This is a time-consuming and labor-intensive task. (Shimizu, 2002) A bioreactor has the potential to reduce culture time and produce thicker cellular sheets.

The goal of this project was to produce stable, totally cell-derived tissue patches, quickly and consistently. The primary objectives were to: provide automated nutrient delivery, develop reproducible results, allow for easy cell sheet removal, produce high throughput patch generation, and be easy to use. The bioreactor needed to perform the following functions: generate a cell sheet, seed cells, feed cells, easily release the resulting patch, and allow for user monitoring. The specifications were that the bioreactor must: generate a cell sheet that is at least 15 mm in diameter, fit in a standard incubator, and produce more than five samples per trial.

In order for the bioreactor to perform these functions, means for both the patch and the bioreactor were determined. To grow cells into a patch, a support that allows and encourages cell self-assembly is necessary. After a review of literature, two different options were decided upon: Transwells and Nitex/polyethylene constructs. Transwells are individual culture columns with porous membranes at the bottom that trap cells inside the column while allowing the passage of media through the filter. The Nitex are similar to Transwells, in that they contain a filter material for cell culture, while allowing the passage of media. Additionally, the Nitex mesh is adhered to porous polypropylene rings that anchor the cells, and include a fibrin gel that acts as a temporary surface, assisting with and encouraging cell attachment and allowing for easier removal of the resulting sheet. Both are accepted cell culture materials were chosen to meet these criteria.

The final design consists of a base which acts as a media reservoir, inserts to hold the chosen culture materials, and a lid which includes a flow distribution system. All of these components were
 machined of polycarbonate. The base is designed to hold enough media to support cell life for an extended period of time (greater than one week), and fit within the incubator.

The lid acts as an irrigation system, containing troughs that distribute the media to each sample. The media is circulated from the reservoir in the base to the lid with a peristaltic pump.

This bioreactor has the ability to culture cell sheets for prolonged periods of time without user intervention. It can be used to test multiple culture materials in one period or produce up to twelve samples per trial. Overall it will provide a method to generate a high number of tissue sheets with minimum effort by the user.
2 Literature Review

2.1 Heart Disease

Cardiovascular disease (CVD) is any disease or defect that affects heart or blood cell function; more than 80 million adults are diagnosed with a form of CVD. Heart disease is the leading cause of death in the United States as nearly 2400 Americans die from it each year. (American Heart Association, 2008) Common symptoms include chest pain (angina), shortness of breath, and pale grey skin. The most common form is myocardial infarction (heart attack) and these occur in 8,100,000 American adults. Also, a significant number of people, 650,000-1,300,000 in the United States, are born with a congenital cardiovascular defect. (American Heart Association, 2008) Treatment options vary depending on the type of CVD that the patient is diagnosed with.

Myocardial infarction occurs when a blockage develops in a coronary artery, preventing the blood from adequately being supplied to the myocardium. Without the necessary oxygen or nutrients to survive, myocytes enter a state known as ischemia, and begin to die. This can lead to a heart attack and progressive heart failure as the heart muscle is not longer able to function. The heart is incapable of self regeneration therefore medical intervention is necessary. (Leor, 2005) If the heart is significantly damaged and life style changes and medication are ineffective, a standard method is to replace the heart tissue, either with a tissue engineered cardiac patch or a complete heart transplant (American Heart Association, 2008) (Atkins, 2002).

An approach to cardiovascular repair involves the implantation of a cardiac patch. An example of this type of procedure is the Endoventricular Circular Patch Plasty or the DOR procedure. This method removes the damaged portions of the anterior wall and septum of the heart. The left ventricle is then reshaped using sutures and a synthetic patch is implanted on the ventricular wall. (Sartipy, 2005) The
primary the goal of these patches is to simply restore the natural shape of the heart, and to repair the continuity of the ventricular wall.

Congenital defects also often require surgery to render improved cardiac function. Approximately 28.3% of ventricular septal defects (a congenital birth deficiency) could also be treated with a vascular patch. (Kofodis, 2002) Currently, clinically available patches are composed of synthetic materials such as expanded polytetrafluoroethylene (ePTFE), or polyethylene terephthalate (PET). Another category of patches utilizes extracellular matrix (ECM) as their basis. These vascular patches are derived from porcine small intestinal submucosa and urinary bladder matrix (UBM), and have undergone preclinical experimentation as vascular grafts. (Robinson, 2005)

Synthetic patches do not promote native cell in-growth. Instead they incite biological reactions including inflammatory response (Kofidis, 2002) and scar tissue formation. (Robinson, 2005) Both of these results are not ideal for improved heart function. The natural material that is currently used is treated bovine pericardium and utilizing animal products in human treatments is also less than ideal. A patch that promoted cell in-growth and was totally cell-derived could be the perfect substitute for natural human tissue.

2.2 Cardiac Patches

There is a considerable need for implantable cardiac patches that could replace damaged heart tissue. The patch must be biologically and functionally comparable to native heart tissue. Scaffolds are often utilized to achieve such patches.

Synthetic scaffolds that have been approved for clinical use in the United States include expanded Polyethylene terephthalate (PET) and Polytetra-Fluoroethylene (ePTFE), commonly known as Dacron and Teflon. Both Gore Medical Products and Bard Peripheral Vascular produce commercially
available ePTFE patches. Bard Peripheral Vascular and Boston Scientific produce PET cardiac patches.

Commercially available patches are shown below in Table 1.

Table 1 - Commercially Available Patches

<table>
<thead>
<tr>
<th>MATERIAL</th>
<th>PRODUCT</th>
<th>FEATURES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expanded Polytetrafluoroethylene (ePTFE)</td>
<td>Gore-Tex ACUSEAL cardiovascular patch (Gore Medical Products, Inc.)</td>
<td>Significant reduction in suture hole bleeding</td>
</tr>
<tr>
<td></td>
<td>Cardiovascular Patch (Bard Peripheral Vascular, Inc.)</td>
<td>Multi-directionally oriented node-fiber structure accommodates cellular ingrowth</td>
</tr>
<tr>
<td>Polyethylene terephthalate (PET)</td>
<td>DeBakey cardiovascular fabrics (Bard Peripheral Vascular, Inc.)</td>
<td>Wide range of configurations from which the surgeon can select for each individual case</td>
</tr>
<tr>
<td></td>
<td>Hemashield Double Velour Fabrics (Boston Scientific, Inc.)</td>
<td>Collagen impregnation provides blood tight seal and optimal tissue ingrowth</td>
</tr>
<tr>
<td></td>
<td>HemaPatch Knitted (DataScope, Inc.)</td>
<td>Coated with cross-linked type I bovine collagen</td>
</tr>
<tr>
<td>Glutaraldehyde treated bovine pericardium</td>
<td>PeriGuard (Synovis Surgical, Inc.)</td>
<td>Improved suturability and handling over synthetic patches</td>
</tr>
<tr>
<td></td>
<td>SJM Pericardial Patch with Encap technology (St. Jude Medical, Inc.)</td>
<td>Proprietary coating minimizes calcification and promotes endothelial covering</td>
</tr>
</tbody>
</table>

(Filipe, 2007)

Dacron and Teflon patches do not degrade and their synthetic origin induces a thick layer of scar tissue around the implant, reducing ventricular contraction. Dacron patches have been seeded with cells to provide a regenerative scaffold environment and achieve better integration with the surrounding tissue. However, the differences in mechanical properties between the patch and natural heart tissue remain a significant issue. (Gaudette, 2006)

Tissue constructs consisting entirely of cell-derived matrix are best suited for implantation because they are biologically and functionally comparable to native tissue. (L'Heureux, 1998) Research has recently focused on generating tissue constructs based on the use of cultured human cells without any synthetic or exogenous biomaterials. This research utilizes the tendency of dermal fibroblasts to self assemble and produce a sheet when cultured with ascorbic acid. Ascorbic acid treatment is also
used during culture since it stimulates collagen production, and improves mechanical properties such as stiffness. (L'Heureux, 2007)

The most significant constraint of cell-derived patches is the time it takes for the cells to produce matrix and self-assemble into a sheet. Many factors can be optimized to reduce the production time: initial cell concentration, culture medium components, and media selection. (L'Heureux, 1998) A method to decrease culture and maturation period is through the use of an appropriate bioreactor which could generate a compliant scaffold to replace the infracted heart tissue. (Martin, 2004) In this instance, compliance is referring to mechanical properties, specifically the material’s ability to withstand the stresses and strains placed on the heart during normal function. (Arrigoni, 2008) It is accepted that static culture is not sufficient to produce tissue that is adequately functional in terms of cell proliferation, cell differentiation, ECM production and tissue organization. (Arrigoni, 2008) Bioreactors seek to address this issue by more closely mimicking the natural environment than static culture.

2.3 Bioreactors

Producing tissue engineered substances can be difficult and costly; therefore devices have been developed to aid this process. Bioreactors are devices that control environmental and operating conditions to aid biological and biochemical mechanisms such as cellular alignment and ECM production. (Zhao, 2004) These devices allow experimental variable control and increased production capabilities. Currently in tissue engineering it is common to utilize a scaffold material in a bioreactor as a surface for seeding cells.

The seeding process in a bioreactor is critical. The cell distribution can have significant effects on the properties of the engineered material. (Martin, 2004) This provides a significant challenge, and multiple methods have been developed to address it. Statically seeding or manually placing the cells in the media is a common and fairly simple method, but it has several disadvantages. Studies have shown
that this method exhibits low seeding efficiencies onto scaffolds due to user error and inconsistencies (Martin, 2004). Another approach to seeding involves a stirred flask bioreactor in which cells suspended in media surround a stationary scaffold. Unfortunately, this method does not allow uniform cell seeding between the surface and the interior of the scaffold. (Martin, 2004) To seed the interior of a surface, cells and media can be directed through the scaffold. This final method is referred to as perfusion seeding. (Martin, 2004)

The basic method of culturing cells culture is static culture but, while it is effective, it is very limited. Nutrient and oxygen delivery is limited by diffusion; any cells outside of this diffusion range become apoptotic. Apoptosis is when cells are unhealthy or problematic, and self destruct. Static culture also does not realistically mimic the mechanical in vivo environment. In vivo, the construct is subjected to forces such as: shear force, compressive stress and tensile stresses. These forces affect tissue growth, and ideally they should be replicated in vitro. Bioreactors can help solve these issues by utilizing dynamic cell culture methods, such as perfusion or rotation. (Martin, 2004)

Perfusion cell culture involves the cell media being forced to flow through the tissue culture chamber. This flow can create excessive shear forces that are detrimental to cells in a tissue culture environment. To address this, the flow rate must be carefully calculated. (Martin, 2004) Studies have shown that perfusion bioreactors can enhance a variety of factors including growth and differentiation, and can deliver a significantly larger cell density than other static methods. (Martin, 2004) Another advantage of a perfusion bioreactor is that cell seeding and nutrient delivery systems can become automated. (Martin, 2004) This removes inconsistencies associated with human error, but has the additional concern of potential mechanical failure. Automated systems are also adaptable for multiple, independent samples. This involves multiple chambers to allow the growth of several engineered tissue constructs in a single experiment. (Zhao, 2005)
A rotating bioreactor is another alternative. This type of bioreactor involves a cellularized construct that is immersed in culture medium, and placed in a rotating chamber. (Arrigoni, 2008) The theory of this device class is that it allows a more uniform oxygenation of the cells than static seeding, but applies significantly lower levels of shear stress than a perfusion bioreactor. It is also mechanically simpler than a perfusion bioreactor, which can be advantageous in a research environment (Arrigoni, 2008). Media delivery is typically not automated, but can be achieved using a simple pipette media exchange method.

While current bioreactors have improved cell culture techniques there are still limitations. They are labor intensive, have long culture periods, and are expensive. Bioreactors commonly use a scaffold to seed cells onto. A bioreactor that is easy to use has a shorter culture time, and produces totally cell-derived sheets.
3 Project Strategy

Initially the team received the following client statement:

*Self-assembled cell sheets have been used to fabricate a variety of engineered tissues, including vascular grafts and patches for cardiac repair. One of the limitations of this approach is the amount of time required to culture sheets of sufficient mechanical integrity for manipulation and transplantation. The goal of this project is to design a novel bioreactor system to rapidly and reproducibly fabricate and culture mechanically stable cell-derived tissue sheets.*

Before the team could formulate a design, an iterative design process was followed. First the stakeholders were identified. From these stakeholders, the project objectives, specifications, constraints, and functions were formulated. These criteria are critical to ensuring that the designed product meets the client’s needs thoroughly. It also establishes the parameters in which the design must stay. From these criteria, a new client statement was formed and provided the basis upon which the design alternatives were formed.

3.1 Stakeholders

The stakeholders were defined as the clients, the designers, and the users as shown in Figure 1.

![Figure 1: Project Stakeholders](image-url)
The main stakeholders in this project were Dr. Marsha Rolle and the design team. These individuals determined the objectives and functions of the new bioreactor and the tissue sheet output.

The primary users of this bioreactor and its tissue sheet products will be Dr. Marsha Rolle and the graduate students working in her lab. Their main concern was that they needed a method to create a cell sheet patch with adequate mechanical properties quickly and easily.

The clients that were considered were the same group as the users. Eventually the clients of this patch may extend to cardiac surgeons implanting the patch in patients, but that is currently beyond the scope of this design project.

3.2 Objectives

Based on the feedback from the stakeholders of this project and conducted research, the team generated design objectives to guide the design process. The main level objectives and secondary level objectives are listed below.

Objectives

- Automated Nutrient Exchange
- Reproducible
  - Mechanical Properties
  - Sheet Dimensions
- Easy to Use
  - Easy to Maintain
  - Easy to Monitor
  - Easy to Assemble
- Easy Cell Sheet Removal
  - Handling
  - Packaging
  - Removal from Bioreactor
- High Throughput
  - Separate Chambers
  - Generates Multiple Sheets
3.2.1 Automated Nutrient Delivery

Automated nutrient delivery was an objective for this project because the cells require constant nutrients to survive. Additionally, automation could increase reliability and reproducibility of results.

3.2.2 Reproducibility

Reproducibility was a critical objective for this project. If the design yields a device that can generate a sheet, but the sheets are not consistent then the bioreactor has not fulfilled its purpose. These sheets could not be used for further research as there would be lot to lot variability. The sheets must be consistently reproducible in terms of mechanical properties and dimensions. Mechanical properties must be uniform in every experiment as to not pose an undue risk of failure after implantation.

3.2.3 Easy to Use

The next major objective focuses on ease of use. If the device is too difficult to actually use in a laboratory setting, it will not be used and will have failed in its purpose. The device must be maintainable as it is designed for long-term use. If it fails and cannot be fixed easily, then research will be unduly slowed down or it will be forced to move in another direction. Failure also introduces financial concerns; the cost of manufacturing becomes superfluous if the machine does not work, replacing parts can become very pricey, and loss of materials can also add up quickly.

The bioreactor should also allow monitoring of the cell sheet during cell culture. It will allow users the ability to check on the progress of sheet growth and notice any unusual developments that would affect the final product.

The bioreactor should also be easy to assemble. More difficult assembly may be acceptable under the condition that assembly is only required to occur once. If repeated assembly is required, then it must be user-friendly. Should the user deem that setting up the device is more difficult than its
potential benefits, they will find alternate means of creating the cell sheet, and this device will not be used.

3.2.4 Easy Cell Sheet Removal
Another major objective is easy cell sheet removal. After removal the sheet must be sturdy enough to handle as it will either undergo further testing or implantation. A sheet that is too delicate for future manipulation is not useful to the user or client. Also, the cell sheet must be removed from the device intact for both research testing and future implantation. If the sheet is compromised during the removal process, then any time and effort spent culturing the sheet was wasted.

3.2.5 High Throughput
Throughput is the ability to produce sheets rapidly or in large numbers. To increase productivity of the bioreactor, it should output more than one cell sheet per culture period. This will decrease variability between batches for research purposes as there will be more sheets cultured under the same conditions. This method will also decrease time needed for experimentation as users will not have to wait separate cell culture periods for multiple sheets. All cell sheets must be consistent in terms of mechanical properties and dimensions. Ideally, multiple sheets will be cultured in separate chambers with an independent nutrient delivery system to all chambers. This would prevent one faulty chamber from ruining every sheet being cultured that cycle.

3.3 Weighted Objectives
Following the development of primary and secondary objectives, the team had to ascertain the importance or weight of each primary and secondary objective. To accomplish this, the team created an objective tree that showed the relationship between the primary and secondary objectives. To establish the importance of each objective, the team met with the client, Dr. Marsha Rolle, and pairwise comparison charts were generated for all primary and secondary objectives which can be seen in
Appendix A. The results of the pairwise comparison charts were analyzed, and the objectives were weighted accordingly. The objectives and their weights can be seen in the objective tree in Figure 2.

The weighted objectives showed the importance of each objective in regards to the other objectives. The weights showed that the objectives should be addressed in the following order:

- Reproducible
- Throughput (>1 sheet)
• Easy Cell Sheet Removal
• Easy to Use
• Automated Nutrient Delivery

3.4 Functions

After analyzing the design objectives, a list of functions was established. As this project is comprised of two major components (the bioreactor and the cell sheet output), a list of functions specific to each were developed. The following lists show the functions the bioreactor must accomplish and the functions the cell sheet must realize in order to achieve the established objectives.

**Bioreactor**

• Generate a cell sheet
• Seed cells
• Feed cells
• Easily release sheet
• Allow user monitoring

**Cell Sheet**

• Produce matrix
• Exhibit enough strength to hold tissue together
• Interface with host body tissue similarly to natural cells

The overall goal of the project was to create a bioreactor that outputs a mechanically strong, thick cell sheet. There are not currently bioreactors that easily output, in one step, cell sheets greater than a few cell layers thick. To accomplish this goal, the bioreactor must be able to perform the established functions. If the bioreactor does not output a cell sheet, then achieving the projects goal is impossible and the design will have failed. A critical function is delivering the cells into the device and retaining them in the chamber. Cells are living and if they are not fed, then they die. Therefore the bioreactor must be able to deliver nutrients. The bioreactor also must not leak or there will be problems with both sterilization and nutrient delivery. The bioreactor must also assemble the cells into tissue or the output will not be a cell sheet. The sheet must be able to be removed from the bioreactor
so the bioreactor’s cell culture chamber must come apart to facilitate cell sheet removal. Also, it must allow monitoring by the user to optimize culture periods and evaluate cell growth over the cell culture cycle.

The sheet must possess appropriate histological organization to achieve adequate tissue integrity. Both cellular alignment and ECM production are necessary to achieve adequate structural stability. If the cell alignment is abnormal then this can lead to functional defects in the tissue. (Grenier, 2005) Also, lacking structural stability will lead to mechanical failure. If the tissue fails mechanical testing, it will not have enough strength to hold tissue together and will not be able to be applied as a cardiac patch. The cell sheet must also interface with host body tissue similarly to natural cells, to prevent cell death, additional damage to native tissue, or rejection of the patch.

3.5 Constraints

In order to reduce the design space for this project and ensure that the client’s needs were met, constraints were established. The constraints were as follows:

**Constraints**

- **Size**
  - The bioreactor must be able to fit inside an incubator
- **Sheet Size**
  - The sheet size must be appropriate to developed testing devices
- **Cell-derived**
  - The sheet must be cell-derived and the final product contain only cells and cell-derived extracellular matrix
- **Implantable**
  - The sheet must be implantable and therefore biocompatible
- **Sterilize-able**
  - The bioreactor must be able to be sterilized
- **Time**
  - The project must be completed by April 2009
- **Budget**
  - The project cannot cost more than the allotted project budget of $624
The constraints were consulted when evaluating alternative designs. If a design violated an established constraint, it was then rejected as undesirable. The bioreactor must be able to fit inside an incubator since cell culture requires specific environmental controls and the best way to maintain this is an incubator. The cell sheets will be tested by a mechanical testing device that has already been developed. (Ahlfors, 2007) In order to use this device, the sheet must be of an appropriate size. Unlike other current products, this sheet must be completely cell-derived. This will eliminate unwanted complications with scaffolds, and allow the research of solely cells rather than scaffolds and cells. The goal of this project was to produce a cardiac patch; therefore the sheet must be sterile, biocompatible and appropriate for implantation. Finally the design must be within budget and completed by April 2009.

### 3.6 Specifications

Establishing specifications was an important step in the design process. Specifications help focus the design process, by assigning numerical values to assist in the realization of objectives. The specifications for the bioreactor design can be found in Table 2. The cell sheet must be at least 15mm in diameter. This size is the minimum diameter that is necessary for mechanical testing protocols. The bioreactor must fit in an incubator with 3 inch shelves. This bioreactor will be used in labs with this size limitation, and for the bioreactor to be useful it must meet this specification.

<table>
<thead>
<tr>
<th>Specification</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Output a Cell Sheet</td>
<td>Greater than 15 mm in diameter</td>
</tr>
<tr>
<td>Bioreactor Size</td>
<td>Must fit in an incubator, 3 inch shelf size</td>
</tr>
<tr>
<td>Sample Size</td>
<td>Must generate greater than 5 samples per trial</td>
</tr>
</tbody>
</table>
The cell sheet must be at least 15mm in diameter. This size is the minimum diameter that is necessary for mechanical testing protocols. The bioreactor must fit in a standard incubator – a fairly standard shelf height is 3 inches. This bioreactor will be used in labs with this size limitation, and for the bioreactor to be useful it must meet this specification. Additionally, to achieve high throughput, each run of the bioreactor must produce at least 5 samples per trial.

### 3.7 Revised Client Statement

The objectives of this design are to design a bioreactor that is:

- Reproducible
- Allows easy cell sheet removal
- High Throughput
- Easy to use
- Automated (nutrient delivery)

The functions of this design are to:

- Generate a cell sheet
- Accept/support cells
- Feed cells
- Assemble cells tissue into a sheet
- Easily release sheet
  - Chamber must come apart
  - Chamber must not leak

The constraints of this design are:

- The bioreactor must be able to fit inside an incubator
- The sheet size must be appropriate to developed testing devices
- The sheet must be cell-derived and the final product contain only cells and cell-derived matrix
- The sheet must be implantable and therefore biocompatible
- The bioreactor must be able to be sterilized
- The project must be completed by April 2009
- The project cannot cost more than the allotted project budget of $624

The specifications of this design are:

- Output a cell sheet in the following dimensions: Diameter greater than 15 mm
- Fit in incubator: shelf height 3 in
Sample size: greater than 5 samples/trial

4 Design Alternatives

After determining the client’s needs and wants, and effectively determining the objectives, specifications, functions, and constraints of the project, it was necessary to begin developing alternate designs. The first step was to determine the means to achieve the specified functions. These means allowed focus of potential materials and methods into different conceptual designs, which were evaluated for feasibility and optimization.

4.1 Means

Once the functions were determined, it was necessary to determine the means to accomplish them. The functions were determined to be that the bioreactor generates a cell sheet, by seeding the cells, feeding the cells, easily releasing the final tissue sheet and allowing for user monitoring. A functions-means table, to list and compare the potential solution elements for each requirement, was created. The results from this can be seen in Table 3.

<table>
<thead>
<tr>
<th>Functions</th>
<th>Means</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed Cells</td>
<td>Perfusion</td>
</tr>
<tr>
<td>Feed Cells</td>
<td>Perfusion</td>
</tr>
<tr>
<td>Generate a Cell Sheet</td>
<td>Surface Modification Patterning</td>
</tr>
<tr>
<td>Easily Release Sheet</td>
<td>Temperature Sensitive</td>
</tr>
</tbody>
</table>

Some of the means defined in the Function-Means Table were immediately dismissed, due to constraints, such as time, expertise, materials, etc. For example, centrifugation and rotation, when considered in light of the constraint that the bioreactor must fit within an incubator, were determined to present more of a challenge than some of the other options. It was determined that creating a
bioreactor that could accomplish those means within the constraints would take unnecessary time.

Similarly, the temperature sensitive material, upon further inquiry, appeared to be more difficult than helpful in the grand scheme of the project.

Additionally, certain means were common to multiple functions and it was determined that certain means for each function were not exclusive, and had the potential to be combined to better solve the problem. Greater weight was given to those means that would more efficiently accomplish the objectives. The common means are highlighted in Table 4.

<table>
<thead>
<tr>
<th>Functions</th>
<th>Means</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed Cells</td>
<td>Perfusion, Rotation, Centrifugation, Degradable Surface Coating, Static/Perfusion, Centrifugation/Perfusion, Column, Static</td>
</tr>
<tr>
<td>Feed Cells</td>
<td>Perfusion, Static Feeding, Osmosis, Column, Gravity</td>
</tr>
<tr>
<td>Generate a Cell Sheet</td>
<td>Surface Modification, Limit Space, Non-adhesive material</td>
</tr>
<tr>
<td>Easily Release Sheet</td>
<td>Temperature Sensitive, Surface Coating, Surface Modification, Non-adhesive Material, Modular</td>
</tr>
</tbody>
</table>

Once the means had been filtered down to the most possible and most efficient, the remaining means were re-considered, compared and weighed, until only the best, most feasible options remained. The means decided upon to satisfy the functions are described in Table 5.

<table>
<thead>
<tr>
<th>Function</th>
<th>Means</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed Cells</td>
<td>Degradable Surface Coating, Perfusion</td>
</tr>
<tr>
<td>Feed Cells</td>
<td>Perfusion, Column</td>
</tr>
<tr>
<td>Generate Cell Sheet</td>
<td>Limit Space, Surface Modification, Non-adhesive Material</td>
</tr>
<tr>
<td>Easily Release Sheet</td>
<td>Surface Coating, Surface Modification, Non-adhesive Material</td>
</tr>
</tbody>
</table>

The final means being investigated were combinations of common means between functions...
and combinations of means within functions that seemed to offer the best options. For the bioreactor to allow the seeding cells into it, a degradable surface coating, such as fibrin gel, would be used concurrently with perfusion. Perfusion would also allow the bioreactor to efficiently deliver media to the cells to accomplish feeding them. A column-like shape would also help make this process more effective, by gathering the cells in one location and taking advantage of gravity to assist with media perfusion. This shape would limit space, assisting with the generation of a cell sheet; by restricting the surface area that the cells can spread out across, density of the cells per unit area is higher. This allows for more cell-to-cell contact, which increases attachment, and generates a thicker sheet. To further allow the bioreactor to generate a cell sheet, surface modification and a non-adhesive material were used, to help encourage the cells to attach to each other instead of the culture material surface. These qualities of that surface, as well as some sort of coating, would also permit easy release of the sheet, with minimal damage, as the cells will have primarily adhered to each other and only minimally to the surface material.

4.2 Conceptual Designs

Once the means were narrowed down, conceptual designs were developed to determine feasibility and materials. With so many potential means to realize the necessary design functions, multiple designs were considered. Each of the alternative designs was developed by examining various combinations of the described solution elements.

Initially, it was necessary to find materials for the cells to be seeded onto that would encourage attachment as well as permit easy release. As defined previously, these materials would need to be non-toxic, sterilizable, and easily release the sheets after culture. Preferably, these materials should also be easy to obtain, commonly used, and fairly inexpensive.
The next step was to design housing for the culture materials. The initial design was a simple horizontal perfusion box, involving horizontal flow of media across the culture materials. A diagram of this design alternative can be seen in Figure 3.

![Figure 3: Conceptual Design - Box](image1)

The next alternative was a column shape. This was designed for a number of reasons. To begin with, the column was determined to be a means that would assist in generating a sheet, while at the same time providing a means to feed the cells – utilizing gravity. Additionally, many common culture materials have a round shape, for example, both the Transwells and NPRs mentioned earlier are round. This alternative can be seen in Figure 4.

![Figure 4: Conceptual Design - Column](image2)
This conceptual design was developed further after referencing the design objectives, specifically automated nutrient exchange and high throughput. Housing with multiple chambers was designed, allowing for a greater number of samples per trial. Additionally, a media distributor was incorporated, to assist with nutrient delivery. This conceptual design can be seen in Figure 5.

**Figure 5: Conceptual Design – Complex**

### 4.3 Materials

In order to move from a conceptual design to an actual design – to move from the theoretical to the physical – it was necessary to determine what materials to use. Given the multiple components of the bioreactor, even in the conceptual stage, the list of potential materials was fairly long. To organize the possibilities, the materials for each component were arranged below, in Table 6. The materials for each component are discussed in the following sections.

<table>
<thead>
<tr>
<th>Component</th>
<th>PC</th>
<th>PP</th>
<th>PMMA</th>
<th>Stainless Steel</th>
<th>Glass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body of Bioreactor</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chamber for media &amp; cells</td>
<td>PC</td>
<td>PP</td>
<td>PMMA</td>
<td>Alumina</td>
<td>Stainless</td>
</tr>
<tr>
<td>Coating to Prevent Cell Adhesion</td>
<td>FEP membranes</td>
<td>Sigmacote</td>
<td>PIPAAm</td>
<td>Cellulose esters</td>
<td>PTFE</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>---------------</td>
<td>-----------</td>
<td>--------</td>
<td>------------------</td>
<td>------</td>
</tr>
<tr>
<td>Degradable Coating</td>
<td>Fibrin</td>
<td>Collagen</td>
<td>Hyaluronic acid</td>
<td>Agarose</td>
<td></td>
</tr>
<tr>
<td>Tubing</td>
<td>Silicon</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Media</td>
<td>DMEM – 10% Bovine Serum &amp; Pen Strep</td>
<td>DMEM +Ascorbic acid</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Acronyms in the above table are as follows: PC (polycarbonate), PP (polymethylmethacrylate), FEP (fluorinated ethylene-propylene), PIPAAm (poly (N-isopropylacrylamide)), and PTFE (polytetrafluoroethylene)

4.3.1 Body of Bioreactor

It was decided that the bioreactor housing was to be manufactured out of polycarbonate and acrylic. These materials were chosen because they can be autoclaved (sterilized), are durable, clear (allowing user monitoring), and reasonably available and priced. (Dynalab Corp.) It was also decided that a flow distributor for media delivery would be manufactured out of a polycarbonate sheet, with multiple holes to distribute the media evenly throughout the bioreactor. Stainless steel screws will be used to attach the cover to the base, because they are strong and sterilizable, as well as easily obtained and fairly inexpensive.

4.3.2 Chamber for Media & Cells

The chamber for media and cells was incorporated into the bottom of the chamber to create a media reservoir. This removed any need for an external media source, instead storing it in the bottom of the base itself. This, as stated above, was to be machined of acrylic and polycarbonate.

4.3.3 Culture Materials

Various coatings and materials which would allow easy cell sheet release were investigated. Fluorinated ethylene propylene has been used in previous studies to prevent cells from adhering to the
surface. Covering the surface with a temperature-sensitive polymer would also allow easy sheet removal and has also been considered. (Matsuda, 2007) (Shimizu, 2002) These were dismissed because their porosity was too low to allow the necessary diffusion of media.

Transwells are plastic cylinders with a permeable membrane at the bottom. The cells are seeded and cultured on the membrane. Mainly Transwells made of PTFE and mixed cellulose esters have been used for more effective cell growth and easy cell sheet removal. Two Transwells considered were a Biopore™ (PTFE) and a MF-Millipore™ (mixed cellulose esters). Each of these options has unique characteristics that better recognize different objectives.

**Biopore™ (PTFE)**

Teflon (PTFE) membranes, specifically, the hydrophilic PTFE with 0.45 micrometer pore size is commercially available under the name BioporeTM from Millipore.

![Figure 6: Biopore™ (PTFE) membrane](image)

It has been selected for its low protein binding properties which are very useful for cell growth. Protein binding is essential for cell attachment; therefore a surface with low protein binding will allow the cells to adhere, but not in an irremovable manner. The rounded dermal fibroblasts have a diameter of 20 μm. This pore size should permit the diffusion of media and should retain the cells inside the chamber but this will have to be further investigated experimentally. (Harley, 2007)

**MF-Millipore™ (mixed cellulose esters)**
MF-Millipore™ membrane filter is composed of biologically inert mixtures of cellulose acetate and cellulose nitrate. The material was selected for its non-adhesive properties which should allow easy sheet removal. The same pore size as for PTFE membrane (0.45µm) was selected based on cell size. However, the pore structure of the Millipore membrane is different from PTFE and the material should be more permeable and allow easier media perfusion.

There are a number of potential challenges with using these membrane filters. First, the pore size (0.45 µm) might be too small to allow sufficient media outflow. Second, the pores in the membrane could potentially become constricted or clogged by the cells, which would completely disable media outflow.

*Nitex/Polyethylene Constructs*

Nitex mesh was also examined as an alternative material. Nitex is a nylon mesh that does not degrade and is autoclavable. It is widely accepted as a material compatible with tissue culture. The pore size (50-350 µm) was large enough to eliminate any potential clogging, however the high porosity could impose some difficulties with cell seeding. To make the Nitex mesh more manageable and help with sheet development, porous polyethylene rings were adhered to either side of circular pieces of Nitex mesh, creating a better culture surface for the cells. Additionally, the porous rings would act as anchors, encouraging the cells to grow into them. This would help reduce contraction when the sample was to
be harvested. A diagram of these Nitex/polyethylene constructs can be seen below, and will be referred to as NPRs from this point forward.

Figure 8: Diagram of NPRs

4.3.4 Degradable Coating

To assist with seeding the cells, generating a cell sheet and also easily releasing that sheet, a degradable coating was considered. The basic idea was that a material would be present at culture that would promote cell aggregation and proliferation from the time of seeding. However, this material would have to be gone by the time the sheet was harvested, to ensure that the result was completely cell-derived. So, the concept of a ‘sacrificial template’ was investigated – a material that would be present at the beginning of culture to help encourage the cells to attach, but would degrade over time, and be completely gone by the time the sheet was release. A fibrin or a collagen gel could serve as such a sacrificial template, under the condition that complete dissolution of the gel would occur by the time the tissue construct was formed.

Nontoxic biodegradable natural materials such as collagen, fibrin and hyaluronic acid have been explored as possible scaffold materials because they do not trigger foreign body response upon
implantation and integrate well with the surrounding tissue. (Zhaodi, 2006) These materials alone are not sufficient to serve as a cardiac patch. It is important to note that fibrin increases the synthesis of both collagen and elastin when seeded with cells. (Zhaodi, 2006) Increased production of collagen and elastin yields tissue constructs with improved mechanical properties. Specifically, compliance is determined by the elastin content of the scaffold. Compliance match between the scaffold and the heart tissue is crucial to maintain the functionality of the heart during contraction. While fibrin is not an acceptable material for the basis of a cardiac patch due to its weak mechanical properties, it could be used as an aid in the culture process. (Ahlfors, 2007) A fibrin gel could be used as a sacrificial template that allows the cells to form a sheet with it initially, but would be digested by the time the sheet was ready to harvest. This would take advantage of the positive properties of fibrin and help resolve the issue of the high porosity of Nitex, while still ensuring that the end product was a totally cell-derived tissue patch.

4.3.5 Tubing

Silicon tubing will be used to perfuse the media and connect the components. Silicon tubing allows for gas exchange within between the closed system and the incubator environment. A peristaltic pump will be used to pump the media through the bioreactor.

4.3.6 Media

Regular media DMEM – 10% Bovine Serum & Pen Strep will be used, as instructed by our advisor. Addition of ascorbic acid has been considered to promote cell proliferation and collagen cross-linking. Adding ascorbic acid to the media will produce a mechanically stronger cell sheet. (Grenier, 2005)
4.4 Design Matrix

A separate list of objectives was established for the culture materials. Each of the identified objectives was ranked as equally important; the chamber must: allow for easy sheet removal, allow media perfusion, and retain cells. A separate design rubric was established for the three different alternative materials for the permeable chamber component. The materials were evaluated for realization of bioreactor chamber objectives, and these results can be seen in Table 7 and Table 8. The three different membrane filter materials being considered – the two different Transwells and the NPRs - were scored with respect to the objectives using the following grading system:

Score:
1- Extremely Difficult
2- Difficult
3- Moderately Difficult
4- Fairly easy
5- Extremely simple

Table 7 - Evaluation of Materials for Realization of Bioreactor Chamber Objectives

<table>
<thead>
<tr>
<th>Objective/Score</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Easy sheet Removal</td>
<td>Biopore (PTFE), MF-Millipore</td>
<td>Nitex+Fibrin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allows media Perfusion</td>
<td>Biopore (PTFE)</td>
<td>MF-Millipore</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retains cells</td>
<td>Nitex+Fibrin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 8 - Weighted Evaluation of Materials

<table>
<thead>
<tr>
<th>Objectives</th>
<th>Biopore</th>
<th>Millipore</th>
<th>Nitex+Fibrin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weight %</td>
<td>Score</td>
<td>Weight score</td>
</tr>
<tr>
<td>Easy sheet Removal</td>
<td>33.33%</td>
<td>0.4</td>
<td>13.32%</td>
</tr>
<tr>
<td>Allows media Perfusion</td>
<td>33.33%</td>
<td>0.4</td>
<td>13.32%</td>
</tr>
<tr>
<td>Retains cells</td>
<td>33.33%</td>
<td>0.6</td>
<td>19.99%</td>
</tr>
<tr>
<td>---------------</td>
<td>--------</td>
<td>-----</td>
<td>--------</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100%</td>
<td>46.63%</td>
<td>66.64%</td>
</tr>
</tbody>
</table>

Although it was determined that the NPRs with a fibrin gel would best accomplish our objectives, this construct requires multiple materials, closing a gap in knowledge/experience (developing the fibrin gel), and assembly. For all these reasons, it was decided that the preliminary design would focus on the use of the commercially available and commonly used Transwells. This would also allow for more accurate comparison of results to published research.

4.5 Preliminary Design

Once the objectives, specifications, functions, and constraints were all determined from the client’s design criteria, different means and materials were weighed, leading to alternatives, which were then compared and modified, resulting in the preliminary design. This selected design had multiple components – a large, hollow base that incorporated the media reservoir (Figure 9) an insert to support culture materials (Figure 10) and a media delivery system (Figure 11). The insert allowed for multiple Transwells to be suspended over the media well, while a perfusion pump would circulate media (and potentially additional cells), distributed by a trough-like irrigation system to each of the Transwells. The bioreactor has small enough dimensions to fit inside an incubator, while maximizing on that space to allow for sufficient media. A picture of this device can be seen below in Figure 12.
Figure 9: Preliminary Design - Media Reservoir

Figure 10: Preliminary Design - Culture Material Support Insert

Figure 11: Preliminary Design - Irrigation Trough Lid
As mentioned, there are multiple components to this design. Each part plays a crucial role in helping to achieve the specifications. The base has a reservoir for media, to ensure cell life and health, and help retain natural mechanical properties. The insert allows for separate chambers that enclose each sheet, encouraging the cells to grow together into individual sheets. The media distributor ensures that equal amounts of media are delivered to each Transwell, and decreases the forces the falling media enacts on the cells and sheets. All of these components have been designed to optimize the health and strength of the cell sheets, while staying inside the size requirements. The media well of the bioreactor is a machined block of acrylic, with the egg-crate insert and irrigation system/lid are machined polycarbonate. Stainless steel fittings and silicone tubing is used to circulate the media. CAD renderings of the device can be seen below in Figure 13.
5 Design Verification

A number of tests were conducted on the final design. These tests were run to determine how well the designed bioreactor system achieves the set objectives and functions to the specifications within the constraints. The tests included fluid flow and functionality testing.

5.1 Fluid Flow Testing

The bioreactor system was run to determine the best flow rate for the system that would deliver sufficient media to all of the cells, while minimizing the forces exerted on the developing tissue sheets. To ensure media was being equally distributed to each sample, flow rate was measure through each distribution port. To test this, media flow was measured through each distribution port for 1 minute periods. The Masterflex peristaltic pump (Model No: 7518-10) was set at a rate of 3.5ml/min for all trials. The media was collected from each distribution port specifically and the volume recorded (in milliliters) was used to calculate the flow rate per port. The results from the initial testing can be seen in Figure 14. As seen in Figure 14, the results were inconsistent in more than one way. There was high
variability between the flow rates across all the ports. There was also high variability within many of the ports, showing inconsistencies over time (trial to trial). The average flow rate delivered to each well was 3.5 ± 2.7 mL/min.

![Flow Rate Testing](image)

**Figure 14: Design 1 Flow Testing**

5.2 Performance Testing

The bioreactor system was tested to ensure that it could be run successfully. The preliminary design was run outside the incubator for 2 days, simply to determine if it could be run effectively while the peristaltic pump was constantly re-circulating media. Effectiveness was determined based on whether the bioreactor ran without leaking or any sort of overflow of media. After this time period, there was no evidence of leakage or flow problems. This test was deemed a success. It was then tested whether or not the bioreactor could be sterilized, that is, withstand the high temperatures of an
autoclave. Unfortunately, the acrylic base deformed in the autoclave, enough that it was no longer a useful component.

5.3 Preliminary Design Conclusions

As the acrylic base deformed in the autoclave, it was determined that re-machining the bioreactor out of a different material was necessary. As the polycarbonate components had the desired properties regarding strength and sterilizability, and also retained their shape after being autoclaved, the new base was to be machined out of that material. As the initial design was successful as far as flow through the system without leaking, the general design of the system could remain the same. However, as the flow testing failed quite badly, it was determined that this component would need adjustments. Upon further inspection, potential reasons for the poor results were discovered. The most obvious drawback of this design was the lack of control afforded the user; there was no mechanism to assist the user in delivering specific amounts of media to the different ports or in priming the system. Additionally, the holes in the ports had been machined using a handheld drill, increasing error in the sizes of the holes and the angles at which they were drilled. While the option of modifying the preliminary media delivery system was available, it was decided that a greater amount of control was desired, and therefore a redesign of this component was necessary.

6 Final Design and Validation

The team was charged with developing a bioreactor system to rapidly and reproducibly fabricate and culture mechanically stable cell-derived tissue sheets. The objectives of this design were determined to be that the bioreactor system easily and consistently generate multiple, healthy cell sheets that have comparable mechanical properties and are easily removed from the system. Consideration of these objectives led to the establishment of a functions list. These functions were
ascertained to be that the bioreactor must generate a cell sheet, accept and support cells, feed said
cells, assemble those cells into tissue, and then easily release that sheet. The realization of these
functions was constrained by the size of the bioreactor, the size of the resulting sheet, the composition
of the final sheet – completely cell-derived and devoid of any synthetic materials, implantability,
biocompatibility, sterilizability, time – completed by April 2009, and money – within a budget of $624.
The specifications of the design were that the bioreactor system must output a cell sheet with a
diameter greater than 15 mm, which will fit in incubator with a shelf height of 3 inches.

6.1 Final Design
After analyzing the results of the validation tests of the preliminary design, it was determined
that many of the results were unacceptable. It was necessary to reexamine how the final design was
going to satisfy the objectives and functions. A detail Bill of Materials for the final design can be found
in Appendix D.

6.1.1 Final Design of Media Reservoir
Given that the reservoir from the preliminary design deformed during the autoclave process, a
new base was fashioned. It was determined that the new base would be machined out of
polycarbonate, for strength, sterilizability and translucence. The base has a lip inside to support the
culture material inserts, and an upper lip to support the media distribution system. The reservoir holds
approximately 500 mL of media – enough to sustain cell health for a prolonged culture period without
manual media exchange.

6.1.2 Final Design of Media Distribution System
As concluded from the results of testing the preliminary design, the media distribution system
needed to be redesigned, as it was failing to consistently deliver sufficient media through each port. To
resolve these problems, increase user control, and improve the overall functionality of the media
distributor, it was redesigned utilizing stainless steel set screws from McMaster. This design feature
allows the flow rates from each hole to be controlled independently. A schematic diagram explaining this can be seen in Figure 15.

![Set Screw Schematic](image)

**Figure 15: Set Screw Schematic**

The set screws can be fine-tuned for each port, to allow the media distributed to each sample to be adjusted to a specific rate. A picture of the new media delivery system can be seen in Figure 16.

![Final Design - Media Distribution System with Set Screws](image)

**Figure 16: Final Design - Media Distribution System with Set Screws**

### 6.1.3 Final Design of Culture Material Supports

The redesign of the media distributor opened up additional options for cell culture. For example, now that specific ports could be closed, static culture and dynamic culture could now be run concurrently. This redesign also led to a redesign of the culture material supports; different inserts were machined. Four inserts were machined, each about the size of a six-well plate. Two of the inserts were created to support six 6-well sized Transwells, and two inserts were created to hold six NPRs. A photo of
one of each insert can be seen in Figure 17. These inserts were designed with the option of using any combination of the different types; each culture can be run with a choice between twelve Transwell samples, twelve NPR samples, or six of each.

![Figure 17: Final Design - Inserts](image)

6.2 Final Design Assembly

As in the preliminary design, the inserts and the media delivery lid all sit inside of the base that contains the media reservoir. Again, the silicone tubing re-circulates the media, using a peristaltic pump, from the media well up through the irrigation system. A photo of the entire system can be seen in Figure 18.
To begin with, the media is poured into the base (Figure 19) before the inserts are set inside (Figure 20). The culture materials are then placed in their respective inserts (Figure 21). The media distributor is then placed on top of the bioreactor, and each individual port is fine-tune adjusted for whatever flow rate is desired for that sample (Figure 22). Finally, the bioreactor is placed in the incubator (Figure 23).
6.3 Testing

The final design of the bioreactor system was again tested to determine how well it achieves the set objectives and functions to the specifications within the constraints. The tests again included fluid flow and functionality testing to validate the newly designed components.

6.3.1 Fluid Flow Testing

The final design of the bioreactor was again run to determine the best flow rate for the system that would deliver sufficient media consistently through all the ports. Flow testing was repeated with the new design, to determine its feasibility, and, ultimately, its superiority to the preliminary design. Again, media flow was measured through each distribution port for 1 minute periods. The peristaltic pump was set at a uniform rate for all trials. The media was collected from each distribution port specifically and the volume recorded (in milliliters) was used to calculate the flow rate per port. The
results from the initial testing can be seen in Figure 24. As seen in the graph, the results even from the first trial were more consistent between ports than was observed with the previous design. The set screws were adjusted between each trial, in an attempt to determine whether the flow could be fine-tuned and controlled through each port. By the third trial, the average flow rate was $8.5 \pm 0.7$ mL/min, and the flow rate for the final trial was $9.1 \pm 0.1$ mL/min. This test – to determine if the flow could be controlled as well as consistent – was determined to be a success.

![Flow Rate Testing - Design 2](image)

**Figure 24: Fluid Flow Testing of Final Design**

### 6.3.2 Performance Testing

The redesigned bioreactor system was tested to ensure that it accomplished all the design goals – meeting objectives, performing functions, etc. To begin with, the system was autoclaved to sterilize all the components. This was completed without any deformations or negative effects to the apparatus. The bioreactor was then tested to determine if it could be run successfully in the incubator. Testing success was determined by whether or not the bioreactor leaked or backed up, and if the media
remained clear and free from contamination throughout the testing period. The bioreactor was run inside the incubator with only media for 4 days. After this time period, there was no evidence of leakage or flow problems, and the media remained clear. This test was also deemed a success.
7 Discussion

This design solved the problem stated in the background, achieving the described objectives and functions. This will allow a user to cultivate up to twelve tissue sheet samples per trial without user intervention for multi-week periods. The controllable flow rate to each port will allow testing multiple materials in one trial. This design has wide-reaching implications as it expands research possibilities.

7.1 Significance

Simplified, the problem is that no device exists that can facilitate the generation of purely cell-derived patches to be used to treat different heart problems. Such a device was created that is easy to use, capable of automated nutrient delivery, produces multiple samples per trial, allows for easy removal of the resulting patches, which have comparable properties. These objectives are accomplished by the bioreactor allowing for seeding of cells into it, delivering media to feed the cells, encouraging the cells to aggregate in order to generate a patch, and then easily release that patch.

The different components of the bioreactor have many advantages, in addition to promoting the generation of these wholly cell-derived patches. The media reservoir base eliminates the need for an additional, external media source. The inserts allow for the use of multiple different culture materials concurrently, if desired. The media delivery system is fine-tunable for each individual sample, allowing for adjustments for different cell types, culture materials, and culture methods. When fully assembled and connected to the peristaltic pump, the entire bioreactor fits within an incubator, on a 3 inch high shelf.

There are limitations to this design. While the bioreactor does allow for the use of common culture materials that have been proven to promote cell aggregation, and can consistently maintain the proper conditions for cell culture, testing of cells in the system has not occurred. Also, while the culture
materials were chosen for their material properties with the intent that they would limit cell adhesion to those surfaces and promote aggregation and attachment within the generated sheet while additionally allowing easy sheet removal, cell culture was not completed, leaving this unconfirmed. These aspects of the design have not yet been validated. There are additional modifications that should be made to optimize this design. For example, 500 mL of media could be too high a volume for certain cultures, and adjustments to reduce this should be available. Also, specific flow rates should be determined for specific cultures.

In order to validate additional aspects of the design, a number of tests should be run. For example, extensive contamination testing should be performed. Once it has been verified that the bioreactor possesses the ability to remain sterile, fluid flow tests with different culture materials should be run, to determine the best flow rates for the different materials. Once this has been completed, cells should be seeded on the different culture materials and the bioreactor should be run for extended periods of time, allowing the cells to aggregate into cell sheets. Removal of the sheets from the culture material should be examined, to determine if the materials properly restrict adhesion while allowing for easy removal. The resultant sheets should then undergo histology and mechanical testing.

7.2 Implications

There are a number of factors to be considered when designing a product for mass production. There can be a number of implications or consequences, specifically concerning a product in the medical sector. Some of these necessary considerations might be economical, environmental, social, political, ethical, or concerning safety, manufacturability or sustainability.

7.2.1 Economics

Tissue engineering is a fast evolving field and “more than $3.5 billion has been invested in worldwide research and development” in the past decade. (McIntire, 2002) Some of the US companies involved in tissue engineering are Synthecon, Lifecell, Interpore, Organogenesis, Cytograft and many
have realized that the bioreactor technology is essential for developing a functional cardiovascular tissue constructs. (ABT, 2003) Synthecon Inc. and Aastrom Biosciences Inc. have been focusing their research on bioreactor development and Synthecon bioreactor has been used as one of the models for our project. Unfortunately, the commercially available bioreactors are expensive and the associated high costs are an obstacle for advancement of tissue culture regeneration. Our design is cost effective and allows fabrication of multiple cell sheets at a time. Our design is based on a commercially available bioreactor and fulfills the same functions but it is cheaper and easier to maintain. It allows production of multiple cell sheets at a time and leads to reduction of lab costs associated with device maintenance and operation.

7.2.2 Environmental Impact
The operation of the bioreactor will not have a negative effect on the environment. The tissue-constructs are made from natural materials and are advantageous over synthetic materials such as Dacron and other synthetic scaffolds.

7.2.3 Societal Influence
The problem of limited supply of donor tissues could be resolved with the advancement of bioreactor technology. Biocompatibility and immunogenicity are the most prevalent issues associated with current implant methods. Our bioreactor allows fabrication of human tissue with patient’s own cells which eliminates these problems. Tissue engineered cardiac patches have many advantages over the current scaffold methods and lack of donor tissue

7.2.4 Political Ramifications
As world’s population ages the demand for improved health care and novel tissue engineered solutions is more evident than ever. The U.S. Federal Government funding “has positioned the United States as a leader” in the tissue engineering industry (McIntire, 2002)and the companies focusing on structural tissue engineering such as skin, bone and cardiac are the fastest growing segment of the
market. (Lysaght, 2001) The US has been the leader in the tissue engineering producing “over seventy percent of the global patents are invented in the US,” (McIntire, 2002) however, while several skin grafts have been patented and received FDA approval, no such cardiac patch has yet been developed. (ABT, 2003) A functional bioreactor is needed to develop a viable cardiac patch and our product is a pre-commercial model which will aid further research and development in the field.

7.2.5 Ethical Concerns

The bioreactor and the cell-derived tissue construct will greatly improve the standard of living. The cells can be grown up to one billion in a culture flask however to improve formation of a cell-derived construct the cells will need to be grown in a bioreactor. If the patch is grown from the patient, then there will not be any ethical issues about the distribution of donor material. Currently there are extensive lists for heart transplants, and if solutions can be grown instead of harvested from donors then the ethical concerns about which patients are more worthy will disappear.

7.2.6 Health and Safety Issues

Cardiac surgery often utilizes foreign material for replacing damaged heart tissue but these materials are subject to failure and can cause “stenosis, thromboembolization, calcium deposition, and infection.” (Shinoka, 2008) The tissue engineered cardiac patch will offer solutions to these health and safety issues. We expect that tissue engineered patches will eliminate infection because the source is human cells as opposed to allogenic or xenogenic cells. Stenosis, thromboembolization, and calcium deposition are expected to be greatly reduced compared to the foreign material patches.

7.2.7 Manufacturability

The bioreactor device is easy to manufacture but close attention must be given to specifications during production. The bioreactor is made of polycarbonate and acrylic body with commercially-available stainless steel fittings and silicone tubing. The materials are easily-accessible and inexpensive and the manufacturing of our bioreactor can be easily reproduced. However, while our bioreactor is
cost-effective and easy to manufacture, it is still on the laboratory, pre-commercial scale and a number of technological modifications will need to be considered.

8 Conclusions and Recommendations

The design of the bioreactor was an iterative process that is still not complete. It has met many objectives established early in the project, but there are components that still needs to be validated and other components where refinement is possible.

8.1 Conclusions

The designed bioreactor functions well and meets the objectives set forth at the onset of the project. The bioreactor fits in an incubator and functions in the incubator without leaking or becoming contaminated. All parts are autoclavable so it is easily sterilizable. In addition flow to all samples is controllable.

This bioreactor offers exciting possibilities for future work. It is designed to allow culture of patches with entirely human material which meets the initial goal of the project. The design is very versatile allowing the use of multiple culture materials as well as adjustable flow depending on the material used. The bioreactor also requires minimal effort. Once the cells are seeded, it can be placed in the incubator and left until the patches are ready to be harvested, which greatly decreases the time and labor required to operate it.

8.2 Design Refinement and Validation

While the bioreactor functions properly there are a few design refinements that could be made. The first is to determine the amount of media required to run the bioreactor for the expected culture time and redesign the base to require less media to run. The second is to incorporate a bleed valve into the irrigation system to assist with priming. In addition to this other inserts could be manufactured to adapt to more culture materials as needed.
Patches need to be cultured in the bioreactor and validated to determine the cell viability as well as the best culture material. The patches will be validated with mechanical testing and histology. Mechanical testing will be performed with the burst pressure method described in Appendix C - Burst Test Method. This test determines thickness, failure tension, failure strain and ultimate tensile strength (UTS). Thickness can also be determined from histology as well as cell viability. Multiple trials need to be run in order to determine lot to lot variability. The design of the bioreactor also allows the use of different cell types that can be tested and compared. Some of the future recommendations can be made regarding our design. Using the same material for the body and the top cover will greatly improve manufacturability of the bioreactor. This will eliminate the issues associated with autoclaving which we encountered during the design testing. We recommend using polycarbonate for the body of the bioreactor because it is more transparent and temperature resistant than acrylic.

In particular, the future research will need to be performed on the methods for introducing multiple tissue layers. A functional cardiac patch would have multiple cell types: “inner layer of endothelium, a layer of smooth muscle cells and an outer layer of connective tissue, produced by fibroblasts”. (McIntire, 2002) A more sophisticated way of cell culture will significantly improve the current design and should be the major factor for other adjustments to the dimensions, the template material and the number of wells.
Works Cited


Glossary

agarose – a polysaccharide gel prepared from agar-agar which is produced from sea-weed

apical (lumen) – apex, top side

apoptosis – programmed cell death

ascorbic acid – vitamin c, a water soluble vitamin

atherosclerosis – a buildup of plaque (fatty substances, cholesterol, cellular waste products, calcium and fibrin) in the inner lining of the artery, thickening and hardening of arteries

basolateral – the base and one or more sides

biocompatible – compatible with living systems (cells, tissue, organs), no risk of injury, cytotoxicity or rejection by the immune system

biodegradable – can be decomposed by biological agents such as bacteria

Biopore – filter material brand name, hydrophilic PTFE, used for: low protein binding, live cell viewing, and immunofluorescent applications

bioreactor - devices that control environmental and operating conditions to aid biological and biochemical mechanisms

bovine pericardium – protective layer surrounding the heart obtained from a bovine

burst pressure testing – test done in an inflation device that can determine thickness, failure tension, failure strain and ultimate tensile strength

cardiomyopathy – general term for myocardial, or heart, disease

cardiovascular disease – a disease of the heart or blood vessels

cell-derived (solely) – consisting only of cells or made from cells or cell material

centrifugation – a process which uses centrifugal forces to separate or concentrate materials suspended in a liquid

chitosan – derived from chitin, a polysaccharide found in shellfish, used in many pharmaceutical applications

collagen – a fibrous protein found in connective tissue – bone, tendons and skin

compliance – inverse of elasticity, measure of the tendency of a hollow organ to resist recoil to its original dimensions upon removal of force
congenital defects – defects present since birth, acquired during fetal development

ddH2O – double distilled water

dermal fibroblast – cells derived from the dermis (skin)

DMEM - Dulbecco’s Modification of Eagle’s Medium, culture media

exogenous – originating outside the body

expanded polytetrafluoroethylene (ePTFE) – microporous polymer, PTFE commercially known as Teflon

extracellular matrix (ECM) – matrix surrounding outside of cells that provides support, abundant in connective tissue

FEP membranes – membranes of fluorinated ethylene propylene

fibrin – an insoluble protein produced in response to bleeding, a major component of the blood clot

fraction collector – collects samples of the same size, usually from a chromatography column

histology – study of microscopic anatomy of cells by staining a thin slice of the cells

hyaluronic acid – major component of the extracellular matrix, contributes to cell proliferation and migration

hydrogel – a colloidal gel with water as the dispersed media

hydrophilic – has a strong affinity to water

in vitro – outside a living organism

in vivo – within a living organism

ischemia - restriction in blood supply resulting in damaged tissue

laminar flow – non-turbulent flow

live-dead assay – two color fluorescence assay that determines number of live and dead cells

lyse – to burst or cut a cell structure

matrigel – liquid gel extracted from mouse tumor cells and is rich in ECM proteins

MF-Millipore – filter material made of mixed cellulose esters, used for: exceptional anatomical and functional polarization

myocardial infarction – a heart attack, occurs when blood supply to the heart is interrupted

nitex – nylon mesh with micron size openings
parafilm coating – a flexible film used in the lab for sealing vessels

PC - polycarbonate, a thermoplastic polymer

perfusion – the pumping of fluid through an organ or tissue

perfusion bioreactor – a bioreactor that uses perfusion to seed and/or feed the cells

peristaltic pump – a pump which compresses a flexible tube between rollers and pushes fluid forward

PIPAAm - poly (N-isopropyl acrylamide), a thermoresponsive polymer

PMMA – poly (methyl methacrylate) clear plastic replacement for glass

dpolyethylene anchor

dpolyethylene terephthalate (PET) – thermoplastic polymer resin, commercially known as Dacron

PP – polypropylene, a thermoplastic polymer

dprolene sutures – monofilament polypropylene non absorbable suture

protein binding – proteins attach themselves to surfaces, such as drugs

pulsatile flow – throbbing or beating flow, as in blood flow in the heart

dsacrificial template – template material used to initially form sheet but will degrade and will not be in the final sheet

SAMs – self assembled monolayers, formed by amphiphilic molecules with a head and a tail that self align

dscaffold – temporary framework material

seeding – the process of introducing the cells to the culture material

Sigmacote – silicone solution in heptanes that readily forms a covalent, thin film on glass, retards clotting, water repellent

strain – deformation of material caused by an applied stress

suture retention test – test performed to determine sutureability of the sheet

systolic – of or relating to systole, the contraction of the chambers of the heart

dtensile strength – strength of the material, greatest longitudinal stress without tearing

throughput – ability to produce sheets rapidly and in large numbers

Transwell – a membrane insert used for cell cultures, allows for feeding from two sides
ventricular aneurysm – localized dilation or protrusion on the wall of the left ventricle occurring after a myocardial infarction
Appendix A – Pairwise Comparison Charts

Table 9 - Pairwise Comparison Chart Primary Objectives

<table>
<thead>
<tr>
<th></th>
<th>Reproducible</th>
<th>Easy to Use</th>
<th>Easy cell sheet removal</th>
<th>Throughput (&gt;1 sheet)</th>
<th>Automated Nutrient Exchange</th>
<th>Total</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reproducible</td>
<td>X</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
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</tr>
<tr>
<td>Easy to use</td>
<td>0</td>
<td>X</td>
<td>0</td>
<td>0</td>
<td>0.5</td>
<td>0.5</td>
<td>.05</td>
</tr>
<tr>
<td>Easy cell sheet removal</td>
<td>0</td>
<td>1</td>
<td>X</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>.20</td>
</tr>
<tr>
<td>Throughput (&gt;1 sheet)</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>X</td>
<td>1</td>
<td>3</td>
<td>.30</td>
</tr>
<tr>
<td>Automated Nutrient Exchange</td>
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<td>0.5</td>
<td>0</td>
<td>0</td>
<td>X</td>
<td>.5</td>
<td>.05</td>
</tr>
</tbody>
</table>

Table 10 - Pairwise Comparison Chart Sub-objective: Reproducible

<table>
<thead>
<tr>
<th>Reproducible</th>
<th>Mechanical Properties</th>
<th>Sheet Dimensions</th>
<th>Total</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mechanical Properties</td>
<td>X</td>
<td>1</td>
<td>1</td>
<td>.67</td>
</tr>
<tr>
<td>Sheet Dimensions</td>
<td>0</td>
<td>X</td>
<td>0</td>
<td>.33</td>
</tr>
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</table>
Table 11 - Pairwise Comparison Chart Sub-objective: Throughput

<table>
<thead>
<tr>
<th>Throughput</th>
<th>Separate Chambers</th>
<th>Template Material No Longer Present</th>
<th>Generates Multiple Sheet</th>
<th>Layers Have Attached/Aligned</th>
<th>Total</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Separate Chambers</td>
<td>X</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>.11</td>
</tr>
<tr>
<td>Template Material No Longer Present</td>
<td>1</td>
<td>X</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>.44</td>
</tr>
<tr>
<td>Generates Multiple Sheet</td>
<td>1</td>
<td>0</td>
<td>X</td>
<td>0</td>
<td>1</td>
<td>.22</td>
</tr>
<tr>
<td>Layers Have Attached/Aligned</td>
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<td>0</td>
<td>1</td>
<td>X</td>
<td>2</td>
<td>.33</td>
</tr>
</tbody>
</table>

Table 12 - Pairwise Comparison Chart Sub-objective: Easy Cell Sheet Removal

<table>
<thead>
<tr>
<th>Easy Cell Sheet Removal</th>
<th>Handling</th>
<th>Packaging</th>
<th>Removal from Bioreactor</th>
<th>Total</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Handling</td>
<td>X</td>
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<td>.42</td>
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<tr>
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<tr>
<td>Removal from Bioreactor</td>
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<td>X</td>
<td>1.5</td>
<td>.42</td>
</tr>
</tbody>
</table>
### Table 13 - Pairwise Comparison Chart Sub-objective: Easy to Use

<table>
<thead>
<tr>
<th>Easy to Use</th>
<th>Easy to Maintain</th>
<th>Easy to Monitor</th>
<th>Easy to Assemble</th>
<th>Total</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Easy to Maintain</td>
<td>X</td>
<td>0</td>
<td>0.5</td>
<td>0.5</td>
<td>0.17</td>
</tr>
<tr>
<td>Easy to Monitor</td>
<td>1</td>
<td>X</td>
<td>1</td>
<td>2</td>
<td>0.67</td>
</tr>
<tr>
<td>Easy to Assemble</td>
<td>0.5</td>
<td>0</td>
<td>X</td>
<td>0.5</td>
<td>0.17</td>
</tr>
</tbody>
</table>
Appendix B – Fibrin Hydrogel Methods

Fibrin Gel Protocol

Fibrinogen stock solution and Thrombin stock solutions were prepared separately and mixed to create a fibrin gel.

**Fibrinogen Stock (Sigma F 4753)**

Materials:

- Sterile H₂O
- HBSS buffer (20 mM Hepes, 0.9% NaCl)
- Bovine Fibrinogen Type IV (Sigma F4753)

Procedure:

- Dissolve 250 mg fibrinogen in 7.5 ml HBSS
- Warm to 37°C in H₂O to aid dissolution
- Shake every 10 minutes until completely dissolved (about 1 hour)
- Filter through syringe filter
- Aliquot into 1.5ml aliquots (5 aliquots) [final conc=30mg/ml]

<table>
<thead>
<tr>
<th>HBSS Volume</th>
<th>Hepes (g)</th>
<th>diH₂O (mL)</th>
<th>NaCl (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>1.193</td>
<td>250</td>
<td>2.25</td>
</tr>
<tr>
<td>0.5</td>
<td>2.383</td>
<td>500</td>
<td>4.5</td>
</tr>
<tr>
<td>1</td>
<td>4.766</td>
<td>1000</td>
<td>9</td>
</tr>
</tbody>
</table>

**Thrombin Stock Solution (Sigma F4648)**

Materials:

- HBSS buffer (20 mM Hepes, 0.9% NaCl)
- Bovine Thrombin (Sigma F4648)
- Sterile H₂O

Procedure:
- Calculate the number of units: \[ \text{mg solid} \times (\text{NIH units/mg solid}) = \text{units per bottle} \]
- Determine amount of liquid needed to reach 25 U/ml
- The liquid added is 10% diH2O and 90% HBSS
- Dissolve thrombin in H2O and HBSS
- Filter through syringe filter
- Freeze in aliquots of 125 \( \mu l \)

<table>
<thead>
<tr>
<th>Volume</th>
<th>Hepes (g)</th>
<th>diH2O (mL)</th>
<th>NaCl (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>1.192</td>
<td>250</td>
<td>2.25</td>
</tr>
<tr>
<td>0.5</td>
<td>2.383</td>
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<td>4.5</td>
</tr>
<tr>
<td>1</td>
<td>4.766</td>
<td>1000</td>
<td>9</td>
</tr>
</tbody>
</table>

**Example:**

\[ 9 \text{ mg solid} \times (106 \text{ NIH units/mg solid}) = 954 \text{ units per bottle} \]

\[ \frac{954 \text{ units}}{25 \text{ units/ml}} = 38.16 \text{ ml} \]

\[ 3.82 \text{ ml H2O} + 34.34 \text{ ml HBSS} = 38.16 \text{ ml} \]

Final amounts:

\[ 9 \text{ mg solid} + 3.82 \text{ ml diH2O} + 34.34 \text{ ml HBSS} \]

Fibrin Gel for use of Flexcell

**Materials:**

- Thrombin aliquot
- Fibrinogen aliquot (3.8 mg/ml)
- 20 mM HEPES in 0.9% NaCl saline solution (HBSS)
- DMEM 1x media
  - 2N Ca++
- Cell Culture Media (FBS + 1% P/S = 1x DMEM)
- Fibroblasts
- Growth Media
- Flexcell Tissue Train 6 well plates

**Procedure:**
The final solution to make fibrin gels (25mm diameter) consists of 2/3 fibrinogen solution, 1/6 cell solution, and 1/6 thrombin solution, with the thrombin always being added last. The final concentrations should be calculated on using 1.5 mL of combined solution if you are making hemisphere gels.

**Fibrinogen:**
- Add fibrinogen aliquot (1.5 ML) to 7.5 mL of HBSS. The concentration is now 5 mg/mL
- Separate fibrinogen aliquots into desired amount of containers
- For one aliquot it is easier to divide the 9mL of this solution into 3 containers each having 3mL.

**Cell Suspension:**
- Spin down cells in centrifuge for 10 minutes @ 1200 rpm.
- Resuspend cells in enough cell culture media (DMEM + 10 % FBS) to give desired final concentration.
- Once desired concentration is achieved, add amount of cell suspension to each fibrinogen container
- Place containers on ice
- For one aliquot it is easier to divide the 9 mL of this solution into 3 containers each having 3 mL.

**Thrombin**
- Add 2mL of DMEM w/o FBS or BCS (just use 1x here) and 7.5µL of 2N Ca++ to 100 µL aliquot of thrombin. Put on ice.

**Gel Preparation**
- Take the container of the fibrinogen and cell suspension and add amount of thrombin needed (0.67 mL for one aliquot of each). Mix the suspension.
- Quickly place 3 ml of the total solution in the center of the Flexcell plate.
- 4 gels can be made with the amount of volume of one container. (If only one aliquot of each is used.
- Repeat steps 3-5 in Gel Preparation until all of the containers are used.
Appendix C - Burst Test Method

Thickness, failure tension, failure strain and ultimate tensile strength (UTS) can be determined with this test. The tissue sheets will be exposed to ddH₂O for one hour to lyse the cells and equilibrated in PBS. This eliminated the natural tension of the fibroblasts but does not decellularize the matrix. The samples are placed in a tissue inflation device with a 1 cm diameter opening. The samples are inflated with PBS at a rate of 1 ml/min to form an approximate spherical cap before failing. The increasing pressure is measured with a pressure transducer and the displacement of the center of the cap is measured with a laser displacement system. The thicknesses of the samples are measured using the laser displacement system before being inflated. (Ahlfors, 2007)

The results will be analyzed using the following equations: (Ahlfors, 2007)

Law of Laplace for a spherical cap: Maximum membrane tension T (N/m):

\[ T = \frac{1}{2}PR \]

where \( P \) (kPa) is the pressure when the tissue bursts and \( R \) (mm) is the corresponding radius at point of rupture.

Radius of curvature \( R \):

\[ R = \frac{(w^2 + a^2)}{2w} \]

where \( a \) (mm) is the radius of the clamp and \( w \) (mm) is the displacement at the center of the sample at failure.

\[ \text{UTS} = \frac{T}{t} \]

where \( t \) is the initial thickness before inflation

Extensibility is defined as Green’s stress at failure:

\[ E = \frac{1}{2}(\lambda_{\text{failure}}^2 - 1) \]

where \( \lambda_{\text{failure}} \) is the stretch ratio as the sample fails.

For a spherical cap the average stretch ratio along a meridian can be estimated using the radius of curvature:

\[ \lambda = \left( R \frac{\arcsin(a/R)}{a} \right) \]
## Appendix D – Bill of Materials

<table>
<thead>
<tr>
<th>Component</th>
<th>Function</th>
<th>Source</th>
<th>Part #</th>
<th>Dimensions</th>
<th>Quantity</th>
<th>Individual Price</th>
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<td>Base</td>
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<td>3” x 7” x 9”</td>
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<tr>
<td>Inserts</td>
<td>Inserts</td>
<td>Plastics Unlimited</td>
<td>456</td>
<td>1/4” x 6”</td>
<td>2</td>
<td>$1.25/lb</td>
<td>$2.50</td>
</tr>
<tr>
<td>Lid</td>
<td>Lid</td>
<td>McMaster-Carr</td>
<td>789</td>
<td>1/8” Fractional Width 3 Feet, 1/4”-20 threaded, 1” length, 5/32” Hex, 150 PSI</td>
<td>1</td>
<td>$11.35</td>
<td>$11.35</td>
</tr>
<tr>
<td>Seal Lid</td>
<td>Seal Lid</td>
<td>McMaster-Carr</td>
<td>123</td>
<td>1/8” x 6”</td>
<td>3</td>
<td>$2.25/lb</td>
<td>$6.75</td>
</tr>
<tr>
<td>Flow Control</td>
<td>Flow Control</td>
<td>McMaster-Carr</td>
<td>456</td>
<td>3/8” x 6”</td>
<td>2</td>
<td>$4.86</td>
<td>$9.72</td>
</tr>
<tr>
<td>Check Valve</td>
<td>Check Valve</td>
<td>United States Plastic Corp.</td>
<td>789</td>
<td>3/4” x 6”</td>
<td>2</td>
<td>$12.10</td>
<td>$24.20</td>
</tr>
<tr>
<td>Connector</td>
<td>Connector</td>
<td>Media Circulation Small Parts Inc</td>
<td>123</td>
<td>1” x 1/8”</td>
<td>2</td>
<td>$5.00</td>
<td>$10.00</td>
</tr>
</tbody>
</table>

**TOTAL:** $182.19