Developing a Decellularized Spinach Leaf Cardiac Scaffold

A Major Qualifying Project Report:

Submitted to the Faculty of
WORCESTER POLYTECHNIC INSTITUTE
In partial fulfillment of the requirements for the Degree of Bachelor of Science

Submitted by:

Jacqueline Berthiaume
Connor Guyette
Nicholas Rath
Karistan Swan

Submitted to:

Professor Glenn Gaudette, PhD,
Department of Biomedical Engineering & Mechanical Engineering

May 6, 2021

This report represents the work of one or more WPI undergraduate students submitted to the faculty as evidence of completion of a degree requirement. WPI routinely publishes these reports on the web without editorial or peer review

Acknowledgements

Our team would like to thank our wonderful MQP advisor, Professor Glenn Gaudette, Ph.D. for all his help, support, and motivation throughout the past year. We would also like to thank William DeMaria for all his help and guidance with our project inside and outside of the lab. We would like to give thanks to Joshua Gershlak and the rest of the initial team that worked on the decellularized leaf, giving inspiration for our project. In addition, we would like to thank Lisa Wall for all her help and suggestions in securing our project materials and getting a lab space. We would also like to thank Anthony Heng, for training us on the Instron at Gateway. Lastly, we would like to thank our friends in the Dominko Lab for housing us and our work throughout our second semester.

Abstract

Myocardial infarction can lead to heart failure resulting in the need for cardiac transplant. To limit the need for a transplant, a suturable cardiac scaffold was designed to be placed on the infarct site. The patch utilizes a decellularized spinach leaf with silicone suture patches and arterial tubing. Tests for strength, biocompatibility, and arterial connection were completed. The data showed that the scaffold has potential to be surgically implantable and encourages further research to be performed to determine its effect on functional recovery of myocardial tissue.

Keywords - myocardial infarction, sew-on patch, decellularized spinach leaf, myocardial cells

Executive Summary

I. Introduction

Background

The major problem that we were presented with in this project was improving treatment for patients who have experienced a myocardial infarction, commonly known as a heart attack. There are over 800,000 cases of MI in the US alone each year, and the damage caused by them is currently not adequately treated [1]. MI causes tissue death and scarring of the heart muscle, which may lead to permanent loss of function in and around the area of the infarct and eventually cause heart failure.

Currently, the only long-term treatment option for this condition is a heart transplant to replace the one that has failed. Our proposed treatment option is a sew-on patch, which would be placed directly onto the infarct site to recover some lost function. Replacing the damaged region of the heart alone will reduce and potentially eliminate the need for a heart transplant for these patients.

Design Problem

Our team started by researching Professor Gaudette's work that used decellularized spinach leaves as a scaffold for cardiomyocytes. Notably, this research has shown that these scaffolds support cell proliferation [2]. These specific scaffolds have a native vascularized structure that may allow blood flow and are flexible enough to contract with myocardial cells. However, the decellularized spinach leaf was just a starting point as it was too fragile to be sutured into the heart and too difficult to manipulate. This spurred our client statement to design a clinically applicable, biocompatible approach for implanting a vascularized, cell-seeded cardiac patch that can withstand the typical load on the heart.

II. Design Process

Objectives for Design

The primary objective of our design was to create a clinically applicable vascularized cardiac scaffold. To do this it needs to be biocompatible, suturable, and able to sustain cell life. It must also be able to contract and expand to mimic the normal cardiac function and not tear under the normal forces experienced within the body.

Design Process

Our design process was driven by the objectives mentioned above and focused on two primary additions to the spinach scaffold, a sewing tab design, and an arterial connection tube.

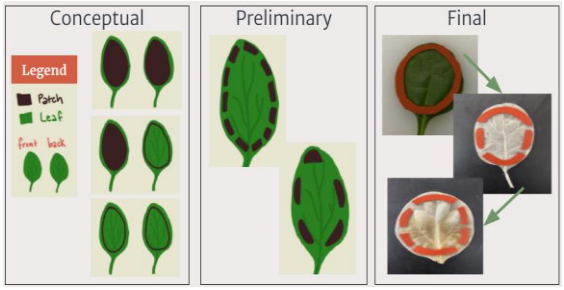


Figure 1: Sewing Tab Design Iterations

Starting with the sewing tab designs, our main purpose was to make the leaf suturable and therefore, implantable. Considering the decellularized leaves' fragility, a biocompatible material patch was required. Our initial conceptual designs focused on large patches and rings that covered both sides of the leaf, but they limited the scaffold's ability to host cells (Figure 1). Moving to our preliminary designs, we theorized that only one side of the leaf needed the additional material, and a tab design would allow the most room for cell growth. Our final design iteration focused on maximizing space for the cells while allowing for the surgeon to suture the edges of the leaf. With that in mind, we created the 6 notched sewing tab design you can see on the leaf in Figure 2.

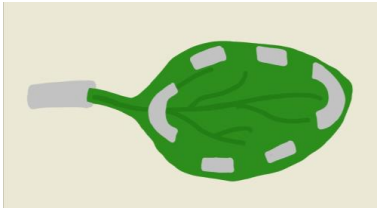


Figure 2: Arterial Connection

The second facet of our design was to create an arterial connection point for the scaffold to receive blood flow. Because the leaf has a natural vascularization and stem, we decided to make that our connection point. The stem, like the leaf, is very fragile and therefore needed extra tubing to make it functional. Our initial designs investigated tubing inserted inside of the stem, tubing wrapped around the outside, and a combination of both.

III. Design Testing

Mechanical Testing

For the mechanical analysis, we performed three separate tests on different potential patch materials including: rubber silicone, polyester sheeting, and medical-grade silicone. The first one performed was tensile testing. This was done to determine the point of failure for each material under a tensile load. Tensile load testing was needed to observe if each material tested would withstand the maximum load within the heart. From there our group was able to eliminate the polyester sheeting from our list of materials based off our initial tensile load testing values collected. Next was the suture retention testing. This was done in order to determine if the material chosen for the sewing tabs would be able to be sutured through and not rip. The thicker materials that were tested showed the highest suture retention strengths.

To attach our sewing tabs onto the leaf before it is anchored with sutures when being used, our group decided that fibrin glue would be a suitable solution. Using various literature on the subject, our group modified a fibrin hydrogel procedure that was already being used in Gaudette Lab to make fibrin glue. In order to test our fibrin glue solution, we developed a third mechanical testing procedure to demonstrate the strength of the fibrin glue while being adhered to a decellularized leaf. Our group cut a piece of both the decellularized leaf and silicone into a dog-bone shape and glued one end of each together using the fibrin glue for each trial. The glue was found to begin to slip at around 0.25 N throughout our testing.

Cellular Testing

Taking 0.015-inch thick medical-grade silicone used for the sewing tabs was tested to see if the material would change the cell growth on the cardiac scaffold. To do this we used hMSC 5's and seeded them directly onto cut-outs of the decellularized leaf as well as cut-outs with a silicone patch attached. After the cells were allowed to grow with cell tracker red, they were stained with Hoechst to identify the nuclei and imaged. As you can see in Figure 3, the cells were able to live on the spinach leaf and were not affected by the silicone.

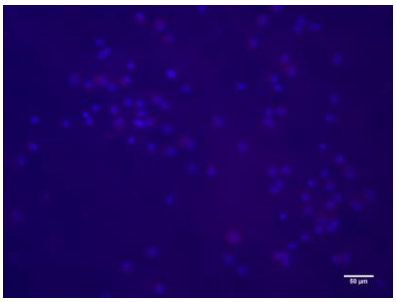


Figure 3: Cells on Spinach Leaf with Silicone

Arterial Testing

We conducted three tests to verify our stem attachment design. In each test, we stabilized a decellularized spinach leaf and ran water through its stem with a syringe to evaluate the connection's viability. The first test was done with the stem alone and resulted in ripping allowing for the water to escape. The second test was done with a biocompatible medical tubing inserted inside the stem, which had a better flow but ripped the stem. The final test was done with the medical tubing inside of the stem and an electrospun material encompassing both the stem and the tube. This test showed minimal leaking and allowed for

the stem to be sutured and receive flow without ripping.

IV. Final Design

Our final design utilized 0.015-inch-thick medical grade silicone to create a 6 notched patch that encircled the edges of the leaf, making it suturable and strong. While this material is transparent it can be seen by the dots in Figure 4. Our final design also made use of the electrospun material encompassing both the natural stem and the medical tubing inside of it to create a point for arterial connection. While the cells are not visible to the eye, they can be seeded and grown from the center to the edges of the scaffold to allow for contraction, potentially recovering the loss of function at the infarct site.

Figure 4: Final Design with all components

Conclusion

Objectives Review

Our scaffold was deemed successful in meeting its objectives. The scaffold is now suturable, strong enough to withstand surgical implantation, supports cell adhesion and survival with the potential for recovered function, and has an arterial connection for blood flow. Conceptually, the spinach scaffold can now be implanted and assist in returning cardiac muscle function.

Further Work

From our testing, we have determined that our patch design has the potential to be used as a biocompatible treatment for myocardial infarction. While our research has shown the scaffold's ability to meet its biocompatible, mechanical, cellular, and arterial needs further research and testing will need to be done to verify the patch's function when sewed into a heart and how well heart function is recovered using the patch after a myocardial infarction occurs.

V. References

[1] Centers for Disease Control and Prevention. (2020, September 8). Heart Disease Facts. Centers for Disease Control and Prevention. https://www.cdc.gov/heartdisease/facts.htm. Hyattsville, MD: National Center for Health Statistics; 2012. Accessed May 9, 2019.

[2] Joshua R. Gershlak, Sarah Hernandez, Gianluca Fontana, Luke R. Perreault, Katrina J. Hansen, Sara A. Larson, Bernard Y.K. Binder, David M. Dolivo, Tianhong Yang, Tanja Dominko, Marsha W. Rolle, Pamela J. Weathers, Fabricio Medina-Bolivar, Carole L. Cramer, William L. Murphy, Glenn R. Gaudette, Crossing kingdoms: Using decellularized plants as perfusable tissue engineering scaffolds, Biomaterials, Volume 125, 2017, Pages 13-22, ISSN 0142-9612, https://doi.org/10.1016/j.biomaterials.2017.02.011. (https://www.sciencedirect.com/science/article/pii/S0142961217300856)

Table of Contents

List of Figures

List of Tables

Authorship

Jacqueline Berthiaume (JB), Connor Guyette (CG), Nicholas Rath (NR), Karistan Swan (KS)

Sections	Author	Editor 1 (if applicable)
Acknowledgments	All	---
Abstract	KS	NR
Executive Summary	All	---
Chapter 1	KS	JB
2.1	KS	KS
2.2	CG	KS
2.3	NR	JB
2.4	NR	KS
2.5	JB	JB
2.5.1	JB	KS
2.5.2	KS	JB
2.5.3	KS	KS
2.5.4	JB	KS
3.1	All	---
3.2	CG	NR
3.2.1	JB	KS
3.2.2	JB	JB
3.2.3	CG	NR
3.3	All	KS
3.4	KS	JB
4.1	JB	KS
4.2	KS	JB
4.3	KS	KS
4.3.1	KS	JB

4.3.2	KS	JB
4.3.3	KS	JB
4.4	KS	JB
4.5.1	CG	All
4.5.2	JB	KS
4.5.3	KS	JB
4.6.1	JB	All
4.6.2	CG	All
4.6.3	KS	All

5.1	CG	KS
5.2.1	NR	CG
5.2.2	NR	CG
5.2.3	NR	CG
5.2.4	NR	CG
5.2.5	NR	CG
5.2.6	NR	CG
5.2.7	NR	CG
5.3	JB	KS
5.3.1	JB	KS
5.3.2	JB	KS
5.3.3	JB	KS
5.4	KS	JB
5.4.1	KS	JB
5.4.1.1	KS	JB
5.4.1.2	KS	JB
5.4.1.3	KS	JB
5.4.2	KS	JB

6.1	KS	JB
6.1.1	KS	JB

6.1.2	KS	JB
6.1.3	KS	JB
6.1.4	JB	All
6.2	CG	All
6.2.1	CG	All
6.2.2	CG	All
6.2.3	CG	All
6.2.4	CG	All
6.2.5	CG	All

7.1	NR	All
7.2	JB	All
7.3	JB	All
7.4	CG	All
7.5	JB	All
7.6	CG	All
7.7	KS	All

8.1	KS	All
8.2	JB	All
8.3	KS	All
Appedicies	NR	CG

Chapter 1: Introduction

The major problem that we were presented with in this project was improving treatment for patients who have experienced a myocardial infarction (MI), commonly known as a heart attack. There are over 800,000 cases of MI in the US alone each year, and the damage that is caused by them is currently not adequately treated (CDC, 2020). MI causes tissue death and scarring of the heart muscle, which may lead to permanent loss of function in the area of the infarct, eventually causing heart failure.

Currently, the only long-term treatment option for this condition is a heart transplant to replace the one that has failed. Our proposed treatment option is a cardiac scaffold with suture patches, which would be placed directly onto the infarct site to recover some lost function. Replacing the damaged region of the heart alone will reduce and hopefully eliminate the need for a heart transplant for these patients.

Our design addresses the need for a biocompatible, easily accessible base for the cardiac patch, which was achieved through a decellularized spinach leaf due to its similar structure to the myocardium. The spinach leaf design was enhanced with the implementation of the following aspects: a stiffer material on the edges to increase suturability, a method of connection between the stem and a functioning artery, and seeded cells to verify the implantability and integration of our design.

Chapter 2: Literature Review

2.1 Healthy Heart Function

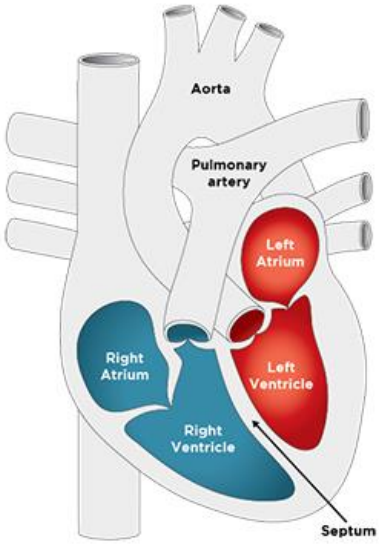


Figure 1: Anatomical Heart (Heart Foundation NZ, 2020)

The average human heart expands and contracts approximately 100,000 times a day to pump 2,000 gallons of blood throughout the body (D.B Tran, 2020). As the primary organ of the circulatory system, the heart pulls deoxygenated blood from the body, supplies oxygen to the used blood, and returns it to the body again to be used. The structure of the heart is made up of four chambers: the right and left atria: the upper chambers, and the right and left ventricles: the lower chambers (Figure 1). Valves between them open in one direction to encourage continuous flow.

The myocardium, or the middle muscular wall layer in the heart, performs the contracting function in the heart producing the heartbeat (D.B Tran, 2020). The cells that make up the myocardium, termed cardiomyocytes, are only found in heart muscle. Cardiomyocytes are striated and uninucleated cells that allow for electrical potential to be spread quickly to tell the myocardium to perform contractions synchronously (D.B Tran, 2020). The myocardium is found in all four chambers of the heart, but the thickness is dependent on the force of contraction needed to pump, causing a thinner wall in the atria where less power is needed and a thicker wall in the ventricles where it is more difficult. An increased need for force to provide adequate contractions may occur due to various heart diseases or from a medical emergency like a heart attack that starves the tissue of oxygen.

2.2 Myocardial Infarction

Myocardial infarction (MI), more commonly known as a heart attack, is an extremely common ailment and is one of the leading causes of death and disability worldwide (Thygesen, K, et al. 2007). Approximately every 40 seconds there will be a victim of MI, with it being responsible for over 800,000 deaths in the United States alone in 2016 (Benjamin EJ, Muntner P, Alonso A, et al, 2019). Myocardial infarction occurs when there is inflammation in the cardiovascular walls restricting the flow of oxygen rich blood to the heart causing a condition known as cardiac ischemia (Figure 2). Cardiac ischemia is a product of the inner walls of arteries in the heart beginning to be clogged and blocked by cholesterol or fatty deposits such as plaque. The process of the plaque and cholesterol build up is known as atherosclerosis. The longer that cardiac ischemia goes untreated the more cardiac muscle tissue dies which can lead to serious medical complications and possibly death from myocardial infarction. It is possible within an artery that has restricted blood flow due to plaque and fatty deposits built up in the artery, the plaque and fatty deposits can break free from the wall and it can possibly form a thrombus. A thrombus is another form of blood clot where the plaque and fatty deposits are flowing in the bloodstream and build up and impede the blood flow causing myocardial infarction.

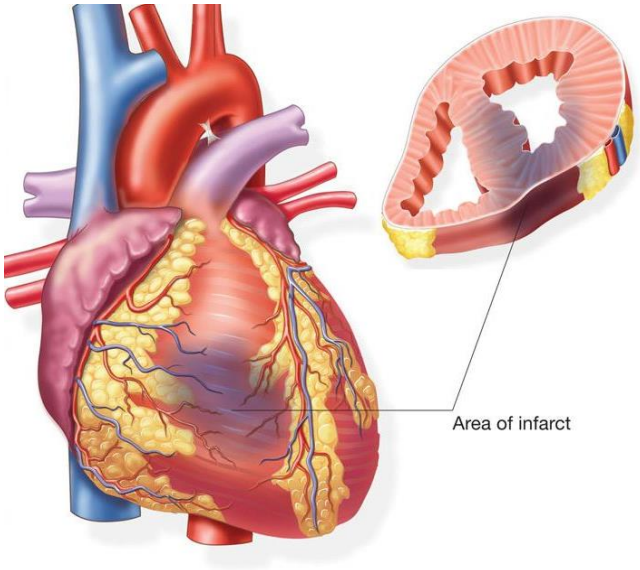


Figure 2: Myocardial Infarction *(Developer, 2020)*

There are a multitude of causes for myocardial infarction. The leading cause is a disease called hypercholesterolemia; this is when an individual experiences an extremely high cholesterol level. High cholesterol leads to the fatty deposits within the arteries that can lead to clotting. Hypertension, or high blood pressure, is another leading cause of MI. If an individual has diabetes or a family history of coronary artery disease, they have a higher risk of experiencing MI. Along with diseases that can lead to MI, poor diet and a lack of exercise can increase the strain on the heart and ultimately increase the chances of MI.

2.3 Impact of MI on Tissue Mechanics

Due to the high variation of effects of myocardial infarction on the heart, quantifying and comparing the mechanical properties of infarcted and healthy myocardial tissue is extremely difficult. For example, the level to which the infarction extends through the myocardial wall as well as the size and location of the infarction can vary, causing discrepancies in heart function from case to case. Based on various studies, however, there have been some similarities seen in mechanical properties of infarcted tissue (E. Romito et al.).

Utilizing various imaging techniques is one way that the mechanical properties of infarcted myocardial tissue can be determined. One study used ultrasound to analyze the strain and dimensions of the left ventricle after MI. The researchers in this study found that patients with an infarcted region that extended throughout the myocardial wall had the highest decrease in strain rate (T. Edvardsen et al.). In another study that utilized magnetic resonance imaging (MRI), researchers found that both normal and shear strains decreased greatly in the infarcted regions of patients (J. Bogaert et al.). An additional study found that bulging that occurs in the left ventricle post-MI actually decreased after about a year (W. Liu et al.). The results of all these studies suggest a stiffening of the myocardial tissue once an infarction occurs.

Since the heart does not have the ability to regenerate cardiomyocytes once they die, there is no way for the body to replace them after MI occurs. These dead cardiomyocytes are then replaced by fibrous scar tissue instead (V. Talman et al.). The buildup of this fibrous scar tissue is the most likely reason for the stiffening of the myocardial wall that was seen in the studies discussed earlier. Due to the changes in mechanical properties of the heart due to this scar tissue, the overall performance of the heart can be significantly altered and inhibited.

2.4 Treatment of MI

Regarding treatment of myocardial infarction, the best and most successful treatment is taking preventative measures to keep the risk as low as possible. If myocardial infarction is discovered and treated in the early stages, then it is possible to reverse it with drug therapies (Peng, Zhou and Wu, 2017). Drug therapy is a noninvasive treatment option where drugs are administered to destroy and break up potential blood clots in arteries within the heart. If a case of myocardial infarction is farther along, then a more invasive treatment, such as percutaneous

coronary intervention (PCI) or coronary artery bypass grafting (CABG), may be needed. Both of these procedures require surgery and either a graft or catheter is set into place at the narrow section of the artery to try to restore proper blood flow to the heart.

For patients in a more severe stage of MI, a heart transplant is necessary. In order to limit the need for a transplant, a new form of cardiac tissue repair can be achieved through the use of cardiac patches. The goal of these patches is to restore blood flow to the diseased parts of the heart. Some patches are used to restore structural strength for the heart as well. The world of cardiac patches is continuously growing and has a prominent future in the repair of myocardial infarction.

2.5 Cardiac Patches

Cardiac patches offer a new and potentially more effective option for treatment of myocardial infarction. Following myocardial infarction, the infarct can expand, resulting in scar tissue formation on the heart (K.L Fujimoto et al, 2007). The heart also has minimal ability to repair itself. Therefore, a solution is needed that can prevent infarct expansion, and in the long term potentially repair and restore the function of myocardial tissue. This solution could also eventually promote proliferation of cardiomyocytes at the site of the infarction. Placing the patch at the infarct site will, in theory, prevent further infarct expansion and limit the formation of scar tissue after myocardial infarction.

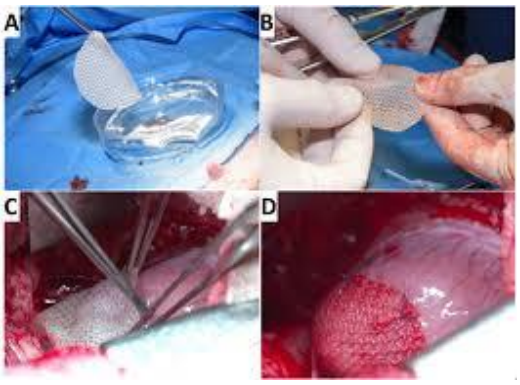


Figure 3: Cardiac Patch Surgery (Lancaster et al, 2019)

2.5.1 Cardiac Patch Types

A variety of cardiac patches have been introduced as potential solutions in recent years. These patches can be either cell-seeded or acellular. Acellular patches do not offer some of the

additional benefits that cell-seeded patches can have, and for the most part just provide necessary structural support to the tissue where it is implanted. However, these patches are more easily stored and used than a cell-seeded patch, and they make up many of the currently clinically available patches (K. Huang et al, 2020).

Cell-seeded patches can be seeded with a variety of different cell types, including human mesenchymal stem cells, human induced pluripotent stem cells, and human embryonic stem cells. In existing patches, human umbilical vein endothelial cells have also been used on patches and showed functionalization similar to that of normal heart tissue (T. Su et al, 2018). One of the goals of cell-seeded patches is to provide the contractile ability that is lost at the infarct site. Another goal of the cell-seeded patch is to have a solution that would fully integrate with the surrounding tissue where it is inserted.

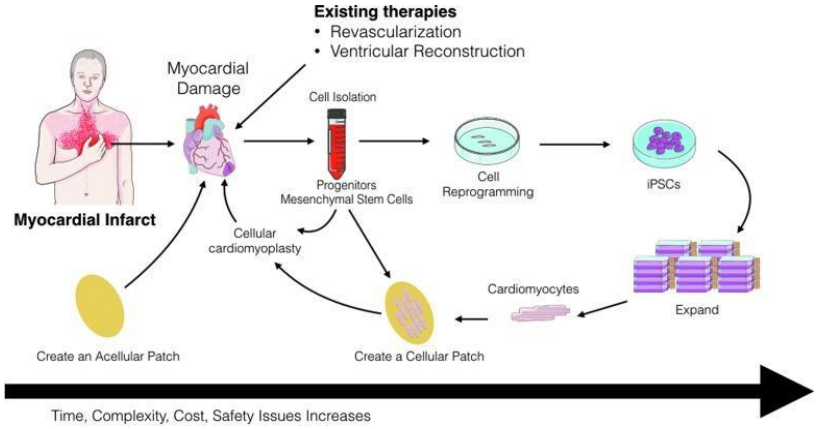


Figure 4: Existing Therapies for Myocardial Infarct (Domenech, 2016)

2.5.2 Cardiac Patch Scaffolds

There are also a variety of different scaffold materials that have been used in cardiac patches and treatments. These include cell sheets, extracellular matrix, and plant-based scaffoldings, Dacron, and PTFE. Using a cell sheet on the extracellular matrix has been done in tissue engineering contexts and can have advantages such as temperature sensitivity and better cell connections (T. Shimizu et al, 2003). Porous extracellular matrix scaffolds can be used to allow for better cell-seeding capabilities of the patch (B.P. Chan and K.W. Leong, 2008). The major advantage of plant-based scaffolding is that the scaffolding is pre-vascularized, which can allow for better blood flow through the patch and better integration (J.R. Gershlak et al, 2017).

Current therapeutic use of cardiac patches has mostly been in treatment of ventricular

aneurysms. Research also suggests that they could be used to treat the infarct and loss of cardiac function associated with myocardial infarction (K.L. Fujimoto et al, 2007). A cardiac patch could allow for the repair of the cardiac tissue at the infarct site, as well as reduce the formation of scar tissue following the myocardial infarction. Cell-seeded cardiac patches also have potential to return the contractile ability of the infarct site through contractability of the cells that are seeded on the patch, which would further return function of the myocardial tissue.

2.5.3 Patches on the Market or in Development

There are currently many different cardiac patches on the market. Each comes with its own defined use, benefits, and shortcomings.

- GORE-TEX Cardiovascular Patch (uses a non-absorbable suture, used for 35 years, ePTFE has multidirectional strength, comes in different sizes) (Figure 5)

Figure 5: GORE-TEX Cardiovascular Patch (Gore Tex)

- GORE Acuseal used in vascular repair and reconstruction (Figure 6)

Figure 6: GORE Acuseal Cardiovascular Patch (Gore Acuseal)

- Pins Fibrin Microthread Cardiac Patch (Figure 7)

Figure 7: Microthread Cardiac Patch (NIH 2010)

- Labcor cardiac tissue matrix PB, xenograft natural structure and biocompatibility (Figure 8)

Figure 8: Labcor Cardiac Patch (Medical Expo)

- 4D Cardiac Patch "expandable microstructure, the specific design has been shown to improve both the biomechanical properties of the patches themselves and the dynamic integration of the patch with the beating heart." hydrogel based (Figure 9)

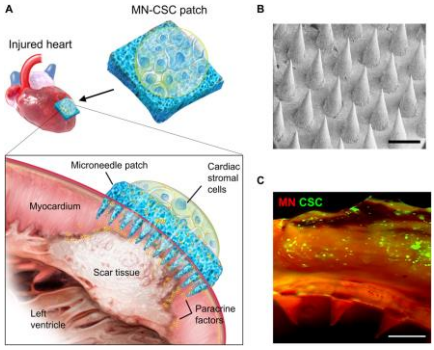


Figure 9: 4D Cardiac Patch (Tang, 2018)

2.5.4 Spinach Leaf Patch Scaffold

Unlike other patches on the market, we opted for a plant-based material. We chose a decellularized spinach leaf, that has many advantages over other types of scaffolding. One of the main drawbacks of other types of scaffolds is that they are not easily vascularized. This lack of vascular structure is one of the major gaps in cardiac patch research and existing scaffolds. Vascularization is important because it allows blood to flow through the patch and sustain the cells growing on top of it. The decellularized spinach leaf overcomes this challenge, because it already has an existing vascular network that can be used for the patch after decellularization of the leaf (J.R Gershlak et al, 2017). Additionally, the materials in the leaf, such as cellulose, have good biocompatibility and other biological properties, making the spinach leaf a suitable candidate for a biological, in vivo, patch (J.R Gershlak et al, 2017). The spinach leaf patch also has good potential ability to support cell-seeding with cardiomyocytes or stem cells. Stem cell-derived cardiomyocytes have been seen to survive and function on the decellularized leaf, and contractile ability has been observed in these cells (J.R Gershlak, 2017).

Practical limitations that exist with the decellularized spinach leaf scaffold are primarily its mechanical properties. The potential for tearing of the patch in its attachment is an issue that needs to be overcome, by creating a suture system that will increase the overall strength of the patch, as well as its adhesion ability to the cardiac tissue. The decellularization process can also have adverse effects on cell-seeding ability of the patch (J. R Gershlak, 2017). The patch also needs to be strong enough and able to withstand contraction of the surrounding tissue after it is implanted, during normal heart function. Despite these limitations, the decellularized spinach leaf scaffold has great potential in its application as a cardiac patch.

Figure 10: Gaudette Decellularized Spinach Leaf (WPI, 2017)

Chapter 3: Project Strategy

3.1 Initial Client Statement

Design a clinically applicable approach for implanting a spinach leaf based cardiac patch

3.2 Design Requirements

For our capstone design process, we focused on 3 main design requirement categories to yield the most successful product. First, there is an overarching main objective, which is to create a clinically applicable vascularized cardiac scaffold. During the duration of the design process there were constraints that had to be factored in. The main constraints are biocompatibility, and suturability. These are our major proponents to our design because if any of these fail during production then it could yield a non-successful product. From there we needed to gauge the functions of our cardiac patch. For this we looked at the capability of the patch being able to expand and contract with existing heart tissue. We also have to make sure that the threshold of the tearability of the patch is greater than any forces experienced within the body. Finally, we focused on the specifications needed for our mechanical testing. We followed ASTM standards while performing the experiments, such as the peel test and the suture retention test, it is important to follow these standards to ensure that our design meets all the benchmarks required.

3.2.1 Objectives and Functions

Our major project objective is to create a cardiac patch using a decellularized spinach leaf as the base for the cardiac scaffold. Our secondary objectives were determined by evaluating the most important mechanical and functional properties of the heart and that the patch will need to be able to withstand. These objectives include implementing a ring or alternative system to allow for easy and functional suturing of the patch into the myocardial tissue, and to increase the overall strength and stability of the patch upon implementation. This is to ensure the patch can be attached to the heart tissue without damaging the spinach leaf scaffold, and without extra difficulty for the surgeon.

Objectives related to the mechanical properties of the patch include ensuring that our scaffold and suturing system have sufficient compliance and mechanical strength compared to that of normal myocardial tissue. This is to verify that our patch can withstand the normal contracting and function of the heart once it is implemented. We also will test our suture system to ensure good pullout strength and puncture results, to prove that the patch is suturable and that the patch and suture system can both withstand the same conditions as myocardial tissue. An additional objective for our patch is to seed it with cells, to improve function and properties of the patch. The objective of seeding the cells is crucial to our end goal because cell life needs to be maintained for the cardiac scaffold to properly work.

3.2.2 Constraints

Two of the most significant constraints on our patch design and testing are time and budget. We have a wide range of objectives that we need to accomplish in completing our cardiac scaffold design. These objectives will require time for design of the patch and the suture system, as well as time for multiple rounds of mechanical and cell survival testing on the patch. Additionally, to test the cell-seeding ability of the patch, the cells will need to be cultured and then seeded several times. All of these tests and processes to meet our objectives need to be completed in the short timeline of a single academic year, adding up to about eight months for completion of the entire project and all of our objectives. Additionally, all of our design processes and materials for creating our design are limited to our allotted budget of $250 for each member of our team, for a total of a $1,000 budget for completing our project. These budgetary and timeline limitations are some of the most important constraints to consider in our design.

Other constraints on our design include the fact that we cannot test our design in vivo. All our tests will be completed on the patch itself outside of the body, so our data will be limited in terms of predicting the behavior of the patch once it is implemented in the myocardium. The ability of the patch to be sterilized prior to implementation, and sterility in the processing of the patch design are also limitations that could cause design constraints.

Additionally, there are three major constraints that are present outside of the production of the cardiac scaffold. The first would be the biocompatibility of the scaffold. It is indicative that the cardiac scaffold is biocompatible with the body because it will be directly sown into the heart with seeded cells to promote the new regeneration of cardiac tissue. The next constraint is

that the cardiac scaffold needs to maintain the cell life that is seeded on it. This is a major proponent to the success of our patch because the cells need to survive the implantation process to successfully start the new growth of tissue. Finally, the suturability of the cardiac scaffold is our last constraint. For this, the scaffold must be able to be sutured into the existing cardiac tissue without rips or malfunctions that are caused by too much force being exerted onto the scaffold during the physical implantation of it.

3.2.3 Specifications

For our project, there are specifications and parameters that need to be met for optimal results from our testing device, the Instron 5544. We will be conducting multiple different tests utilizing the same Instron machine, so it limits human error within the experiment. The way the initial specifications and parameters were determined was through our literature review. We focused and looked at previous experiments that were done using a similar machine testing along with similar cardiac properties. The main tests that are being conducted are suture retention strength, suture puncture strength, and peel off strength. The specifications that were determined by the team for suture retention testing was a speed of 10mm/min. The suture must be placed at a minimum of 2mm from an existing edge to promote structural integrity per the specific ASTM Standards. Regarding suture puncture testing, the specifications that are going to be initially used are a rate of 10mm/min and the needle being placed 3-5 mm above the surface of the material. The material will be pulled taught with no more than a 2-3 newton force applied horizontally. The final mechanical test we will be conducting is the peel-off strength test. This test is aimed to gauge the strength of our fibrin glue adhering to the decellularized leaf. The way this test is going to be performed is the leaf will be cut into a dog bone shape and attached to the top grip of the Instron. The bottom grip of the Instron will be securing the excess silicone material. The dog bone shaped leaf and silicone piece are adhered together with our fibrin glue. This is a tension test and will be performed at 10mm/min.

3.3 Design Standards

As this product is developed, design standards used throughout the industry must be followed. The most widely accepted standards used are the International Organization for Standardization (ISO) and the American Society for Testing and Materials International

(ASTM). The following standards will be applied to this project, copies of each standard can be seen in Appendix A:

- ISO 10993-5:2009 - Biological evaluation of medical devices — Part 5: Tests for in vitro cytotoxicity
- ISO 11737-2:2019 - Sterilization of medical devices — Microbiological methods — Part 2: Tests of sterility performed in the definition, validation and maintenance of a sterilization process
- ISO 10993-1:2018 - Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process
- ASTM STP1173 - Biomaterials' Mechanical Properties
- ANSI/AAMI/ISO 7198:1998/2001/(R) 2004 - Cardiovascular implants— Tubular vascular prostheses

The ISO 10993-5:2009 standard discusses ways to determine the biological response of cells *in vitro* with a medical device. This will be useful in determining the response of the myocardial cells grown on the spinach leaf to the cardiac patch. The ISO 11737-2:2009 standard discusses how to ensure that a medical device can be sterilized which is important in ensuring that the cardiac patch will be sterile. The ISO 10993-1:2009 standard evaluates risks involved in biological medical devices and how to assess their safety. The ASTM STP1173 standard outlines the mechanical properties of various biomaterials which will be useful when determining materials that must be used in the device. For the purposes of this project, the ANSI/AAMI/ISO 7198:1998/2001/(R) 2004 standard serves as an outline for performing suture retention testing.

3.3 Revised client statement

Design a clinically applicable, biocompatible approach for implanting a vascularized cell seeded cardiac patch that is able to withstand the mechanical loads experienced by cardiac tissue.

Acknowledging the time and budgetary restraints imposed upon this project the primary goal that is to be completed in this client statement is the design and testing of a vascularized, spinach leaf based cardiac patch. The cardiac patch must be mechanically interchangeable with our patches on the market and must withstand the normal function of the heart. The cardiac patch

design will be seeded with a set of cells similar in function to the endothelial cells to assess biocompatibility and potential for implantation.

3.4 Management Approach

There are four core tasks in this project. The different tasks are split up to make the project team accountable for work completed and meeting project goals.

The initial task was to research: studying the current cardiac patches on the market, assessing their values and what would make them better, and finding a corner of the market that is needed. In our case this missing function is an easily accessible, biocompatible cardiac patch that is formed through naturally occurring material: spinach leaves. In this background research must be conducted to evaluate the mechanical and structural standards of a cardiac patch, their comparison to a spinach leaf patch design and if implementation of therapeutic cells to revitalize myocardial function is possible.

The secondary task of the cardiac patch project was design development. The core points of the project design is the spinach leaf integration, a supportive edge attachment to increase weight and stability of the patch without impeding flexibility, the construction of the stem stent that allows the patch to be attached to an existing artery to allow for access to oxygenated blood, and finally the design process of attaching cells to the cardiac patch. The design development is dependent on the research conducted on current mechanical standards and the objectives listed in the design requirements.

The third and primary task of this project was device testing. The cardiac patch design must be tested for strength, compliance, and contraction to match industry standards. The edge design must be evaluated for its attachment properties and its impact on compliance and strength. The spinach patch will be evaluated for suture puncture and retention in order to see how easily it could be attached to a human heart. The design will also be tested for cell biocompatibility through cell culture and seeding with the patch. Additionally, testing will be done to determine if the leaf’s natural stem can be used for arterial connection or if it needs supplemental strength from another material

The final task of this project is the review and editing process of the project. This is critical to the project as it encourages thorough design evaluation and testing. This is to be done from the first day of the project, August 31st to the final due date of May 6th.

Chapter 4: Design Process

4.1 Needs Analysis

The primary focus of our patch design is the requirement of mechanical properties that reflect those of native myocardial tissue. Our design should be able to withstand the same mechanical stresses and other properties that cardiac tissue undergoes on a day to day basis and in extreme circumstances in order to be applied clinically. As the heart is constantly contracting, the patch needs to be flexible and able to undergo a range of mechanical loads dynamically. Inability to maintain these properties could lead to failure of the patch, which in practice could be extremely dangerous for the patient.

Another need of our patch is for it to be suturable, and thus, implantable. Without the ability to be sutured into the cardiac tissue and stay securely in place after implantation, the patch will not function. The sutures need to be able to withstand pulling at a rate of 1 mm per second with a force of approximately 2N in order to prove that the suture strength and location on the patch are adequate for holding in place in cardiac applications.

Additionally, our patch needs to be biocompatible, to prevent danger to the patient in clinical applications. Since the patch itself is decellularized, this should not pose problems for biocompatibility, but the materials chosen need to be biocompatible and applicable in medical uses. Another aspect of our design needs is that cells can be seeded on the patch to test for cell survival and proliferation. This will help to verify biocompatibility of the device by showing its potential ability to integrate into the cardiac tissue after implantation. Another design componet is to connect the stem of the spinach leaf to an artery, to take advantage of the vascular properties of the leaf for cardiac applications.

4.2 Design Requirements

For our vascularized cardiac patch, a decellularized spinach leaf makes up the base of our scaffold, chosen for its availability, biocompatibility, and structure. Upon decellularization, the spinach leaf is incredibly flexible and lightweight causing it to bend and stick to itself when moved and to tear if pulled or punctured. While this flexibility is ideal for cell mechanics and

viability, allowing them to bend and contract the leaf to mimic myocardium, it is impractical for surgeons to use. In addition, the spinach leaf's low resistance to tension limits its potential for surgeons to safely move the patch and place it without tearing.

In order to combat these flaws, the spinach leaf requires an additional material to supplement its areas of weakness. For our bio scaffold design, we are looking to increase resistance to tearing, mechanical strength, and stability when moving. With an additional material being integrated into the design we also need to consider the flexibility of the combined spinach leaf and material to allow for the myocardial cells to be able to contract on the surface of the scaffold. It is also necessary that the additional material does not impair the biocompatibility for its later bio-integration. Blood flow through the vascularized spinach leaf may not be compromised through the additional material. The following qualities must be evaluated for a satisfactory design.

- Stability: the patch must not fold upon itself and be capable of surgical transfer
- Strength: the patch must resist tears when moved
- Suturability: the patch must be capable of suturing without tearing
- Flexibility: the patch must still support cell contraction
- Biocompatibility: the additional material must be biocompatible and not impede cell viability
- Blood flow: capacity of blood flow through vascularization cannot be compromised

Our design must meet all these requirements in order to be considered as a cardiac patch. Concepts on the addition of a material backing to the spinach leaf are discussed in section 4.3.

4.3 Preliminary Designs

There are three initial designs that are evaluated against the design requirements described in section 4.2. These include a full material backing on the side of the leaf that does not touch the heart, a ring design that covers all edges of the spinach leaf, and a notched ring design that leaves the center and pieces of the outer edge exposed.

4.3.1 Full Patch Backing Design

Using an additional material cut to match the spinach leaf a backing is attached with a fibrin glue. In Figure 11 this design is depicted, the left shows the front of the leaf and the right shows the patch covering. The six design requirements are evaluated in Table 1.

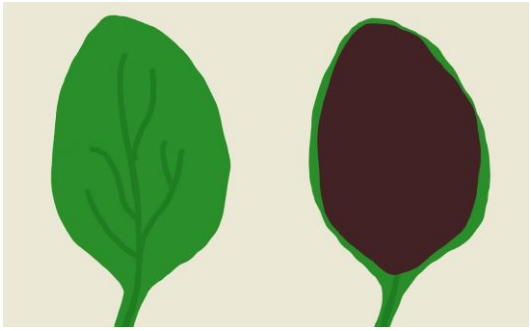


Figure 11: Full Patch Design

Table 1: Full Patch Design Impact

Design Requirement	Design Impact
Stability	A full backing on the backside (seen on the right of Figure 11) of the leaf increases the scaffolds resistance to folding upon itself and allows the scaffold to be handled easily by surgeons
Strength	Overall scaffold strength increases with a material backing as it lends its mechanical properties to the leaf
Suturability	The surgeon can suture through the material backing and leaf combination without causing any tearing
Flexibility	The full backing design restricts the spinach leafs flexibility and does not allow for the cells to contract as needed
Biocompatibility	With an additional material covering the full spinach leaf the cells are unable to maintain the same level of viability on the leaf
Blood Flow	Blood flow is restricted by the materials compression on the leafs vascularization

While the full backing design allows for increased stability, strength and suturability it does not meet three of the six design requirements needed to be successful making this an ineligible patch design.

4.3.2 Outer Ring Design

Using an additional material cut to match the outer edge of the spinach leaf a ring is attached to the leaf with fibrin glue. In Figure 12 and Figure 13 this design is depicted in two forms, a ring is on a single slide of the leaf and a double ring design that covers both sides. The six design requirements are evaluated in Table 2.

Figure 12: Single Side Ring

Figure 13: Double Side Ring

Table 2: Outer Ring Design Impact

Design Requirement	Design Impact
Stability	By attaching an outer ring the spinach leaf is less likely to fold onto itself and can be easily separated if needed using the edges as grips
Strength	The ring increases the mechanical strength of the edges of the leaf
Suturability	The scaffold can be sutured through the ring attachment
Flexibility	The full ring prohibits the cells from contracting the edges of the

	leaf
Biocompatibility	The ring disrupts the cells and has potential negative impact on cell viability
Blood Flow	By using a full ring the edges of the leaf are unable to allow for blood flow

While the ring design allowed for better flexibility, biocompatibility and blood flow than the backing design was able to, it is still unsatisfactory in meeting all the design needs to be an eligible cardiac patch.

4.3.3 Notched Ring Design

In the same method mentioned in 4.3.2 an additional material is cut to match the outer edge of the spinach leaf; the ring is then notched to allow for edge exposure and is attached to the leaf with fibrin glue. In Table 3 this design is depicted in four variations. The six design requirements are evaluated in Table 4.

Table 3: Notched Ring Designs

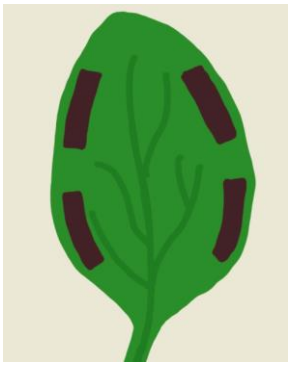
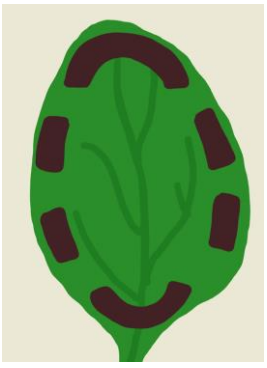
5 Patch Rounded Design	Micro Patch Design	4 Patch Design	6 Patch Design
			

Table 4: Notched Ring Design Impact

Design Requirement	**Design Impact**
Stability	Stability is increased with the small material patches and deters the leaves from folding upon itself
Strength	Strength is increased by the smaller material patches on top of the spinach leaf
Suturability	The cell patch can be sutured through the additional material without ripping the edges
Flexibility	Flexibility is maintained with lesser resistance on the spinach leaf
Biocompatibility	The primarily exposed leaf allows for greater biocompatibility than other designs and poses the least interference in cell viability
Blood Flow	Blood flow will be least affected by notched ring

The notched ring design allows for the spinach leaf to maintain its flexibility while increasing stability and strength as necessary in our design requirements. This product design meets the six requirements and is therefore eligible for patch integration.

4.4 Final Design Selection

For our final design our cardiac patch will utilize the notched ring approach to adapt the spinach leaf to be usable by surgeons and meet design needs. In order to leave the maximum amount of spinach leaf exposed for cell compatibility and flexibility a four to six patch design is used. A ring to match the outer edge of the leaf is used as a guide and is notched to reflect the design shown in Table 4. The leftmost leaf shows the unchanged leaf front that touches the heart, the middle image shows the four-patch design, and the right shows the six-patch design. The number of sewing patches needed is determined by leaf size, the smaller leaves needing 4 patches and the larger needing 6. This design also utilizes an additional material around the stem in order to make it suturable for arterial connection. This is represented by the grey tubing in the

figures included in Table 5. Moving forward with this design, further tests for mechanical properties, biocompatibility and arterial flow will be done.

Table 5: Final Design Options

Leaf Front	4 notched patch design	6 notched patch design
		

For an additional visual of this design we have included what the leaf would look like with extra material we have provided images of the spinach leaf, decellularized, and with an orange material on top of it. This can be seen below in Figure 14, a 4 notched sewing patch design, and Figure 15, a 6 notched sewing patch design. Table 5 can be found below these images to categorize final design impact in terms of our objectives

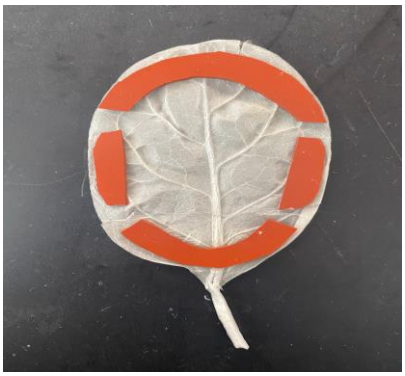


Figure 14: 4 Notched Patch

Figure 15: 6 Notched Patch

Table 6: Final Design Impact

Design Requirement	Design Impact
Stability	Stability is increased with the small material patches and deters the leaves from folding upon itself
Strength	Strength is increased by the smaller material patches on top of the spinach leaf
Suturability	The cell patch can be sutured through the additional material without ripping the edges
Flexibility	Maximum flexibility is maintained with limited material addition
Biocompatibility	The primarily exposed leaf allows for greater biocompatibility than other designs and poses the least interference in cell viability
Blood Flow	Blood flow will be enhanced using a supporting material around the natural stem to increase strength and suturability

4.5 Materials

4.5.1 Mechanical Materials

Material selection for the cardiac patch is a fundamental step in the design process of creating a clinically applicable cardiac patch. For the initial testing that was done there were two materials that were selected to be tested on and a third material that was to be applied for our final patch design. For the third material, we tested different thicknesses to determine which would be most applicable and best suit our design and yield the desired results. One of the main concerns is that the material that is used for the cardiac patch is highly biocompatible. If the material that is chosen in the end does not have a high biocompatibility rate it cannot be used. When choosing what materials would best fit this design, we wanted a range of different types of materials. A range of different properties for the materials allowed us to see what would work

best with the dynamics of the decellularized spinach leaf. Along with being able to stretch and contract with the spinach leaves and the heart we had to consider the suturability of the material.

The fist material that was chosen was 40A silicone rubber that has a thickness of 0.03 inches. This material was chosen because in the research that we conducted, we found that most materials used in cardiac applications are easily manipulated and don't have much resistance on the heart to let it work as efficiently as possible. The chart below shows a durometer rating of the hardness of silicone rubber (Figure 16). The hardness of the material relates directly to its

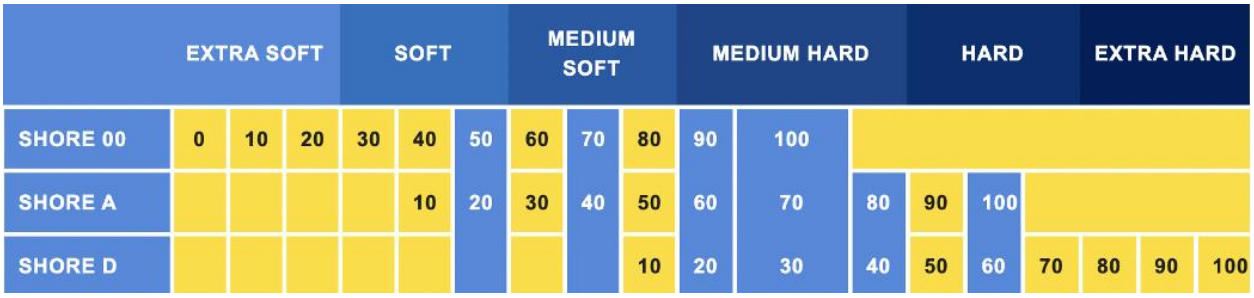


Figure 16: Durometer scale chart *(Mykin, 2019)*

flexibility and manipulability, with softer materials being more ideal for physiological applications, especially cardiac.

Our silicone rubber is rated as a 40A, this means it is a soft flexible silicone rubber. It is also rated a Shore 00 which means that it is the softest version of that specific type of silicone rubber. This material is on the thickest material that we chose to test but it is extremely elastic and there is no deformation to the material after being stretched and then returned to its original state. This material is very soft and flexible, but the thickness also allows it to have good suturable properties. As a team, we believed that this material would be a good fit for our initial tests that are being performed due to its elasticity. When the heart is beating and pulsating there will be forces in all directions that are being applied to the patch, so having a material that can stretch and deform with those forces but still creating enough stability and reinforced for the decellularized leaf is crucial to the success of the patch.

The second material that we acquired for our initial material testing was a sheet of polyester fabric. This material is 0.015 inches thick, which is half of the thickness of the silicone rubber that we chose. This material has very different properties than silicone. With the polyester only being 0.015 inches thick allows for it to be very flexible. These materials vary the most when looking at the elastic properties of both. The polyester is woven threads of polyester, the

woven construction of the material allows for little to no elastic deformation to happen to the material. This means that when it is tested it will not stretch much until it is about to yield and then it will rip. We chose this material because we want to see if the flexibility properties of the polyester fabric would be suitable to use for a cardiac patch. The only drawback of this material is that it is not biocompatible.

The third material that we used was a medical grade, non-reinforced silicone rubber. We got this material in a range of thicknesses that we believed could be compatible with our design, the thicknesses we got were 0.007, 0.01, 0.015, and 0.02 inches thick. This material bridges the gap between the 40A silicone sheeting and the polyester fabric. This material is purely silicone, making it less elastic than the silicone rubber but when stretched there is no deformation when it returns to its original state. This material has a 40A durometer reading which is the same hardness as the original rubber but has a much more rigid structural support system and will allow for easier suturibility. We plan on using this material for our final design but due to the cost of the material preliminary testing will be done with our other materials.

4.5.2 Cell Survival

In order to induce contraction of the cardiac patch and ensure proper integration when sutured into the body cells must be seeded onto the patch. The cells will force contraction and help pump blood in the heart to replace the function of the damaged muscle. Cells have previously been shown to grow on the leaf scaffold, but we needed to ensure that the cells would still grow with our added sewing patch material.

During our cell survival and growth testing, we used Passage 5 (P5) Human Mesenchymal Stem Cells (HMSCs) seeded onto cutouts of our leaf scaffolds and patches in a 12-well cell culture plate. During the cell culture process, the cells were seeded and fed with media made from Lonza's MSCGM Mesenchymal Stem Cell Growth Medium BulletKit (catalog #: PT-3001). In order to observe cell survival over the course of the experiment, the cells were stained using control tracker red, and then with Hoechst, because the red fluorescence of the leaves made it difficult to see the cells under the microscope.

4.5.3 Arterial Connection

In order to make use of the natural vascularization of the spinach leaf we plan to utilize the stem for the arterial connection point. In our final design mockup we theorized an additional material to be added to surround the stem to make it stronger. However, we collected two materials for the arterial connection point. One to be inserted within the stem to help maintain its structure and prevent changes in flow, and another to be wrapped around the outside of the stem to reinforce it for suturing.

Our interior material is a polyethylene micro medical tubing (catalog #BB31695-PE/5) from Scientific Commodities Inc. It has an interior diameter of 0.86 mm and an exterior diameter of 1.32 mm, allowing it to fit inside the average leaf stem size. This tubing is already biocompatible allowing us to reach our objective of biocompatibility. The exterior material is an extro spun tubing. This material comes in a range of interior diameters that can be chosen to best fit around the stem leaf as it does vary from leaf to leaf. It is made with a biocompatible polymer and is able to be sutured, meeting our design goals.

4.6 Product Fabrication

4.6.1 Spinach Leaves

The first step in our design process was completing decellularization of spinach leaves to use as our patch scaffold. This process was completed repeatedly over several weeks and adjusted to continually improve decellularization each time. The decellularization process took approximately one week, with one batch taking closer to ten days to ensure the leaves were completely decellularized. Small to medium sized baby spinach leaves with a strong stem were chosen for decellularization. This size requirement was chosen for the comparative ease of product assembly with smaller leaves, and for consistency in product design.

The decellularization process began with cannulation of leaves and then tying with sutures. This was followed by an optional manual washing of the leaves with hexanes and then PBS, repeated once, washing each leaf until a dark green color was seen. In the first batches, leaves were decellularized with and without hexane washing and cannulation prior to being placed into SDS solution. After this batch, it was determined that hexane washing speeds up the

decellularization process and promotes more complete decellularization, so the decision was made to hexane wash and cannulate all leaves prior to SDS washing for later batches.

The leaves were then placed in 50 ml conical tubes with 10 ml of 10X SDS solution and approximately 35 ml of DI water and placed on the rotator. After approximately 48 hours in SDS solution, this solution was replaced with a solution of 0.1% Triton-X and 10% Clorox bleach. This was added to the tubes to about the 45 ml mark and the tubes were returned to the rotator. This solution was created at a ratio of 2000 ml DI water: 48 ml Clorox bleach: 20 ml Triton-X.

After a day in this solution, the leaves were observed to see if decellularization was progressing successfully at this point. When leaves were observed to still be green in some batches, they were left in Triton bleach for an additional day. One batch of leaves was still not showing enough decellularization after this point and were returned to SDS solution again before being given another day in Triton bleach. This switch back to SDS was to reduce the risk of the long exposure to Triton bleach damaging the mechanical properties of the leaf scaffold.

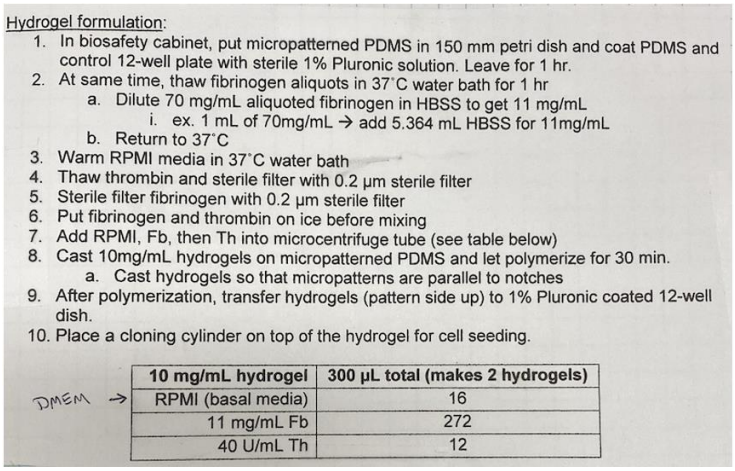
When leaves progressed in decellularization and were ready to be taken out of Triton bleach, this solution was removed from the tubes and the tubes were filled to the 45 ml mark with DI water and placed on the rotator for another day. Following DI water, the tubes were filled with a Tris buffer solution for the last day of decellularization. This buffer was made of 500 ml of DI water and approximately 607.5 g of 0.1 mM Tris buffer. This solution was brought to a pH of 9.0 by adding HCl and NaOH as needed by drop while stirring.

After a day in Tris buffer, the decellularization process was complete and the leaves were removed from the conical tubes and placed on weigh boats, with 2-4 leaves per weigh boat, based on how many could fit without overlapping. The leaves were lightly patted dry when placed in the weigh boat. This was done to ensure that there were no large drops of liquid that would freeze onto the leaves. The weigh boats were taped closed and frozen for several days prior to being lyophilized. After lyophilization, the leaves were ready for mechanical testing.

4.6.2 Sewing Patch

The second step in our product design was to come up with an adhesive that has good biocompatibility and durability. It was decided that the best adhesive for our product would be a fibrin glue. There are many different formulations and recipes to create a fibrin glue to tailor to a specific design. In the literature, two of the main components of fibrin glue are fibrinogen and

thrombin. These two enzymes were used in our formulation of fibrin glue that we created based off of the hydrogel formulation and the formulation that was found in the literature. The hydrogel formulation can be seen in Figure 17 below.



Hydrogel formulation:

1. In biosafety cabinet, put micropatterned PDMS in 150 mm petri dish and coat PDMS and control 12-well plate with sterile 1% Pluronic solution. Leave for 1 hr.
2. At same time, thaw fibrinogen aliquots in 37˚C water bath for 1 hr
   a. Dilute 70 mg/mL aliquoted fibrinogen in HBSS to get 11 mg/mL
      i. ex. 1 mL of 70mg/mL → add 5.364 mL HBSS for 11mg/mL
   b. Return to 37˚C
3. Warm RPMI media in 37˚C water bath
4. Thaw thrombin and sterile filter with 0.2 µm sterile filter
5. Sterile filter fibrinogen with 0.2 µm sterile filter
6. Put fibrinogen and thrombin on ice before mixing
7. Add RPMI, Fb, then Th into microcentrifuge tube (see table below)
8. Cast 10mg/mL hydrogels on micropatterned PDMS and let polymerize for 30 min.
   a. Cast hydrogels so that micropatterns are parallel to notches
9. After polymerization, transfer hydrogels (pattern side up) to 1% Pluronic coated 12-well dish.
10. Place a cloning cylinder on top of the hydrogel for cell seeding.

10 mg/mL hydrogel	300 µL total (makes 2 hydrogels)
DMEM → RPMI (basal media)	16
11 mg/mL Fb	272
40 U/mL Th	12

Figure 17: Hydrogel formulation

The modification that was made to the hydrogel formulation was according to the literature calcium chloride was used as a binding agent for the fibrin glue. (S. Alston, 2007). With the knowledge of the literature, the cell (basal) media used in the hydrogels was substituted with calcium chloride.

The first step was to calculate the proportions that were needed to create the adequate amount of fibrin glue. We used 300mL of the fibrin glue per patch that was created. According to the literature we needed to use 9mM of the calcium chloride solution which equated to .1g per 76mL of DI water (S. Alston, 2007). This step was modified by increasing the amount of calcium chloride from .1g to .2g per 76L of DI water. While that is being mixed on a stir plate, the frozen fibrinogen and thrombin were placed in a 37°C water bath to defrost for about 10 minutes. Once the fibrinogen and thrombin are one minute from being defrosted, we fill a small styrofoam cooler and fill it with ice, the reason for this is keeping the mixture cold. Once the thrombin is put in the mixture it will become a gel liquid quickly, so the ice prolongs the process and keeps it a liquid longer.

With the calcium chloride being mixed and the thrombin and fibrinogen defrosted the formulation can be made. In the conical tube that is set in ice, 16 mL of the calcium chloride DI water mixture was added to it. After that is added, 272 mL of the fibrinogen is added to the same conical tube as the calcium chloride mixture. Once both of those are mixed together, it is ready

for the thrombin to be added. 12 mL of thrombin is then added to the mixture. This mixture of 16mL of calcium chloride mixture, 272mL of fibrinogen, and 12mL of thrombin will equal 300mL of fibrin glue. 300mL is the amount that we use per patch, it is crucial that the thrombin is added at last due to its rapid congealing rate. The final steps are to coat the decellularized leaf with the fibrin glue and secure the material backing to it to reflect the design shown in section 4.4 and leave them to dry for 24 hours.

4.6.3 Stem Stent

In order to create an arterial connection point, we added additional tubing material inside and outside the spinach stem, to access the natural vascularization of the leaf. The interior material is slid inside the stem, acting as a stent, and waits for stabilization from the exterior material. The electrospun tubing that surrounds the outside of the stem is cut to create a vertical slit down the back, letting it be opened to fit to the outside of the stem. Fibrin glue is applied around the stem and the electrospun tubing is wrapped around the stem. This is held together until the glue is dry. Following this a surgical knot is tied around the top of the stem encompassing all three materials: the electrospun tubing, the stem itself and the microtubing. This will stabilize the stem for arterial connection and hold the materials in place.

Chapter 5: Design Verification

5.1 Design Testing

Initial design testing was done with regular spinach leaves, which consisted of using the fibrin glue that we formulated and quickly pipetting it onto our preliminary ring patch design and then laying the spinach leaf over the top of the ring patch. During the first design test the leaves were left to dry and set for 30 minutes. This was not enough time to let the glue dry and was still wet and was not adhering to patch when we lifted the leaf by the stem only. They were left to dry overnight to see if a 12-24 drying period would perform better or if the glue was not adhering properly. After 24 hours the leaves were still not adhering to the patch. We then decided to modify the amount of calcium chloride that was originally set at .1g to .2g as stated above. Then another set of patches was made with the fibrin glue that had a higher concentration of calcium chloride, these were left to set for 24 hours as well. When this set of patches was picked up by the stem only it was adhered to the ring patch, as seen in Figure 18 below.

Figure 18: Spinach leaf attached to patch with 18mM calcium chloride solution in fibrin glue

After the formulation of the fibrin glue was finalized, we were able to test it with the lyophilized decellularized spinach leaves seen in Figure 19 on the following page. For the lyophilized leaves we used the same 300 mL formulation for each of the leaves and were left to set and dry for a 24-hour period.

Figure 19: Lyophilized spinach leaves attached to ring patch with fibrin glue

The last step in our initial design testing process was to see how the lyophilized leaves and fibrin glue would withstand being soaked in PBS, which is a solution that rehydrates the spinach leaves before testing. The patches were soaked in a petri dish of PBS for 2-4 minutes and were being rotated in the solution consistently to ensure all portions of the leaf were rehydrated. The leaf and the glue withstood the rehydration and were ready for mechanical testing.

5.2 Mechanical Analysis

5.2.1 Tensile Testing

The initial set of testing that was performed was tensile testing on the potential materials that were to be used in the design. Plain polyester 0.015 inch thick sheets, 0.03 inch thick 40A Duro Silicone rubber sheets, and multiple thicknesses of 40A Matte non reinforced medical grade silicone sheets ranging from 0.007 to 0.02 inches were tested. Rectangular pieces of varying dimensions underwent a simple tensile test using the Instron 5544 machine. Three trials were done for each type of material. The data were recorded to determine the point of failure, or the ultimate tensile strength of each material. The experimental setup can be seen in Figure 20: where rectangular slices of silicone (left) and polyester (right) were placed within the grips of the Instron. The images show the point of failure for the polyester and the point where testing was stopped for the silicone.

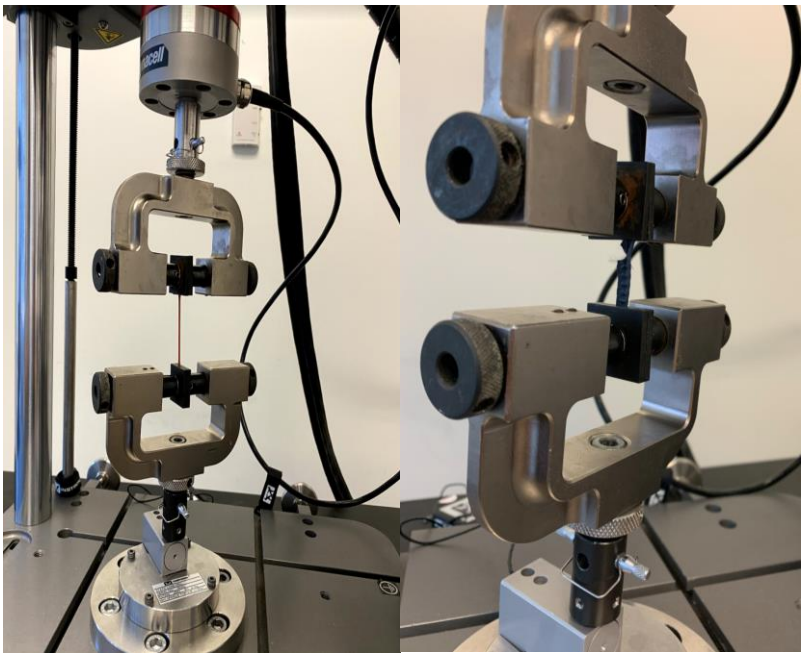


Figure 20: Material testing setup using the Instron 5544.

5.2.2 Data for Tensile Testing

All of the silicone materials showed similar ultimate tensile strengths. The data collected in each trial for each material can be seen in Appendix B. Throughout this testing, the silicone sheets showed similar results. The only exception was that the 0.007 inch medical grade silicone and the non-medical grade 0.03 inch showed lower points of failure than the other silicone sheets tested. Additionally, when the polyester was tested, it reached failure much more quickly. This could be due to the way that the polyester was manufactured. It appeared as if the failure for each slice of polyester ripped along the larger stitches of the polyester. If a more uniform polyester was used, the results could have been different. For the initial testing, the polyester did not have the tensile strength which we believed was needed for our final design, so we were able to eliminate this as an option and move forward with testing on only the silicone sheets.

5.2.3 Suture Retention Testing

The next set of testing was to do suture retention testing of each material. The ANSI/AAMI/ISO 7198:1998/2001/(R) 2004 standard, referenced in appendix A, was used as an outline to set up this test. The suture was threaded through the silicone at a minimum of 2 mm from the edge, and the grips of the Instron were used to secure both the suture and the silicone. Tests were conducted until complete failure was seen in the material. The experimental setup can

be seen in Figure 21. Testing of a control group made up of only the leaves was attempted but the suture would rip through the leaves before it could even be set up on the Instron.

Figure 21: Suture retention testing setup using the Instron 5544

5.2.4 Data for Suture Retention

During the suture retention testing a total of three trials were performed on each material. When looking at the points of failure, the thicker materials always withstood a higher load than the thinner ones during out testing. However, the medical grade silicone had a higher suture retention than the generic silicone that was tested. One observation seen in most of the trials is that the material would rip slightly before the suture would tear completely through the material. An example of this can be seen in Figure 22, and the rest of the data can be seen in Appendix C. In Figure 22, the orange arrow indicates a slight tear in the material caused by the suture that was seen in most of the trials.

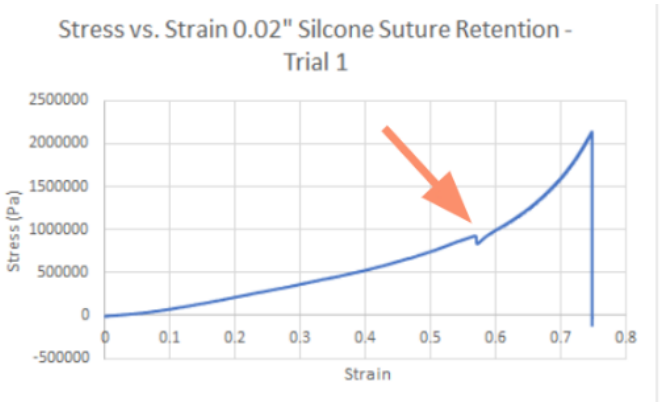


Figure 22: Stress vs. Strain plot of trial 1 of suture retention on the 0.02 inch thick silicone

5.2.5 Peel-Off Testing

The next set of testing was to do peel testing of the silicone with the decellularized spinach leaf attached to it with the fibrin glue that was discussed in Section 5.1. This test was performed to determine the strength of this fibrin glue solution to see if it would be a viable option to attach the decellularized leaf to the silicone tabs. Only one of the biocompatible, medical grade silicone sheets was used for this test since the only difference between all of the materials is the thickness. The leaf and the silicone were glued together on each end and then were cut into a dog-bone shape to ensure that the stresses the specimen would undergo would be around the midline instead of near the grips. Each end of the glued leaf and silicone were then placed in the grips of the Instron. The experimental setup can be seen in Figure 23 on the following page.

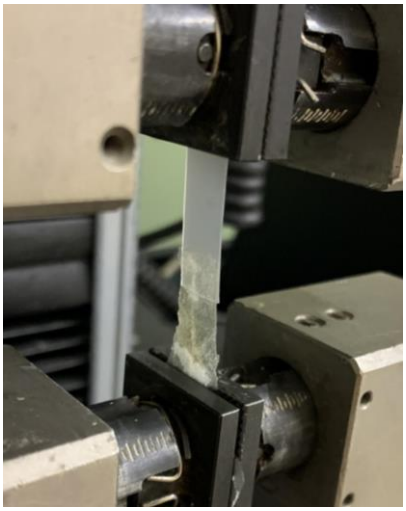


Figure 23: Peel-off Testing setup using the Instron 5544

5.2.6 Data for Peel-Off Testing

In each of the trials, slippage between the leaf and the silicone can be seen at around 0.25 N. Once slippage began, the silicone and leaf slowly peeled from each other until they became unattached. None of the trials surpassed 0.3 N before slippage was seen. The data graphs for peel off testing can be seen in Appendix D.

5.2.7 Results

Taking into consideration all of the data from these tests, our group decided to move forward with the 0.015 inch medical grade, non-reinforced silicone rubber sheets for the sewing tabs. This thickness was chosen because it provided the most well-rounded results of tensile and suture retention strength.

5.3 Cell Survival Testing

Cell survival testing was performed on the decellularized spinach leaves to verify the ability of cells to live on the leaf, and to measure the effects of our suture patch material on the survival of these cells. Prior research from Gaudette's team had shown that cells could be seeded onto the decellularized spinach leaves and continue to survive, but our team wanted to compare results from seeding on the decellularized leaf to results from seeding on a decellularized leaf that had our chosen patch material attached to it with fibrin glue. This comparison has implications in regard to the overall biocompatibility and integration capabilities of our design,

as our patch would be unlikely to successfully integrate into the heart tissue if cell survival was inhibited by the patch materials.

5.3.1 Cell Seeding on Decellularized Spinach Leaves

To begin our cell survival testing, we first seeded human mesenchymal stem cells (HMSCs) onto decellularized spinach leaves. This cell culture experiment was performed to establish a baseline for how well cells would survive on the leaves themselves, so this could be compared to the later testing to be performed on the leaves with the patch materials attached. The HMSCs used for seeding in the first round of survival testing were P9 cells. The cells were thawed in a water bath, and then introduced to a flask with 10 ml of complete HMSC media and incubated for two days prior to seeding on the leaves. After one day during the incubation period, the media was aspirated, and the cells were fed with 10 ml of new media.

The decellularized leaves were punched out using a metal hole puncher to create small circles of decellularized leaf that would fit easily into the well plates where the cells were seeded. Prior to seeding the cells onto the leaf cutouts, the cutouts were sterilized. The cutouts were placed into a cell culture dish, which was first filled with tris buffer, covered, and rotated on a rotating plate for about 20 minutes. The tris buffer was then carefully removed from the dish, and the dish was filled with DI water, covered, and rotated for an additional period of about 20 minutes. After this period, the DI water was carefully removed from the dish, and the dish was then filled with ethanol, covered, and rotated for 20 more minutes, after which point the leaves were sterilized and prepared to enter the BSC to have the cells seeded on them.

After incubation in the flask, the cells were seeded onto the sterilized leaf cutouts. Six leaf cutouts were used in this round of testing and were placed into a 12 well plate with one leaf cutout per well and a cloning well in each on top of the leaf cutout. Two additional wells were used as control wells and were seeded with cloning wells and cells without any decellularized leaf cutouts present, in order to compare cell survival on the plate to on the cutout.

Prior to seeding the cells onto the prepared well plate, the media was aspirated from the flask where the cells were incubated. The cells were then trypsinized and incubated for 5 minutes to ensure that they were free and moving. After adding more media to the flask, the cells and media were moved to a 15 ml conical tube and centrifuged. At this time, a small sample of the

cell solution was taken in order to perform a cell count. This was done so that the cells could be ensured to be adequately split between the wells during seeding.

Following centrifugation, the media solution was aspirated, more media was added to the conical tube, and the cell pellet was adequately mixed into the media. After this point, the media and cells were divided such that each well would receive approximately the same cell count, and the cells were then pipetted into the cloning well directly onto the leaf cutout, or onto the plate through the cloning well for the control wells. The well plate was then covered and incubated for three days. After one day during the incubation period, the wells were aspirated and fed with new media.

After the incubation period was completed, the cells were stained in order to observe the survival of the cells on the leaves and the well plate. After staining was completed, the cells were observed under a fluorescent microscope. For this set of leaves, there were some cells observed after seeding and incubation that were still surviving on the cutouts, but the quantity was small in comparison to what was seen on the control plates, and the cells themselves were smaller.

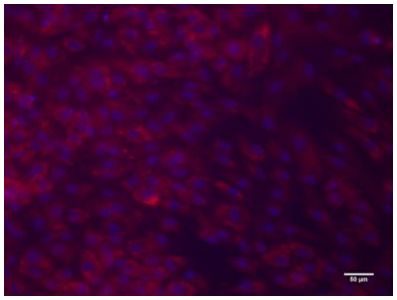
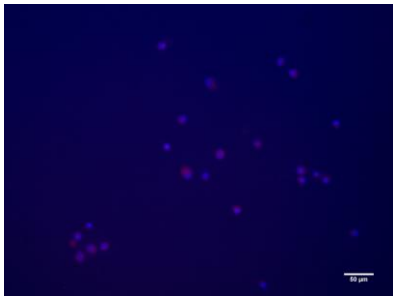
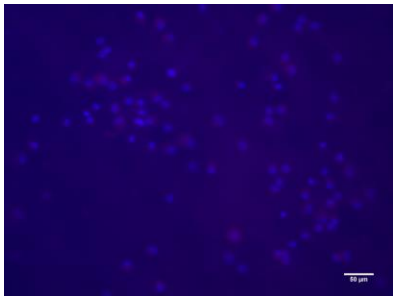
5.3.2 Cell Seeding on Decellularized Spinach Leaves with Patch Materials

After concluding the seeding and staining process for the decellularized spinach leaf cutouts, we began a second round of cell survival testing that included leaf cutouts with our full patch design material and structure implemented on them prior to seeding, to determine if the patch material impeded cell growth or survival. The leaf sections were cut out in the same method as in the first round of testing, and the silicone patch material was then attached. In order to do this, fibrin glue was made inside the BSC to ensure sterility, and quickly applied to the leaf cutouts, followed immediately by premade cutouts of the silicone patch material. These cutouts were made using the same tool as the leaf cutouts but using the smallest possible circle size.

The same incubation process was followed for the cells as in the first test, except that during this test, the HMSCs that were used were P5. After incubation, the cells were trypsinized, counted, and seeded per the same process as in the first test. During this testing, the 12 well plate was set up such that six wells contained a leaf cutout with a silicone cutout fibrin glued to its surface, three wells contained leaf cutouts without the patch material, and three wells were seeded as control wells, with no leaf cutouts at all. These cells were seeded and pre-stained so that they could be observed following their three day incubation period for survival. Control

tracker red was used to track the cells, along with Hoechst to stain the nuclei blue for easier visibility. The cells were observed under the fluorescent microscope and the control wells, wells with leaf cutouts, and wells with leaf cutouts and silicone patches, were compared for cell survival. The images taken for each type of well during this test can be seen in Table 7 below:

Table 7: Cell Survival Images with Silicone Patches Attached with Fibrin Glue

Control Well	**Leaf Cutout (No Silicone Patch)**	**Leaf Cutout (Silicone Patch)**
		

5.3.3 Results and Implications

Similarly to what was seen in the first round of cell survival testing, Table 7 shows that the cells survive much more favorably on the control wells than on the leaf cutouts. A much greater quantity of cells can be seen on the control well, and the cells that are seen there are larger and healthier in appearance than those on the two types of leaf cutout wells. This was to be expected.

However, as can be seen in the images, cells were able to survive on the leaf cutouts, both with and without the silicone patches attached. The leaf cutout with the silicone patch that was imaged actually has more cells surviving than the imaged leaf cutout without the silicone patch. Based on what was seen during this test, the silicone patch and fibrin glue did not have an adverse effect on the ability of cells to survive on the decellularized spinach leaf. This indicates that our patch design should not have a negative effect on the biocompatibility or the ability to integrate of the spinach leaf patch as a whole.

However, in future research, the survival of the cells overall on the leaf would need to be improved in order to eventually promote cell growth on the patch to improve its overall

functionality as a treatment for myocardial infarction. Future work on the cell survival on the scaffolds could also be expanded to look into potential for regain of some function lost due to myocardial infarction, such as contractability and tissue growth.

5.4 Arterial Connection Testing

5.4.1 Flow Test

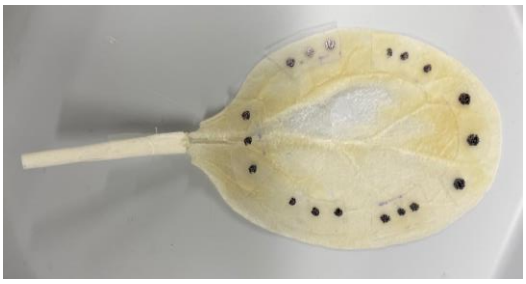


Figure 24: Final Design Product

In order to test for the viability of using the stem as an arterial connection point we did a simple flow test for proof of concept. Three tests were performed to determine what tubing created the best possible design to limit tearing and overflow (Figure 24 shows stem set up). Using a syringe filled with water we pushed 1.5 ml of water at a steady rate through the stem. We focused on the stem section and checked for ripping or leaking during and after the water being pushed through.

5.4.1.1 Testing the Natural Stem

We started with testing the stem alone to determine how well the natural structure handled flow without additional material (Figure 25). When testing we saw that the stem allowed water to pass through the leaf but the stem leaked from underneath and allowed water to drip through. This indicated that another material would be necessary to stabilize the stem and prevent leakage.

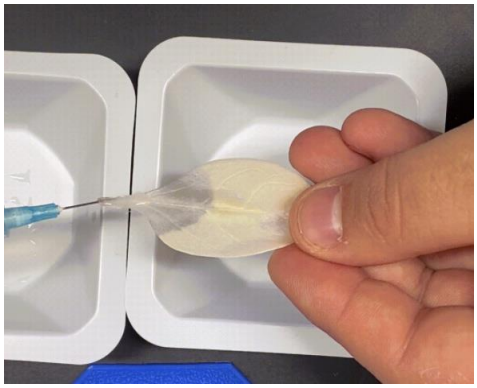


Figure 25: Flow Test with Spinach Stem Alone

5.4.1.2 Testing the Natural Stem with Interior Microtubing

In order to prevent leaking we added the interior medical microtubing. This material was intended to push the flow through the stem to the leaf. When testing flow we saw that leakage from the stem stopped but with the additional pressure the microtubing tore at the base of the leaf creating an additional opening for the flow to go to rather than the vascularized leaf. This indicated that an additional material encompassing the stem would be needed in order to prevent tearing under pressure. In Figure 26, the tear can be seen where there is a red circle.

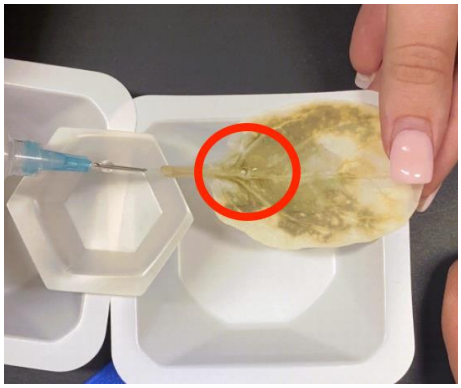


Figure 26: Flow Test with Micro Tubing

5.4.1.3 Testing the Natural Stem with Interior and Exterior tubing

Our final flow test was done utilizing both the interior microtubing from 5.4.1.2 and an additional electrospun material. With the additional material surrounding the stem the water was able to flow freely to the leaf and there were no tears at the stem or base of the leaf after the test was completed (Figure 27).

Figure 27: Flow Test with Micro Tubing and Electrospun material

5.4.2 Results

With the flow test completed, we found that the stem alone is unable to be used as an arterial connection point as there is leakage. We also saw that the stem and microtubing limited leakage but still experienced tearing. Our final test using the microtubing and electrospun material gave the best results with no leakage or tearing occurring after 1.5ml of water was pushed through it. This experiment gave us proof of concept that the stem can be utilized as a connection point with the additional materials but further testing according to blood flow standards would need to be done to verify compatibility.

Chapter 6: Final Design and Validation

6.1 Final Design

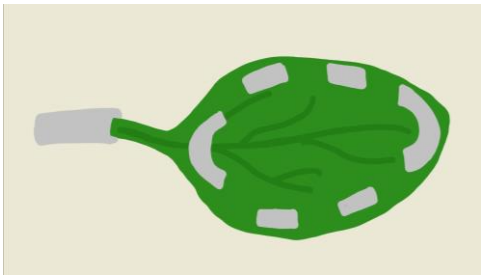


Figure 28: Final Design Sketch of Cardiac Patch

The final design of our cardiac patch has four primary components. The spinach leaf that makes up the base of our scaffold. The sewing patch materials that allow for the spinach leaf to be suturable. The stem extension that makes arterial attachment possible, bringing blood flow to the cells on the scaffold. Lastly, the cells that adhere to the scaffold making it contract and expand with the myocardial tissue. The full design sketch can be seen above in Figure 28.

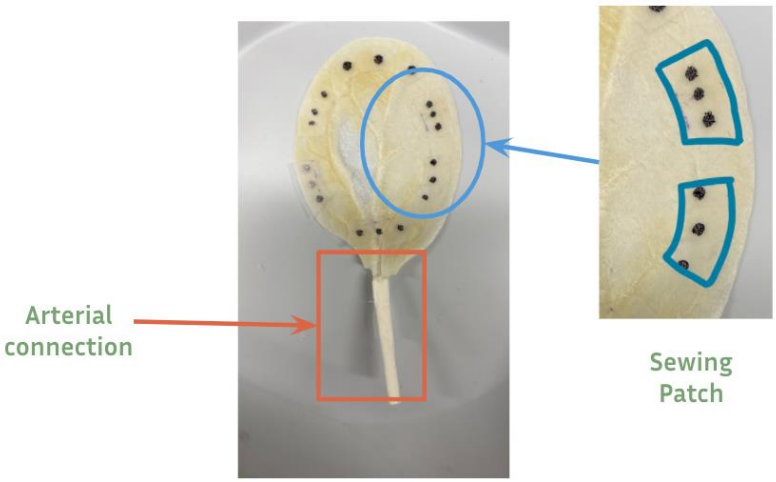


Figure 29: Full Design Mockup

Above in Figure 29, the full design mockup is visualized with the materials and decellularized leaf. The sewing patches are identified by three black dots, noting the location of each clear patch. An enhanced image of the patches can be seen on the right with each patch outlined in blue. The arterial connection is shown on the bottom half of the mockup, only the exterior material can be seen enclosing the leaf, but the interior tubing is inside the natural stem.

Each of these components, from the stem to the arterial connection material will be discussed in the following sections.

6.1.1 Spinach Leaf

The spinach leaf is the base component of our design. In order to produce a patch to assist in myocardial infarction repair we decided to expand upon Professor Gaudette's research in using decellularized spinach leaves as scaffolds for myocardial tissue growth. Gaudette's patch looked great on tv and had a clear vascularized structure but it had some big limitations (Figure 30). The leaf was too flexible and folded on itself when hydrated. It also was insuturable because of the limited strength causing it to rip at the introduction of a needle. In order to combat that we need to make some changes to the design. Utilizing Professor Gaudette's procedure for decellularization, our scaffold is completely devoid of its cells and maintains its vascularization.

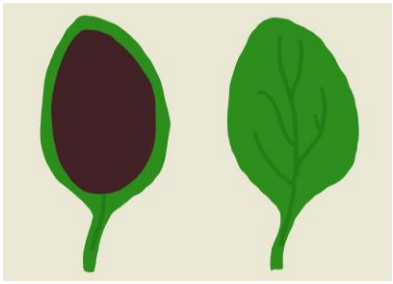
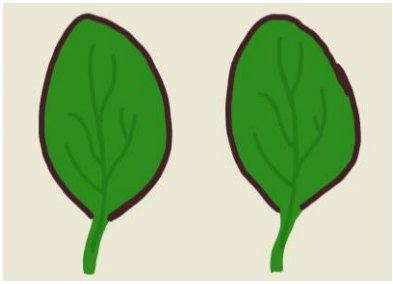
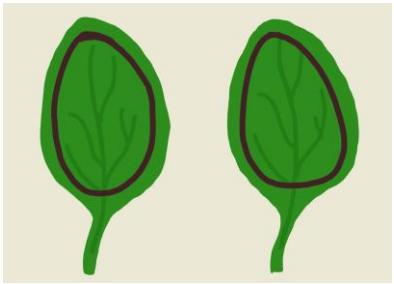
Figure 30: Decellularized Spinach Leaf

6.1.2 Sewing Patch Material

In our engineering design process, we started with brainstorming different sewing patch designs that allows for the spinach leaf to be sutured. We started by thinking about large material patches on either side of the leaf, on one side by itself and even one side with the large patch and another with a ring design. These designs had too much material and limited the leaf from being able to contract movement because they were too stiff, an example of this is shown in the first column of Table 8. We also worked on an encapsulation design where the material would hug the edges of the leaf and cover both sides, shown in the middle column of Table 8. This design concept was also limiting in motion but was also too complicated to reproduce. In order to make the design more easily reproduced and allow for movement we discussed a double o ring design,

shown in the last column of Table 8. This design was the best of our original concepts and led to our optimized design.

Table 8: Initial Sewing Patch Material Designs

Large Patch	Encapsulation	Double O Ring
		

Taking from our first design concepts we realized that the sewing patches were only needed on side to allow for suturing. We initially worked with the O ring design that you saw in the past slide. It is the most easily reproduced design but it made the edges of the leaf very stiff. In order to combat that we moved to a notched patch design that allowed for surgeons to suture in the edges but allowed for cells to potentially grow all the way around the patches and contract the leaf to match the natural cell movement in myocardial tissue. The progression of this design can be seen below.

Table 9: Progression Sewing Patch Material Designs

Ring Patch	4 Notched Patch	6 Notched Patch
		

The final material chosen was the 0.015 inch thick medical grade silicone. It had the best performance in terms of tensile testing, suture retention and peel off testing. The 4-6 patch

design allows for maximum area for the cells to contract while still allowing the patch to be sutured. This final material is clear, and thus, harder to image. Figure 31 below shows the final sewing patches in the clear material, indicated by the three black dots and blue outline.

Figure 31: Clear Sewing Patch

6.1.3 Stem Attachment

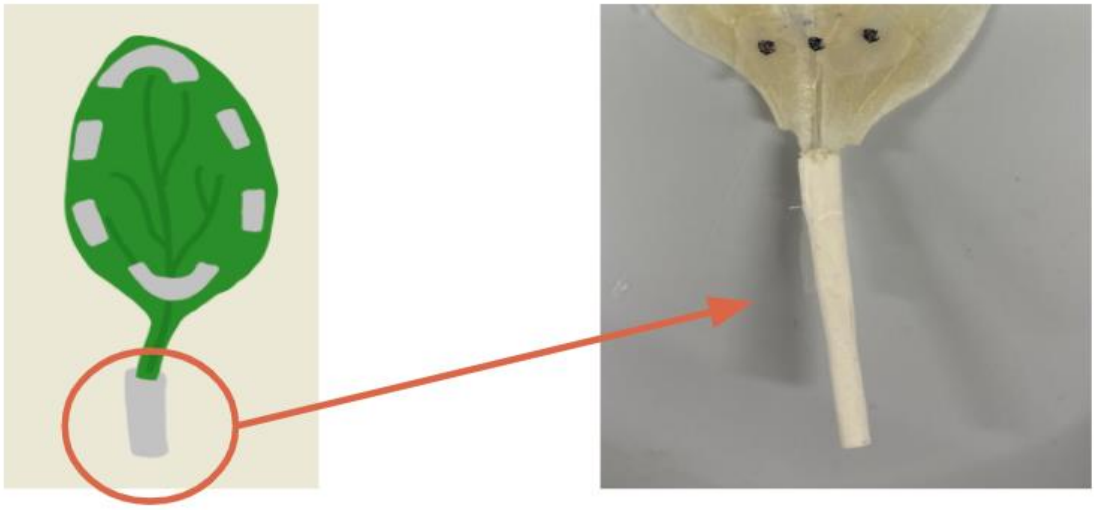


Figure 32: Arterial Attachment (left: sketch) (right: final product)

One constraint that we needed to solve when thinking about implementing this cardiac patch in the body was how we were going to get blood flow to the leaf. We need an arterial connection to the natural stem of the leaf that can be sutured in. The spinach stem itself, like its leaf, is very fragile and cannot be sutured into without ripping the stem. In order to combat this, we need an additional tubing material that can be sutured into that allows blood to flow into the leaf. We utilized an interior and exterior tubing in order to maximize flow and make the stem attachable (Figure 32). The interior tubing is a polyethylene microtubing that stabilizes the inside of the stem and prevents collapse. The exterior tubing wrapping around the stem is an

electrospun material that helps to prevent tears and leaking. Through our testing we verified that the stem could sustain flow without tear indicating a probable point of connection.

6.1.4 Cell Adhesion and Growth

Figure 33: Silicone Patches for Cell Culture Testing

The final component of our cardiac patch design is the cells that need to adhere to the spinach scaffold. The cell growth will allow for the patch to contract and expand with the surrounding heart muscle. The cell culture experiment proved that hMSCs are able to grow on the spinach leaf scaffold and are unaffected by the silicone patch (Figure 33).

6.2 Evaluation of Design Criteria

6.2.1 Objectives

There were 3 major objectives for the cardiac patch, and they were able to maintain the cell life that was seated on it, it also needed to meet our mechanical requirements for testing. And most importantly it has to be clinically applicable and be able to be used during surgery. Our final design was able to meet all three of these objectives. Our cell culture experiment showed that cell life can be sustained on our spinach base and sewing patches. The patch also met the mechanical requirements to make it compatible with recovering the function of the infarct tissue. Lastly, the patch is able to be used in surgery because it can now be handled by surgeons and withstand suturing.

6.2.2 Constraints

There were also constraints that needed to be addressed with this design. Working with the spinach leaves, they are very fragile and can fold over and rip with minimal contact. Another constraint that we have is reproducibility. Due to the different shaped leaves and the various sizes, they come in no patch will be exactly the same so being able to try to recreate and reproduce patches that are exactly the same is a challenge.

These constraints were very present during our design process. The spinach leaves maintained their fragility but with the addition of the sewing patches they became easier to manipulate. The edges are weighed down with the silicone limiting the patch from folding in on itself. The irregularity in the sizes of leaves, however, is still a constraint that could be improved upon in the future. A stamp tool could be used in future work to punch out the silicone to the same size, but it needs to be within the correct range for the spinach leaves. In order to do this the samples for the scaffold must be constricted to a range of sizes. In its current state the patches must be cut individually to match the size of the leaf.

6.2.3 Functions

The cardiac patch needed to be able to perform two functions in order for our team to consider the design successful. These two functions included the capacity to sustain cell growth and to be suturable. Both of these functions are achieved by our final patch design.

6.2.4 Specifications

The overarching specification that needs to be maintained for our patch design is the leaf must be able to withstand forces up to 6 newtons at all times to be able to function with the cardiac tissue it is sewn into. The patch also needed to meet the specifications for the peel test and suture retention test while abiding by and following all of the ASTM Standards for each of the respective mechanical tests. These specifications were met using 0.0015 inch thick medical grade silicone sewing patches.

6.2.5 Capstone Design Evaluation

In total our cardiac patch design was successful in achieving its objectives, overcoming its constraints, meeting its functions and its specifications. The patch design shows potential for replacing the loss of function experienced by myocardial infarction and reducing the need for total heart transplants for patients who experience a myocardial infarction.

Chapter 7: Discussion

7.1 Economics

There are many different economical concerns when it comes to trying to create a device that has a significant impact on the consumer's life. This device was developed around being able to be reproducible and scalable in the years to come. The way that the decellularized spinach leaves are stored via a freezing technique called lyophilization, this will allow for production and storage costs to be much lower. The production aspect of this device is built around a simple but effective design to allow the decellularized leaf to host and grow new cardiac tissue. In turn, this means that the production costs of this device will be lower than most cardiac tissue regeneration devices. Another key aspect to this design is the capability of having an extended shelf life. Due to the process the device goes through in its production phase of being lyophilized, allows for the device to be stored and used on an as needed basis. This means that there does not have to be continuous production of this device which could potentially lower the overall total cost of the production process for it.

7.2 Environmental Impact

Our patch design, as it is made of a spinach leaf as its primary scaffold, would have minimal impact to the environment. The leaf itself can continue to be grown as it already is, as it is decellularized and sterilized before being applied in its intended use as a cardiac patch. The leaf itself also has no real potential to harm the environment in the event of needing to dispose of it, as the scaffold is made of natural material. Since this scaffold is a naturally occurring material, it can be reproduced without harming the environment in any unexpected ways, as it is already grown around the world for agricultural purposes and occurs naturally in some environments and without undue damage in others. Overall, compared to many other medical devices, our proposed patch treatment using a spinach leaf scaffold would have minimal influence on the environment, and is a sustainable and naturally based treatment method for myocardial infarction.

7.3 Societal Influence

Myocardial infarction is a significant societal problem in the United States and around the entire world. Cardiac disease is a leading cause of death worldwide, and each year there are hundreds of thousands of cases of myocardial infarction that occur in the United States alone. Currently, there are very few effective treatment options for this condition, particularly in the case of a severe infarction, where the common treatment methods such as reperfusion are not viable options due to the extent of the damage. In a clinical application, our patch may fill this treatment gap, as it is intended as a treatment to be used particularly for these severe cases with significant infarct damage. This would vastly improve treatment and outcomes for patients who have experienced a severe myocardial infarction, which is a prevalent condition across today's society. In a clinical application, this treatment would be intended to increase the overall health and quality of life of these patients following their myocardial infarction, where current treatment options for these patients are limited and the consequences of these events can be severe.

7.4 Ethical Concerns

There are essentially no major ethical concerns relating to the development and implementation of this product. When using stem cells, there are usually concerns regarding embryonic stem cells that end up destroying human embryos. However, hMSCs do not have these ethical issues as they are not derived from embryos and therefore have no ethical problems associated with them.

7.5 Health and Safety Issues

As with any device that is designed to be implanted into the body, there are safety concerns associated with our patch design. One of the most significant concerns with the spinach leaf as the scaffold for our design is the fragility of the leaf under normal physiological loads. Our silicone patch design combats this concern by adding strength to the patch as a whole so that it can withstand these loads, as well as increasing the suturability of the patch, as the leaf on its own cannot hold sutures without tearing. However, despite the improved properties of the leaf with our silicone patch design, there is still a risk of the patch failing, as there is with any

implanted device or surgical repair. In the case of our patch, where the repair occurs directly on the heart muscle, and is used to treat myocardial infarction, the consequences of the device failing would be severe. Any damage to the heart can cause very serious, immediate complications for a patient, and the cardiac tissue at the site of an infarct, where our patch will be implanted, is already severely damaged and fragile prior to treatment, so device failure would be a very serious concern for a patient. However, based on our mechanical testing of our design with the silicone patches, we expect that our patch would not fail under normal physiological loads and conditions, reducing the safety concerns associated with the treatment.

7.6 Manufacturability

In order to improve the manufacturability of this patch, molds to cut out the shapes of the sewing tabs must be made. These must be able to cut through a sheet of silicone to be able to cut out the correct shape of each sewing tab cleanly. Different cutouts would have to be made for the tabs on each end and the sides of the leaf due to their slightly different shapes. Additionally, a uniform size would have to be decided on since the silicone tabs would not fit on spinach leaves that were either too large or too small.

7.7 Sustainability

This design utilizes a spinach leaf, medical grade silicone, cardiomyocytes and electrospun tubing. Spinach can be sustainably grown and harvested by leaving the roots to regrow after the spinach is cut (Almanac, 2021). The other materials are used in minimal amounts and are implemented for long term use.

Chapter 8: Conclusions and Recommendations

8.1 Final Review

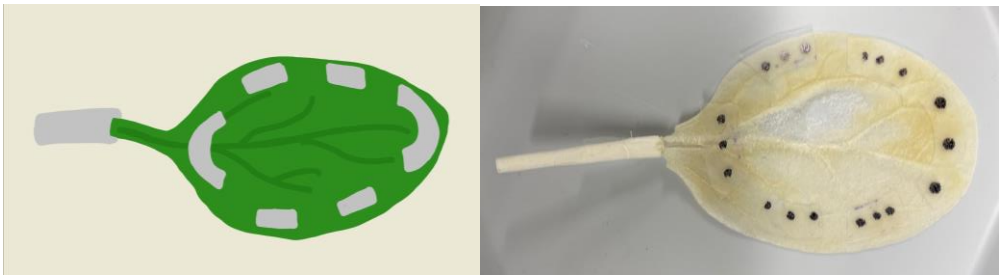


Figure 34: Final Product Design of Final Product

At the start of the design process we saw that in order to make a sew-on patch utilizing a vascularized structure, like a spinach leaf, changes needed to occur. The spinach leaf itself needed to be less fragile and easier to handle for surgical use, which we corrected with our material additions to create a supportive backbone for the decellularized leaf. The strength of the leaf was increased along with it being made suturable with the addition of our silicone patches This material can be seen in our suture retention and tensile tests. In regard to supporting cell growth, we saw that our cells survived on the leaf with the sewing patches suggesting the potential for biocompatibility. And finally, our arterial connection experiment showed that with the addition of the medical tubing and the electrospun material liquid can go through the stem, creating a probable point for connection. Overall, our final product met our initial objectives for the project and leads to future implications and suggestions for further research as a sew on cardiac patch (Figure 34).

8.2 Implications

Overall, our testing indicates that our final, cell seeded, patch design may be a biocompatible treatment for myocardial infarction that is mechanically strong enough to be sutured into the heart muscle. Mechanical testing indicated that our chosen material was the most suited for enhancing suturability of the spinach leaf scaffold out of all of those that we tested. Additionally, cell seeding on the patch indicated that the chosen material did not reduce the survival of cells on the scaffold, and our arterial connection experiment enhanced the ability of

the stem to withstand flow similar to what it would need to do in a clinical application. Therefore, this treatment method could be successful at providing a solution for treating the damage caused by myocardial infarction directly at the site of the infarct, and more effectively than existing options. This could improve recovery from a myocardial infarction for patients and reduce the risk of heart failure and the potential need for a heart transplant in the future for patients.

8.3 Recommendations for Future Work

While our cardiac patch design shows promise as an implantable, biocompatible patch there is still much work to be done. Our testing set the foundation of the patch showing the suture patches had little effect on the cell growth and that liquid can flow through the stem. In order to further prove that additional testing is required. Further repetitions of our cell culture test are needed, including the process of making the leaf fully sterilized and seeded. Along with the cell culture, more in-depth biocompatibility testing needs to be done to determine if our cardiac scaffold would have a positive or negative immuno-response when implanted. Also, testing of the leaf's contraction in comparison to normal myocardial tissue must be conducted. More in-depth arterial connection testing must be done including a flow test with the same viscosity of blood as well as the pressure and rate a typical heartbeat would bring. Further research may need to be done to determine if the patch is able to handle normal blood flow or if blood thinners might be needed. An experiment where the patch is sewn into a pig heart can be conducted to determine how well the leaf holds up through a surgery and how difficult the arterial connection may be. Further work on a reproducible stamp to cut out and apply our sewing patches would be ideal in making our patch reproducible. Finally, more mechanical testing procedures would have to be performed to completely understand if the cardiac scaffold can withstand all the forces of the heart. For example, a future test could be a cyclic loading test which could show a more accurate representation of what values are needed for a successful scaffold and design. While our patch laid a foundation for the overall design, much work is needed to bring it to a patient in need.

References

Almanac: Sustainable Cut: Giving Spinach a Second Chance. Evolution Fresh. (2021). https://www.evolutionfresh.com/blog/article/sustainable-cut-giving-spinach-second-chance/#:~:text=After%20the%20spinach%20is%20cut,can%20regrow%20after%20being%20mowed.&text=Using%20sustainably%2Dcut%20spinach%20does,we%20work%20with%20happy%2C%20too.

Benjamin, E. J., Muntner, P., Alonso, A., Bittencourt, M. S., Callaway, C. W., Carson, A. P., Chamberlain, A. M., Chang, A. R., Cheng, S., Das, S. R., Delling, F. N., Djousse, L., Elkind, M., Ferguson, J. F., Fornage, M., Jordan, L. C., Khan, S. S., Kissela, B. M., Knutson, K. L., Kwan, T. W., … American Heart Association Council on Epidemiology and Prevention Statistics Committee and Stroke Statistics Subcommittee (2019). Heart Disease and Stroke Statistics-2019 Update: A Report From the American Heart Association. Circulation, 139(10), e56–e528. https://doi.org/10.1161/CIR.0000000000000659

B.P. Chan and K.W. Leong. *European Spine Journal* **17** (2008) 467-479

Centers for Disease Control and Prevention. (2020, September 8). Heart Disease Facts. Centers for Disease Control and Prevention. https://www.cdc.gov/heartdisease/facts.htm. Hyattsville, MD: National Center for Health Statistics; 2012. Accessed May 9, 2019.

D.B. Tran and C. Webber. *Anatomy, Thorax, Heart Muscles* (2020)

Developer. (2020, October 3). Myocardial Infarction (Heart Attack). Top Neuro Docs. https://www.topneurodocs.com/myocardial-infarction-heart-attack/.
Domenech PMC, E. (n.d.). Europe PMC. https://europepmc.org/article/PMC/PMC3325606.
Image 4 (cell seeded vs acellular) https://europepmc.org/article/pmc/pmc5124749 (2016)

E. Romito et al, *Applied Physiology* **123** (2017) 728-745

GORE ACUSEAL Cardiovascular Patch. HC21. (2020, July 13). https://www.healthcare21.eu/product/gore-acuseal-cardiovascular-patch/.

GORE-TEX® Cardiovascular Patch. Gore Medical Asia Pacific. (n.d.). https://www.goremedical.com/ap/products/cardiovascularpatch.

Heart Basics. (n.d.). Retrieved October 18, 2020, from https://www.hoag.org/specialties-services/heart-vascular/conditions/heart-basics/

Heart Foundation NZ. (n.d.). https://www.heartfoundation.org.nz/your-heart/how-the-heart-works. (2020)
J. Bogaert et al, *Journal of the American College of Cardiology* **35** (2000) 1525-1534

J.R. Gershlak. *Biomaterials* **125** (2017) 13-22

K. Huang et al. *Science Translational Medicine* **12** (2020)

K.L. Fujimoto et al. *Journal of the American College of Cardiology* **49** (2007) 2292-2300

Lancaster, J. J., Koevary, J. W., Chinyere, I. R., Daugherty, S. L., Fox, K. A., & Goldman, S. (2019, September 17). *Surgical treatment for heart failure: cell-based therapy with engineered tissue*. Vessel Plus. https://vpjournal.net/article/view/3218. Image 3 (cardiac patch)

M. Chrobak et al. *ACS Biomaterial Science and Engineering* **3** (2016) 1394-1403
Medical Expo Cardiac Tissue Matrix PB. (n.d.).
https://www.medicalexpo.com/prod/labcor/product-84175-534465.html

NIH funds WPI Project to Develop a Heart Muscle Patch. WPI. (2010, December 9).
https://www.wpi.edu/news/nih-funds-wpi-project-develop-heart-muscle-patch-0.

S. Alston et al. *New method to prepare autologous fibrin glue on demand* **149** (2007) 187-195

Tang, J. et al (2018, November 3). AAAS.
https://advances.sciencemag.org/content/4/11/eaat9365 Image 9

T. Edvardsen et al, *Circulation* **106** (2002) 50-56

T. Shimizu et al. *Biomaterials* **24** (2003) 2309-2316

T. Su et al. *American Chemical Society* **10** (2008) 33088–33096

Thygesen, K. (2007, October 1). Universal definition of myocardial infarction: Kristian Thygesen, Joseph S. Alpert and Harvey D. White on behalf of the Joint ESC/ACCF/AHA/WHF Task Force for the Redefinition of Myocardial Infarction .
https://academic.oup.com/eurheartj/article/28/20/2525/416363.

V. Talman et al, *Cell and Tissue Research* **365** (2016) 563-581

W. Liu et al, *Magnetic Resonance in Medicine* **52** (2004) 1282-1290

WPI Team Grows Heart Tissue on Spinach Leaves (2017, March 22). WPI
https://www.wpi.edu/news/wpi-team-grows-heart-tissue-spinach-leaves

Appendices

Appendix A: ISO Standards

ISO 10993-5:2009 - Biological evaluation of medical devices — Part 5: Tests for in vitro cytotoxicity

Introduction

Due to the general applicability of *in vitro* cytotoxicity tests and their widespread use in evaluating a large range of devices and materials, it is the purpose of this part of ISO 10993, rather than to specify a single test, to define a scheme for testing which requires decisions to be made in a series of steps. This should lead to the selection of the most appropriate test.

Three categories of test are listed: extract test, direct contact test, indirect contact test.

The choice of one or more of these categories depends upon the nature of the sample to be evaluated, the potential site of use and the nature of the use.

This choice then determines the details of the preparation of the samples to be tested, the preparation of the cultured cells, and the way in which the cells are exposed to the samples or their extracts.

At the end of the exposure time, the evaluation of the presence and extent of the cytotoxic effect is undertaken. It is the intention of this part of ISO 10993 to leave open the choice of type of evaluation. Such a strategy makes available a battery of tests, which reflects the approach of many groups that advocate *in vitro* biological tests.

The numerous methods used and endpoints measured in cytotoxicity determination can be grouped into the following categories of evaluation:

— assessments of cell damage by morphological means;

— measurements of cell damage;

— measurements of cell growth;

— measurements of specific aspects of cellular metabolism.

There are several means of producing results in each of these four categories. The investigator should be aware of the test categories and into which category a particular technique fits, in order that comparisons be able to be made with other results on similar devices or materials both at the intra- and interlaboratory level. Examples of quantitative test protocols are given in annexes. Guidance for the interpretation of the results is given in this part of ISO 10993.

ISO 11737-2:2019 - Sterilization of medical devices — Microbiological methods — Part 2: Tests of sterility performed in the definition, validation and maintenance of a sterilization process

Introduction

A sterile medical device is one that is free from viable microorganisms. International Standards that specify requirements for validation and routine control of sterilization processes require, when it is necessary to supply a sterile medical device, that adventitious microbiological contamination of a medical device from all sources be minimized. Even so, medical devices produced under standard manufacturing conditions in accordance with the requirements for quality management systems (see, for example, ISO 13485) can, prior to sterilization, have microorganisms on them. Such products are non-sterile. The purpose of sterilization is to inactivate the microbiological contaminants and thereby transform the non-sterile products into sterile ones.

The kinetics of inactivation of a pure culture of microorganisms by physical and/or chemical agents used to sterilize medical devices can generally best be described by an exponential relationship between the numbers of microorganisms surviving and the extent of treatment with the sterilizing agent; inevitably this means that there is always a finite probability that a microorganism might survive regardless of the extent of treatment applied. For a given treatment, the probability of survival is determined by the number and resistance of microorganisms and by the environment in which the organisms exist during treatment. It follows that the sterility of any one item in a population subjected to sterilization processing cannot be guaranteed and the sterility of a processed population is defined in terms of the probability of there being a viable microorganism present on a product item.

Generic requirements of the quality management system for design and development, production, installation and servicing are given in ISO 9001 and particular requirements for quality management systems for medical device production are given in ISO 13485. The standards for quality management systems recognise that, for certain processes used in manufacturing, the effectiveness of the process cannot be fully verified by subsequent inspection and testing of the product. Sterilization is an example of such a process. For this reason, sterilization processes are validated for use, the performance of the sterilization process is monitored routinely and the equipment is maintained.

International Standards specifying procedures for the development, validation and routine control of the processes used for sterilization of medical devices have been prepared [see ISO 11135, ISO 11137 (all parts), ISO 14937, ISO 14160, ISO 17665-1 and ISO 20857]. An element of validation might consist of exposing medical devices to the sterilizing agent with the extent of treatment being reduced relative to that which will be used in routine sterilization processing, in order to provide a knowledge of the resistance to the agent of the microbial contamination as it occurs naturally on medical devices. The reduced exposures applied in these instances are often called fractional exposures or verification doses. Subsequent to this reduced exposure, medical devices are subjected individually to tests of sterility as described in this document. Examples of the use of such tests are in:

a) establishing a dose for sterilization by radiation,

b) demonstrating the continued validity of an established sterilization dose, and

c) establishing a cycle for sterilization by evaluating the product's naturally occurring bioburden.

Product that has been exposed to a terminal sterilization process in its final packaged form has a very low probability of the presence of a viable microorganism; such as one in one million or 10^{-6}. As such, performing a test of sterility on product that has been exposed to the complete sterilization process provides no scientifically usable data and is not recommended.

Annex A of this document gives guidance on the techniques used and on practical aspects of the requirements.

ISO 10993-1:2018 - Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process

Introduction

The primary aim of this document is the protection of humans from potential biological risks arising from the use of medical devices. It is compiled from numerous International and national standards and guidelines concerning the biological evaluation of medical devices. It is intended to describe the biological evaluation of medical devices within a risk management process, as part of the overall evaluation and development of each medical device. This approach combines the review and evaluation of existing data from all sources with, where necessary, the selection and application of additional tests, thus enabling a full evaluation to be made of the biological responses to each medical device, relevant to its safety in use. The term “medical device” is wide-ranging and, at one extreme, consists of a single material, which can exist in more than one physical form, and at the other extreme, of a medical device consisting of numerous components made of more than one material.

This document addresses the determination of the biological response to medical devices, mostly in a general way, rather than in a specific device-type situation. Thus, for a complete biological evaluation, it classifies medical devices according to the nature and duration of their anticipated contact with human tissues when in use and indicates, in a matrix, the biological endpoints that are thought to be relevant in the consideration of each medical device category. See also 3.14, Note 1 to entry.

The range of biological hazards is wide and complex. The biological response to a constituent material alone cannot be considered in isolation from the overall medical device design. Thus, in designing a medical device, the choice of the best material with respect to its biocompatibility might result in a less functional medical device, biocompatibility being only one of a number of characteristics to be considered in making that choice. Where a material is intended to interact with tissue in order to perform its function, the biological evaluation needs to address this.

Biological responses that are regarded as adverse, caused by a material in one application, might not be regarded as such in a different situation. Biological testing is based upon, among other things, *in vitro* and *ex vivo* test methods and upon animal models, so that the anticipated behaviour when a medical device is used in humans can be judged only with caution, as it cannot be unequivocally concluded that the same biological response will also occur in this species. In addition, differences in the manner of response to the same material among individuals indicate that some patients can have adverse reactions, even to well-established materials.

The primary role of this document is to serve as a framework in which to plan a biological evaluation. A secondary role is to utilize scientific advances in our understanding of basic mechanisms, to minimize the number and exposure of test animals by giving preference to *in vitro* models and to chemical, physical, morphological, and topographical characterization testing, in situations where these methods yield equally relevant information to that obtained from *in vivo* models.

It is not intended that this document provide a rigid set of test methods, including pass/fail criteria, as this might result in either an unnecessary constraint on the development and use of novel medical devices, or a false sense of security in the general use of medical devices. Where a particular application warrants it, experts in the product or in the area of application concerned can choose to establish specific tests and criteria, described in a product-specific vertical standard.

ISO 10993 series is intended for use by professionals, appropriately qualified by training and experience, who are able to interpret its requirements and judge the outcome of the evaluation for each medical device, taking into consideration all the factors relevant to the medical device, its intended use and the current knowledge of the medical device provided by review of the scientific literature and previous clinical experience.

Informative Annex A contains a table that is generally helpful in identifying endpoints recommended in the biocompatibility evaluation of medical devices, according to their category of body contact and duration of clinical exposure. Informative Annex B contains guidance for the application of the risk management process to medical devices which encompasses biological evaluation.

ASTM STP1173 - Biomaterials’ Mechanical Properties

Introduction is not available for public viewing

ANSI/AAMI/ISO 7198:1998/2001/(R) 2004 - Cardiovascular implants— Tubular vascular prostheses

1 Scope

1.1 This International Standard specifies requirements relating to testing, packaging, labeling, and terminology for sterile tubular vascular prostheses intended to replace, bypass, or form shunts between segments of the vascular system in humans.

This International Standard addresses vascular prostheses that are made wholly or partly of materials of: biological origin; synthetic textile materials; and synthetic nontextile materials. In addition, guidance for characterization of compound and composite prostheses is provided. It specifies the designation of materials of manufacture and the construction, and specifies the designation of sizes and dimensions of vascular prostheses. It refers to biological requirements of the materials of construction and of the finished product, taking into account the appropriate part of the horizontal International Standard ISO 10993.

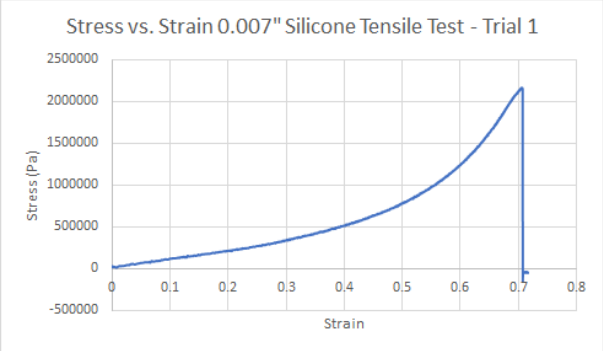
This International Standard also specified the designation of mechanical properties. It describes methods for the measurement and verification of the dimensions and mechanical properties declared by the manufacturer. It refers to sterilization of prostheses and specifies requirements for labeling and packaging. It also provides definitions of terms in common use.

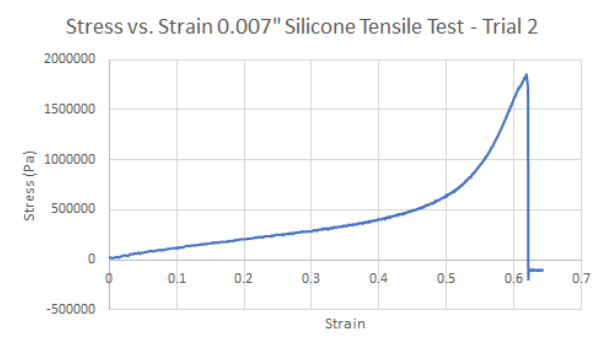
1.2 This International Standard does not specify all the performance or dimensional characteristics, but it does include methods for verifying that the nominal values disclosed by the manufacturer are within the permitted tolerances. These recommendations do not purport to comprise a complete test program.

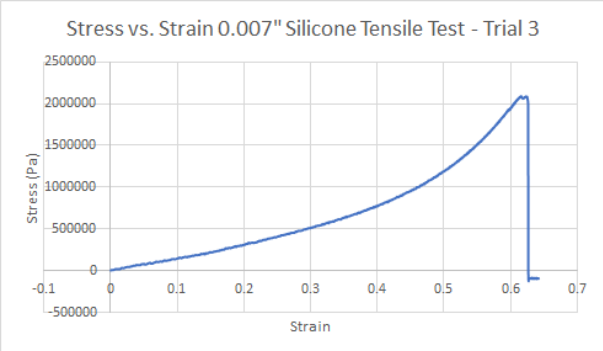
1.3 For the purposes of this International Standard, the disclosure of test methods, results, and other information on request shall relate solely to requests from a National Regulatory Authority with responsibility for surgical implants.

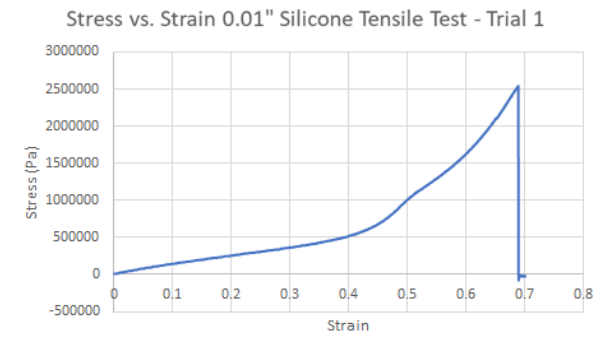
This International Standard does not apply to human donor tissue devices such as cryopreserved vessels. Also excluded are all patches, pledgets, and stents.

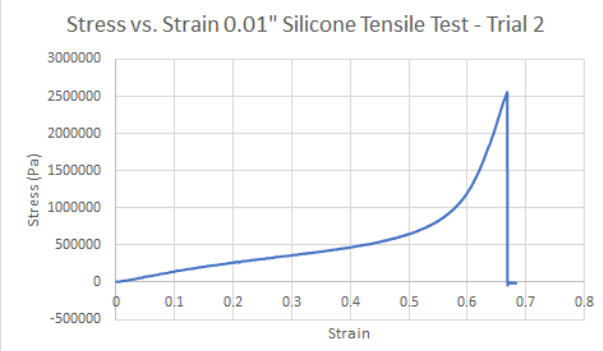
Appendix B: Tensile Testing Data

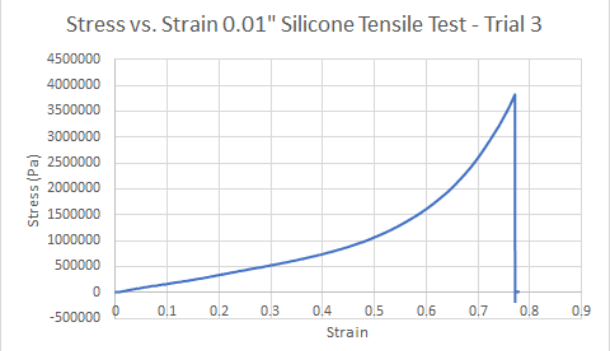


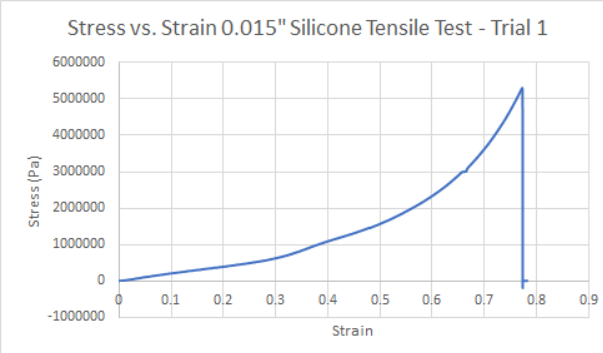


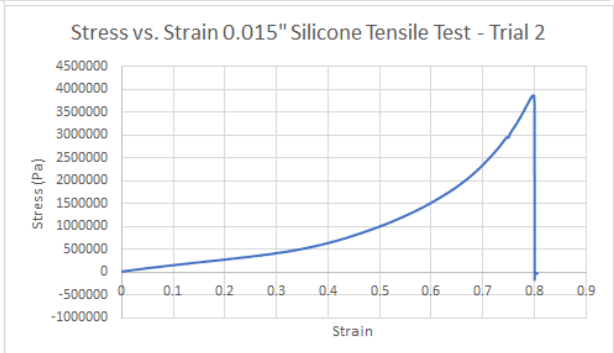


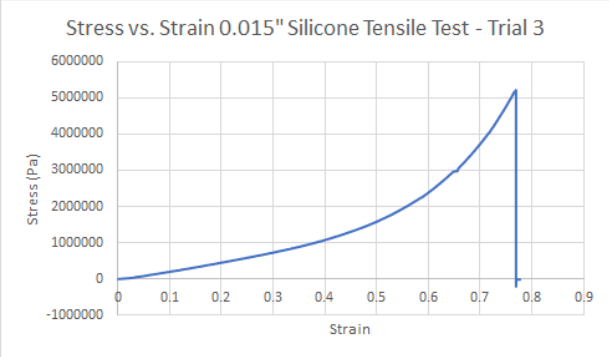


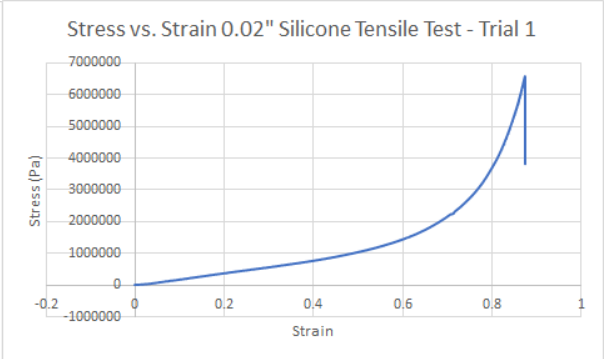


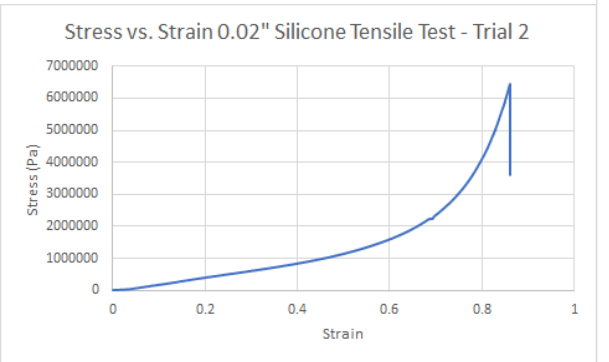


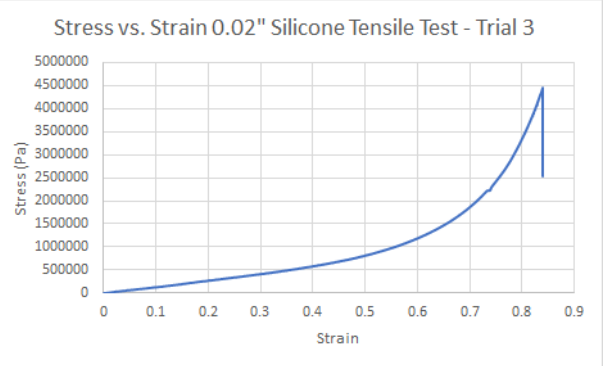


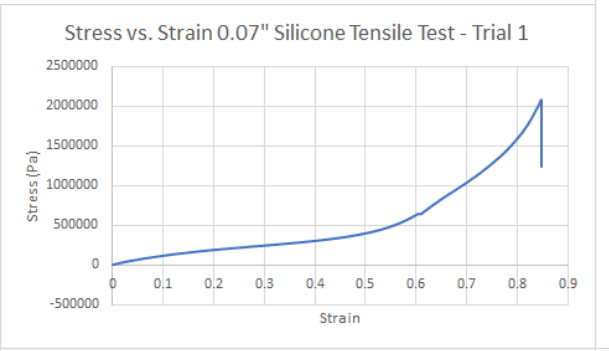


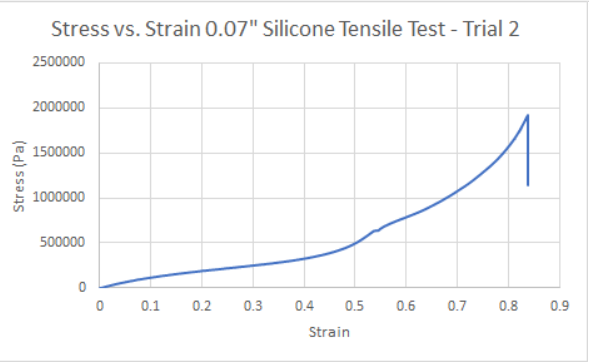

Stress vs. Strain 0.015" Silicone Tensile Test - Trial 3
Stress (Pa)
6000000
5000000
4000000
3000000
2000000
1000000
0
-1000000
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
Strain

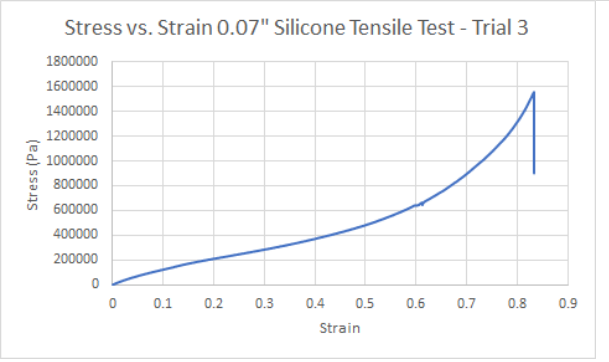

Stress vs. Strain 0.02" Silicone Tensile Test - Trial 1
Stress (Pa)
7000000
6000000
5000000
4000000
3000000
2000000
1000000
0
-1000000
-0.2
0
0.2
0.4
0.6
0.8
1
Strain


Stress vs. Strain 0.02" Silicone Tensile Test - Trial 2
Stress (Pa)
7000000
6000000
5000000
4000000
3000000
2000000
1000000
0
0
0.2
0.4
0.6
0.8
1
Strain

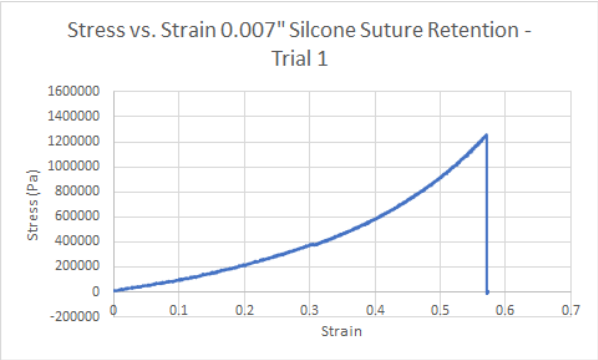

Stress vs. Strain 0.02" Silicone Tensile Test - Trial 3
Stress (Pa)
5000000
4500000
4000000
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
Strain

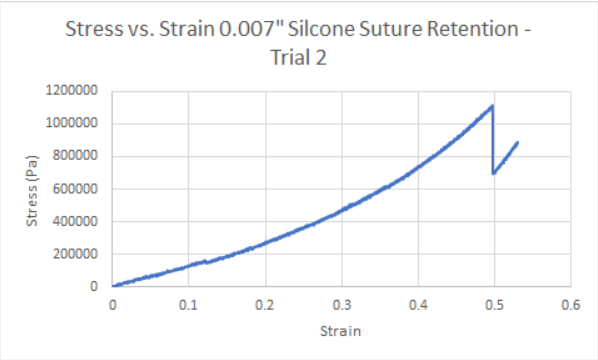

Stress vs. Strain 0.07" Silicone Tensile Test - Trial 1
Stress (Pa)
2500000
2000000
1500000
1000000
500000
0
-500000
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
Strain

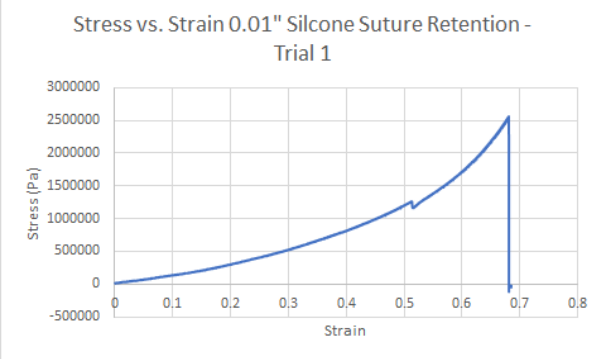

Stress vs. Strain 0.07" Silicone Tensile Test - Trial 2
Stress (Pa)
2500000
2000000
1500000
1000000
500000
0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
Strain

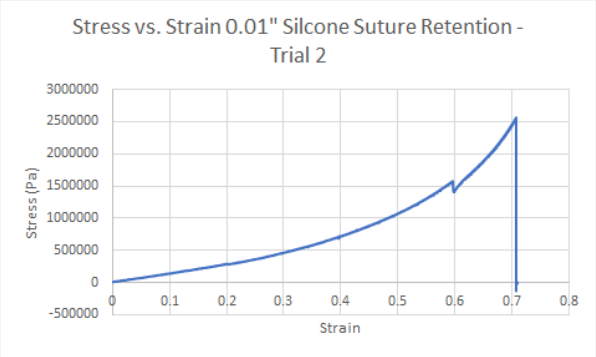

Stress vs. Strain 0.07" Silicone Tensile Test - Trial 3
Stress (Pa)
1800000
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
Strain

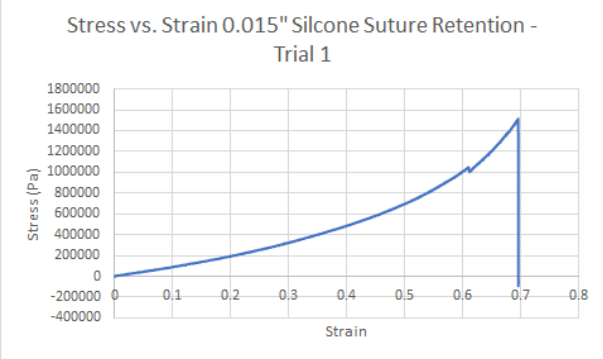
Appendix C: Suture Retention Testing Data

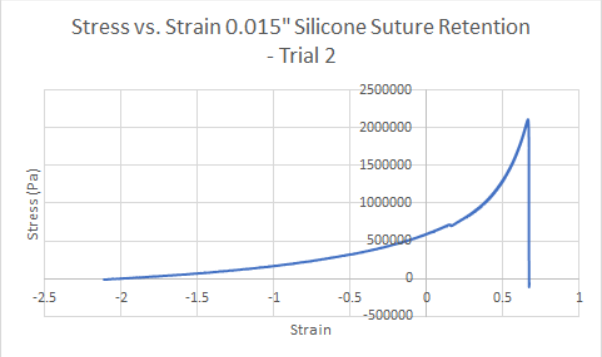


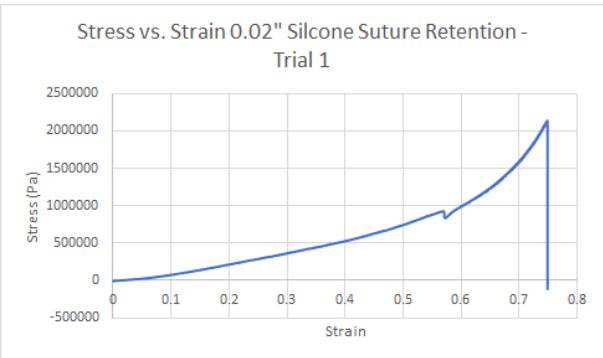


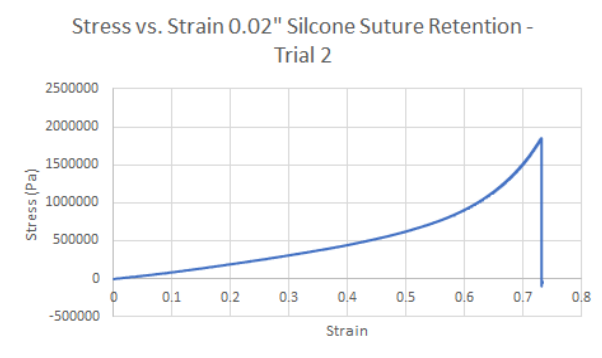












Appendix D: Peel-Off Testing Data