BioPacer: Electrospun Polyurethane Scaffold Containing hMSCs for Autonomous Cardiac Pacing

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A Major Qualifying Project Report

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Authorship

All team members contributed equally to all aspects of the project.

Abstract

The purpose of this project was to develop a scaffold to contain hyperpolarization-activated cyclic nucleotide gated (HCN) channel modified human mesenchymal stem cells (hMSCs), which would couple with cardiac myocytes, forming a biological pacemaking unit. The primary objectives of the scaffold were to prevent hMSC migration out of the scaffold, allow contact with neighboring myocytes to form gap junctions, and protect the hMSCs from damage during and after implantation. To do this, we designed a scaffold and chose materials that would meet these objectives. Through a detailed design process, our final design components included a stent-like structure made of Nitinol to provide structure yet remain flexible in the heart and an electrospun polyurethane sheath to encapsulate the hMSCs but still allow gap junction formation. To characterize the thickness of the polyurethane we completed a migration assay, which resulted in an ideal thickness achieved between 30 and 45-minute spin times. Our work on the design of the BioPacer has made progress towards achieving the above-mentioned objectives and autonomous cardiac pacing.

Chapter 1: Introduction

In a 2010 update on Heart Disease & Stroke Statistics the American Heart Association reported 831,300 deaths related to heart diseases within the past year. The United States alone spent $316.4 billion on expenses related to cardiovascular disease. Many of these problems are related to the electrical functions of the heart and are currently remedied by implanting an artificial pacemaker into the heart.[1,2]

Although electrical pacemakers do remedy many cardiovascular problems, they still have many limitations. Electronic pacemakers have a limited battery life, which ultimately leads to battery replacement and thus repeated surgeries. Electronic pacemaker could be severely displaced from their implanted location and could also be functionally impeded by machines such as MRI or CT scan equipment. Infections due to the pacemaker could also lead to removal of the pacemaker. A patient implanted with an electronic pacemaker also has restrictions in their day-to-day activities. Pacemakers are not a replacement for the autonomic pacing of the heart and thus create limitations on the physical exertion a patient can go through. It is also important to remember that pacemakers have to grow with the person. This becomes a concern for pediatric patients, whose age and size create several problems. Thus it is important to note that electrical pacemakers are a palliation rather than a permanent cure.[3]

Biological pacemakers represent an alternative that is much more biologically inert and has the potential to be a cure for the life of a patient. Biological pacemakers could be autonomically responsive in that they could have the ability to change with the patient's physiological and emotional demands. The use of human mesenchymal stem cells (hMSCs) has great potential as a biological pacemaker. hMSCs transfected with a particular HCN gene have been proven to combine with cardiac myocytes to create a pacing unit that could aptly substitute for the natural pacing of the heart.[3]

However before stem cell therapy can be used as a cure to cardiovascular diseases, several obstacles must be overcome, such as the design and deliver of the cells. A scaffold must be designed that addresses issues such as cell migration, gap junction formation, mechanical strength, and compliance with surrounding tissue, autonomic response, and a minimally invasive implantation mechanism. To properly address these issues the team designed several experiments including: biological assays such as migration assays and Connexin 43 assays, mechanical tests such as uniaxial load testing and fatigue testing, and conducted significant research. Through their work the team developed a final design for a scaffold, which could be implanted through a minimally invasive procedure, to hold hMSCs in place and allow for the formation of gap junctions between the hMSCs and cardiac myocytes while complying with the hearts physiological changes.

Chapter 2: Literature Review

The Heart

Structure and Function:

The heart is a hollow organ that is located between the lungs and is positioned posterior to the sternum and the rib cartilages. The organ is divided into four sections as follows: The right and left atria, and the right and left ventricle.[4] These four chambers are connected to several blood vessels. The structure of the heart is seen in Figure 1 below.[5]

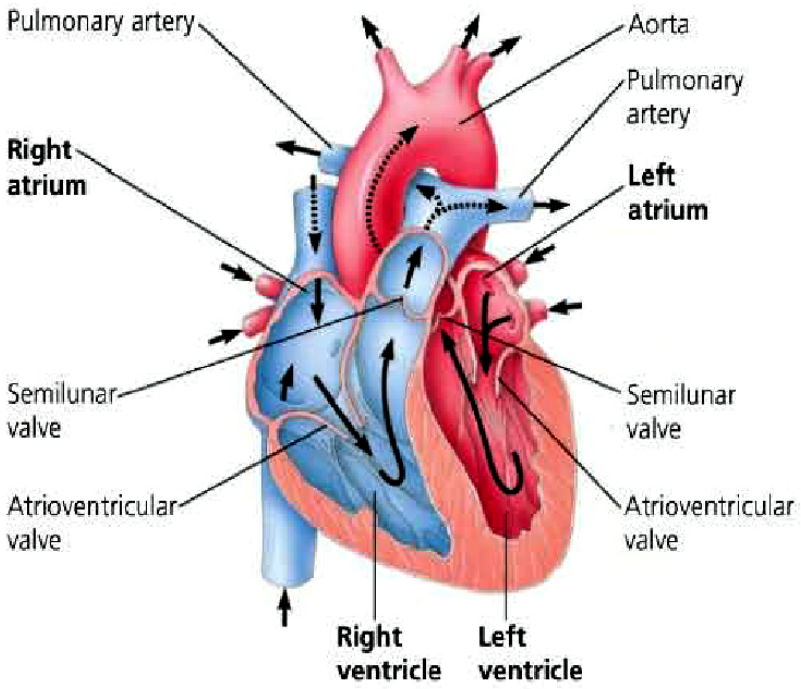


Figure 1: The chambers and valves of the heart.[5]

The chambers, vessels, and valves of the heart control the flow of blood through the heart and to the rest of the body. Deoxygenated blood first enters the heart through the inferior and superior vena cava and empties into the right atrium. The blood then flows through the atrioventricular valve and into the right ventricle. As the heart contracts it pumps the blood from the right ventricle through the pulmonary valve into the pulmonary artery to the lungs where it becomes oxygenated. The oxygenated blood enters back into the heart's left atrium through the pulmonary vein, and the subsequent opening of the mitral valve allows the blood to empty into the left ventricle. Another contraction causes the left ventricle to pump the oxygenated blood through the aortic valve, into the aorta and out to the rest of the body.[6]

Aside from the structure and blood flow of the heart it is also important to consider the mechanical functions of the heart. Every day, an average adult heart pumps approximately 2,000 gallons of blood. The heart also beats an average of 100,000 times per day. Through the course of seventy years this adds up to more than 2.5 billion beats and 31 million gallons of blood.[7] These numbers could vary from person to person based on the person's blood pressure, which is the pressure that the blood exerts on the walls of blood vessels as it circulates around the body. This pressure is controlled by three factors: blood volume, the peripheral resistance, and the cardiac rate. As the heart experiences systole (contraction) and diastole (relaxation) the pressure rises and falls respectively. When the heart undergoes diastole the average systemic pressure is 80 mm Hg (millimeters of mercury) and the pressure averages 120 mm Hg during systole.6 Higher or lower than average blood pressure rates could drastically impact the wear of a person's heart.

Electrical Function

The heart contracts and relaxes in response to the electrical stimuli generated within the hearts unique electrical system. This system is controlled by the flow of ions though cells which results in an action potential. To better understand the mechanism of the majority of heart cells observe Figure 2.[8]

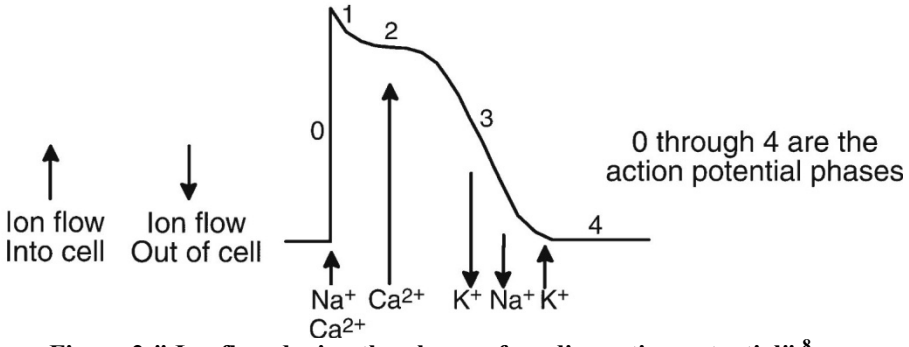


Figure 2:" Ion flow during the phases of cardiac action potential".[8]

Phase 4 represents the normal resting state of 99% of cardiac myocytes. The cells remain in this potential stage until they are excited by a neighboring cell. Phase 0, which is the excitation of the myocyte, occurs due to the influx of sodium ions (Na^+) as fast Na^+ ion channels open. There is a higher concentration of Na^+ ions on the outside of the cells and thus the opening of these channels triggers what is called the depolarization phase as the ions flow into the cell. This phase causes the cell to fire an action potential.[9,10]

Phase 1 occurs when the Na^+ channels are closed and K^+ ions flow to the outside of the cell. This process re-polarizes the cell slightly; the cell does not further depolarize due to the inward flow of Ca^{+2} ions that negates the outward flow of the K^+ ions. Phase 3, which is the rapid polarization of the cell, occurs when there is no longer an influx of Ca^{+2} ions while K^+ ions are flowing out. Once the cell has gone back to its resting potential the K^+ are closed and the cell

returns to Phase 4. The remaining 1% of heart cells, known as pacemaker cells, has the ability to generate their own action potentials.[9,10]

The autorhythmic pacemaker activity of these cells is created by a similar flow of ions as mentioned above. Figure 3B represents the flow of ions and there representative effects on the on the current within the cells.[11] The key differences to note are that these cells do not have a resting potential like the other cells, this difference can be seen by comparing Figure 3A and 3B below. Figure 3B also shows that these cells gradually depolarize after an action potential and fire again once they have crossed their threshold potential. The ability of these cells to keep depolarizing and re-polarizing autonomously controls the natural pacemaker activity of the heart.[11]

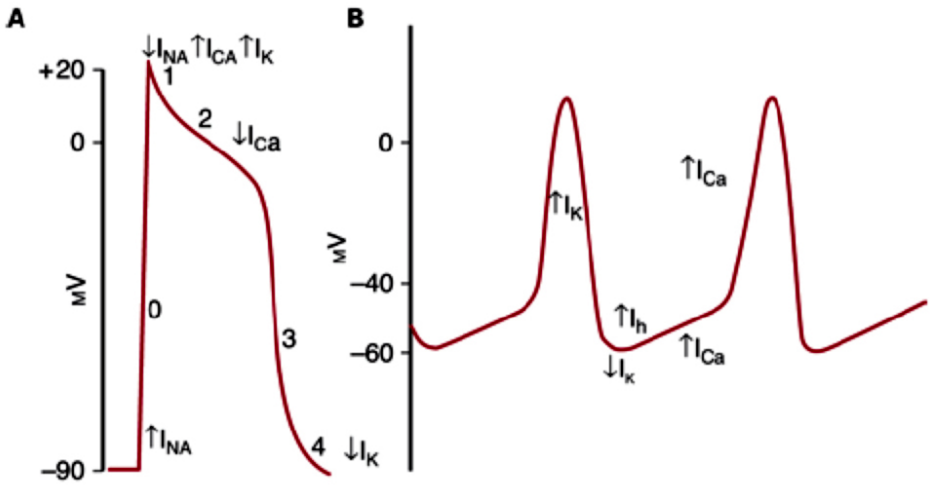


Figure 3: "Comparison of action potentials in ventricular muscle and diagram of the membrane potential of pacemaker tissue."[11]

This pacemaker activity mentioned above originates at the Sinoatrial node (SA node) in healthy hearts. The SA node is located in the right atrial wall close to the superior vena cava. The function of the node is to fire an action potential approximately 70 to 80 times per minute. The action potential from the SA node first travels to the contractile cells in the two atriums. From there it propagates to the Atrioventricular node (AV node).[9,10]

The AV node is another pacemaker region in the heart, often referred to as the secondary pacemaker. The AV node only generates an action potential 40 to 60 times per minute. Following the AV node the signal that propagated from the SA node travels down through the Bundle of His, through the Purkinje Fibers and around the ventricles.[9,10] The conduction system of the heart in Figure 4 below.[6]

Similar to the AV node, the Bundle of His and the Purkinje Fibers all fire at slower rates individually when compared to the SA node. However, since gap junctions connect them all, the AV node, the Bundle of His, and the Purkinje Fibers assume the same rate as the SA node. Gap junctions allow for electrical impulses to be passed from one cell to another without interruption through an electrical coupling of the cell. Gap junctions are channels that enable the flow of ions from one cell to another. This keeps the contraction and relaxation of the heart functioning in unison.[6, 9, 10]

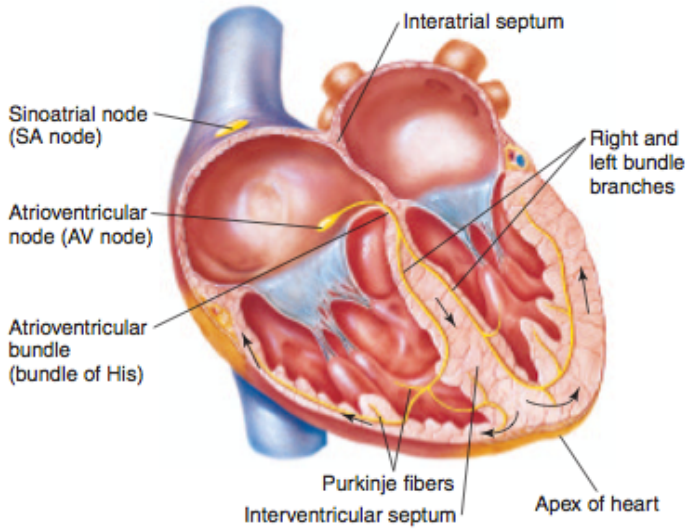


Figure 4: Conduction system of the heart.[6]

The SA node maintains a regular rhythm while still being able to adapt to physiological and emotional changes. Thus the malfunctioning of the SA node or a disturbance in the communication between the SA node and the AV node can lead to severe issues regarding the heart.

Cardiac Arrhythmias

Several of the cardiovascular diseases that affect many Americans are cardiac conduction disorders. The heart contracts in response to electrical stimuli that the heart's electrical system delivers in a sequential manner to facilitate the pumping of blood. In some patients however, the functionality of the heart's electrical system is disturbed causing erratic heart rates and rhythms as opposed to the regular, synchronized rates and rhythms caused by correctly sequenced conduction in a healthy heart.[12] These abnormal heart rates and rhythms are called *arrhythmias*.

A variety of scenarios can result in an arrhythmia. The Sinoatrial (SA) node is the heart's natural pacemaker as it contains the cells that can independently depolarize the fastest. When the SA node fires at an abnormal rate or rhythm, the rest of the heart also follows that same abnormal pattern resulting in an arrhythmia. In some cases, the normal conduction pathway in the heart is interrupted or "blocked" making it impossible for the electrical signal to propagate correctly through the heart. An arrhythmia can also occur after a myocardial infarction, where a portion of the heart that would normally be responsible for acting as a pacemaker or for propagating the electrical current dies, so the heart adopts an irregular rate or rhythm.[12]

Unfortunately, thousands of people in the United States alone are affected by arrhythmias. The latest estimate by the American Heart Association reports that in 2006, more than 36,800 death certificates in the United States mentioned cardiac arrhythmias as a primary or underlying cause of death, and more than 835,000 patients were discharged from hospitals for conditions relating to cardiac arrhythmias. In that same year, over $3.1 billion was paid to Medicare beneficiaries for treatment relating to cardiac arrhythmias.[13]

Many arrhythmias are life-threatening, for instance, ventricular tachycardia and ventricular fibrillation are extremely rapid or chaotic rhythms that completely impair the heart's ability to pump blood, ultimately causing damage to organs including the heart and brain.[12] In rhythms such as atrial fibrillation, the quivering of the upper chambers of the heart does not allow the blood to flow from the atria to the ventricles as quickly as it should. As a result, clots can form in the stagnant blood, which can migrate from the heart into the lungs or brain causing a pulmonary embolism or a stroke, both of which are potentially fatal.[12]

Artificial Pacemakers

Many arrhythmias can be treated using an artificial electronic pacemaker. In addition to treating arrhythmias such as symptomatic bradycardia, ventricular tachycardia, atrial fibrillation, and the collection of arrhythmias known as sick sinus syndrome. Artificial pacemakers are also being used to treat conditions other than arrhythmias such as hypertrophic cardiomyopathy, neurocardiogenic syncope, and chronotropic incompetence[14]. Currently, pacemakers are also being used to assist patients with congestive heart failure or patients who have recently had an acute myocardial infarction.[15]

Pacemakers have two distinct parts: the pulse generator and the leads. The pulse generator is about the size of two half-dollar coins and typically weighs slightly more than one ounce. It contains a small computer and a battery that generates small electric impulses that can be delivered to the heart in order to stimulate it to contract. The impulses are delivered to the heart by thin, insulated wires called leads. Electrodes on the ends of the leads sense the heart's electrical activity and deliver the electrical impulse when the heart's rate and rhythm are abnormal.[16]

Pacemaker Implantation: Minimally Invasive Surgery

Over 600,000 new pacemakers are surgically implanted each year. The most common method for pacemaker implantation in adults is the endocardial or transvenous approach, which can take anywhere from two to five hours.[15] In this method, the patient is given an antibiotic and a relaxant through an IV and is then given a local anesthetic to numb the area where the device will be inserted. A three to four inch incision is made underneath the patient's left clavicle and a small incision is made into a large vein, usually the subclavian vein. The leads are inserted into the vein, and the surgeon uses fluoroscopy to guide the leads to the heart and attach them to the muscle in the appropriate chamber of the heart. The other ends of the leads are then attached to the pulse generator, which is then tucked into the incision under the clavicle. The incision is then closed and the patient is monitored in the hospital overnight. Typically, after a period of two to three weeks, the patient is able to return to their normal physical activities.[15]

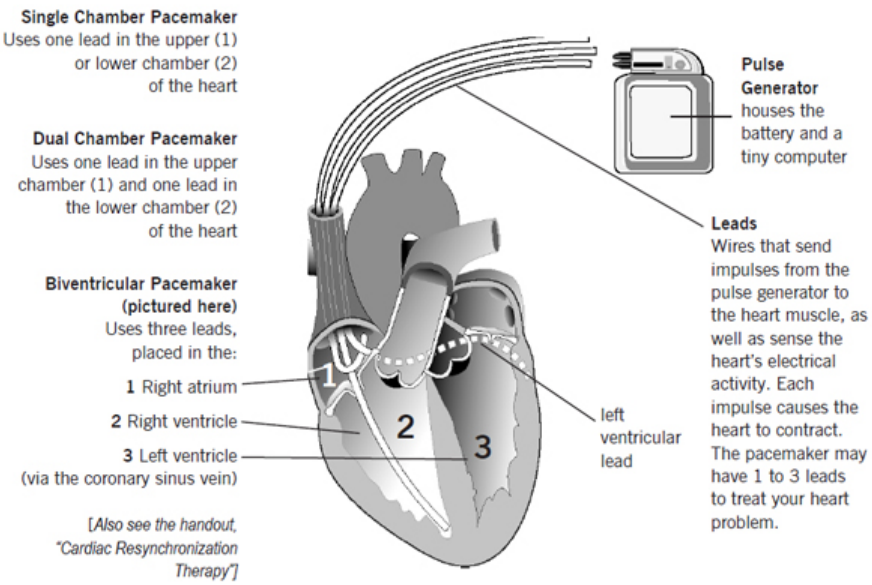


Figure 5: Types of pacemakers and their parts.[17]

Pacemaker implantation is an example of a minimally invasive surgery. The term "minimally invasive" was coined in 1984 when John EA Wickham included it in an article for the British Medical Journal. Since then, it has come to mean any surgery that is less invasive than an open surgery used for the same purpose or when there is minimal damage to the tissues at the point of entrance of the surgical instrument(s). Instead of exposing the organs, it is typically done through the skin or through some body cavity or natural orifice. Like pacemaker implantation, many other minimally invasive surgeries are carried out endovascularly. Endovascular surgeries are designed to access many regions of the body, including the heart, via major blood vessels. While endovascular surgeries were originally developed for diagnostic procedures, the development of balloons, stents and catheters have allowed for therapies in addition to diagnoses. These procedures involve the introduction of a catheter through a small puncture in the skin and into a large blood vessel. By injecting a radio-opaque dye, the surgeon can see the catheter and the blood vessels and can advance the catheter to the particular area of interest. Endovascular procedures can be performed by radiologists, neurologists, neurosurgeons, cardiologists or vascular surgeons.

Minimally invasive procedures have many obvious benefits. For example, unlike open surgeries, minimally invasive procedures only occasionally require the patient to undergo general anesthesia. Most procedures, including pacemaker implantation, only require localized anesthesia. As a result, patients who endure minimally invasive surgeries require shorter hospital stays or are even allowed to go home the very same day. The recovery time is less than that of traditional surgeries and, in general, the patient is subject to less pain and scarring and fewer post-surgery complications. Of course, all of these things do vary based on the specific procedure. Similarly, the implantation procedure and the recovery are dependent upon the type of pacemaker the patient receives.

Pacemakers can have anywhere from one to three leads, depending on the type of pacemaker (Figure 5: Types of pacemakers and their parts.).[17] Single-chamber pacemakers use one lead to stimulate either the atrium or the ventricle, while dual-chamber pacemakers use two leads (one in the atrium and one in the ventricle) to coordinate the function of both chambers. Biventricular pacemakers use three leads, one in the atrium and one in each ventricle. This is particularly useful in patients with heart failure, bundle branch blocks, or a history of cardiac arrest. Until recently, pacemakers were set to monitor the heart rate and begin pacing only when the heart rate fell below a predetermined rate (typically somewhere around 70 beats per minute). Now, most artificial pacemakers are capable of adjusting the pacing rate in response to exercise and other stresses by sensing differences in the patient's motion, breathing, temperature and other physiologic conditions. These are called rate responsive pacemakers, and while they are significantly more advanced than the pacemakers of fifty years ago, they often do not respond to the patient's needs appropriately. For instance, variations in breathing or body temperature may be signs of physical exertion, or it may just be a hot summer day.

Pacemakers also have other limitations that require further advancement. Many of these problems can be attributed to the limitations of the leads and the pulse generator. More specifically, the typical battery life is approximately eight to ten years; once the battery begins to fail, the entire implantation procedure must be repeated to replace the pulse generator. Furthermore, the leads may fail for many reasons including the loss of the insulating material, displacement of the electrodes, loss of or inappropriate stimulation, or fracture of the wires.[14] Perhaps the most important inadequacy is the fact that even pacemakers that incorporate the most recent technologies are often incapable of responding appropriately to physically or emotionally stressful situations.

To address these shortcomings, many studies have recently been done to look into the feasibility of making a biologically-based alternative to today's electronic pacemaker.

Biological Pacemakers

Numerous recent studies have been published that collectively illustrate a shift in the way researchers are looking to advance cardiac conduction therapies. Instead of investigating ways to improve current electronic therapies, many studies have been published recounting efforts to develop a biologically-based pacemaker to supplement, or ideally replace, artificial pacemakers. These methods have been aimed at either stimulating the heart's natural pacemaker (the SA node) or generating an ectopic focus (a group of cells other than the SA node that act as a pacemaker for the heart). So far, studies have generally employed one of three main strategies[14]:

- gene transfer to existing myocytes
- cellular transplantation
- delivery of genetically modified cells to the heart

Many of the more successful studies utilize some variation of the third strategy in which cells that are modified to spontaneously produce pacemaker activity are delivered to the heart. In

particular, several studies have shown promising results by delivering cells that are genetically modified to over-express the hyperpolarization-activated cyclic nucleotide-gated (HCN) channel HCN2.[18,19,20] Studies have also shown that the use of genetically modified human mesenchymal stem cells (hMSCs) to create this spontaneous pacemaker activity has the potential to open up a whole new realm of possibilities for the treatment of cardiac conduction disorders. [14,21]

Mesenchymal Stem Cells

To address the issues surrounding artificial pacemakers, (human) mesenchymal stem cells (hMSCs) have been explored as a basis for the creation of a biological pacemaker cell. hMSCs are a type of stem cell that are characterized by their self-renewal and multipotency, meaning they have potential to differentiate into a number of cellular lineages such as osteocytes, chondrocytes, myocytes, adipocytes, stromal cells, and fibroblasts.[22] Sources for MSCs include isolation from cartilage, periosteum (membranous surface on certain bones), synovium (non-cartilaginous soft tissue lining), tendons, adipose tissue, muscle, fetal tissue, placental tissue, and umbilical cord blood.[23,24,25] However, the most common site of MSC harvesting occurs at the bone marrow, where isolation from bone marrow aspirates is very efficient compared to other harvesting sites.[26] Additionally, MSCs have low antigenicity as research has indicated that in experimentally transplanted hMSCs there was a lack of immune response and clearance.[27] The combination of these characteristics, in addition to the absence of ethical considerations associated with embryonic stem cells restrictions has made MSCs an appealing option for use in cell or gene therapies, tissue regeneration, and anticancer treatments.[28]

The use of MSCs as a pacemaker cell was driven by research indicating that forced expression of a hyper-polarization activated cyclic nucleotide-gated channel (or HCN) in an in vivo model could generate a pacemaker current (I_f). This current is largely responsible for the diastolic depolarization and rhythm of the Sinoatrial node (SA node), 100 to 1000 fold greater than the native I_f.[29,30] Additional research that supported the potential for MSCs for pacemaker function illustrates their ability to transfer dye and electrical current to other cell types (including myocytes), as well as the ability to form functional gap junctions among themselves and myocytes as indicated by their expression of the protein Connexin 43.[31] This led to research that used hMSCs, transfected by electroporation using a viral vector, that is a genetically engineered virus used to delivery genetic information to a cell, containing an isoform of the HCN family (murine HCN2), to create a cardiac pacemaker.[32] Specifically the HCN gene family is a set of protein channels that allows the passage of both sodium and potassium ions.[33] In a normal cardiac pacemaker cell, such as those of a normal sinus node cell, phase 4 depolarization, or the spontaneous depolarization (pacemaker potential) that triggers the action potential will be propagated through the conducting system of the heart and cause cardiac contraction. The genetically modified MSCs with an HCN channel are unexcitable like these sinoatrial nodal cells because they do not express all the factors needed to generate its own action potential. These MSCs can be coupled with adjacent myocytes through gap junctions that provide a depolarizing

current and drive the myocytes toward an action potential. Ultimately, this produces a coupled pacemaker unit between the engineered MSC and the myocytes.[32]

Though this provides a platform for the use of modified undifferentiated MSCs as a biological pacemaker there are concerns that need to be addressed for its advancement. Little is known about longevity of the pacemaker function and the MSCs' ability to remain in an undifferentiated state. In addition, cell migration is a key issue as it is important to keep the delivered cells to the appropriate region. Should the cells migrate and delocalize from the target area then the pacemaker function may be compromised. [34]

Electrospinning

The problem of stem cell migration can be addressed by using a scaffold that contains the stem cells. There are many types of scaffolds and many methods to create them, however electrospun scaffolds are becoming increasingly popular. Electrospinning is a method of polymer processing that uses an electrically charged jet of polymer solution to create fibers with diameters in the nanometer to micrometer range (Figure 6). The polymer solution is placed into a syringe and a high voltage is applied to the tip of the needle (typically anywhere from 0 to 40

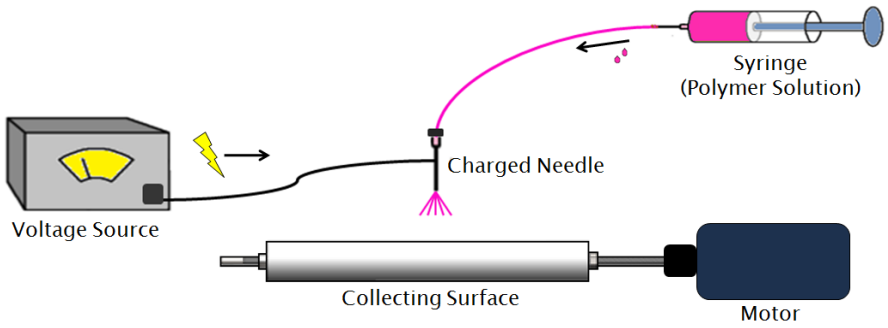


Figure 6: Schematic of the electrospinning process.

kV). The polymer liquid flows at a set rate from the syringe and becomes charged as it leaves the needle. These charges cause the droplets to repel one another resulting in the formation of cone at the tip of the needle where the polymer is "whipped" and stretched out into long fibers. This whipping motion creates many thin fibers that continue being stretched until they are deposited onto a grounded collecting surface, or mandrel, to which they are attracted.

There are many factors that control the diameter of the fibers and the porosity of the material. For instance, adjusting the distance from the tip of the needle to the mandrel or adjusting the applied voltage will both affect the diameter of the fibers. In addition, longer spin times create thicker materials with smaller pores. Although electrospinning is an attractive option for the creation of porous materials that still have a certain degree of structural integrity, it is not always consistent in that the same spinning conditions can sometimes create materials with different properties (fiber diameter, porosity, thickness, etc.).

Still, there are many advantages to electrospinning. First, the random mesh-like structure formed by the electrospun nanofibers closely resembles the natural extracellular matrix which then enables cell attachment (Figure 7).[35] Second, as previously stated, the porous materials can

be created in a relatively efficient and inexpensive way. In addition, a variety of solvents allow for electrospinning to be completed at room temperature as opposed to the high temperatures required for melt spinning. This eliminates the problem of degradation, either of the polymer itself or any drug or biomolecules that may be incorporated into the polymer. Finally, electrospinning offers flexibility by using mandrels of different shapes that result in materials that vary in size and shape, such sheets and tubes. These qualities make electrospinning a viable option for the creation of a scaffold for use in a biological pacemaker.

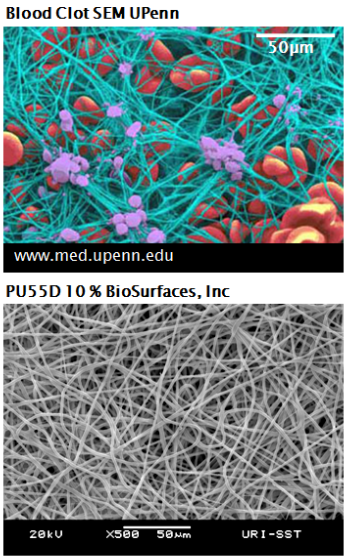


Figure 7: SEM images comparing electrospun polyurethane (bottom) to ECM (top).[35]

Polyurethane

One of the major aspects of designing a scaffold is deciding on the material to be used. The material must be able to comply with the contraction of the heart tissue, yet strong enough to withstand the cyclic loading without excessive permanent deformation or breakage. Furthermore, the pore size of the material must be controlled so that it is large enough to allow gap junction formation between the hMSCs and the myocytes, which exist on opposite sides of the material, but also small enough so that it does not allow cell migration into or out of the scaffold. The chosen material must also be biocompatible to minimize the immune response incited by the implantation. In addition, having a non-degradable material will increase the long-term durability of the scaffold, another necessary quality. A material that exhibits all the necessary qualities for this function is polyurethane.

Polyurethane has an extensive history dating back to 1937 when Otto Bayer's team discovered the first polyurethane synthesis reaction. The first biomedical application of polyester-urethane foams was used for breast implants by Pangman in 1958. Also in 1958, Mandrino and Salvatore used rigid polyester-urethane foam called Ostamer for bone fixation. Initially, cardiovascular applications of polyurethane did not yield favorable results due to its

hydraulic instability. Further research explained that all polyurethanes could not be categorized as one major class of material, but rather the properties of the polyurethane depend on many factors including chemistry and manufacturing.[36]

The properties of polyurethane may differ depending on the type of polyurethane and the processing mechanism but generally, all polyurethanes have some common qualities. One particular study on polyurethane proved that even after 42 days, cells had over 90% viability on the polyurethane scaffold and there was never enough change in the DNA structure of the cell to be statistically significant.[37] Studies have also shown that polyurethane supports cardiomyocyte gap junction formation.[38] In regards to the biocompatibility of polyurethane, studies have shown that polyurethane does not induce cytotoxicity. Furthermore, polyurethane does not release cytotoxic contaminants when degrading or interacting with its surroundings.[39] Polyurethanes have also exhibited positive results when testing blood compatibility.[36]

The mechanical properties of polyurethane also make it an attractive material choice for use as a scaffold material. Although the mechanical properties of electrospun polyurethane vary depending on the conditions under which it was created, polyurethane generally exhibits good mechanical strength while maintaining flexibility. Polyurethane is available in two major classes, thermoplastics and thermosets. For the purpose of scaffolding design, thermoplastic polyurethane has more desirable qualities. Mechanically, thermoplastic polyurethanes are elastomeric, meaning, it will return to its original shape when flexed. Furthermore, thermoplastic polyurethanes are resistant to microorganisms, which will help with the biocompatibility of the scaffold. They also have a high level of hydrolytic stability.[40] However, while there are many advantages to using polyurethane as a scaffold, one of the major challenges is the fact that the material used for the biological pacemaker would need to be extremely thin so as to allow gap junction formation across the scaffold. At such thicknesses, the material is very flimsy and would likely need to be reinforced in some way.

Cardiovascular Stents

Atherosclerosis, a condition in which the arteries are clogged with plaque, is typically treated using a minimally invasive procedure called angioplasty. This procedure uses a catheter with a deflated balloon on the tip which is inserted into the vasculature and guided to the site of the plaque buildup. Once it is in the correct location, the balloon is inflated pushing the plaque back against the wall of the arteries thus improving blood flow. The balloon is subsequently deflated and removed. According to the American Heart Association, stents are used in conjunction with angioplasty roughly 70% of the time. A sent is essentially a mesh tube that is placed on the tip of the catheter over the balloon. As the balloon inflates inside the blood vessel, the catheter is forced to expand. As the balloon in deflated and removed, the stent remains in its expanded form and remains in the blood vessel permanently acting as a sort of scaffolding to hold the vessel open.

Although all cardiovascular stents traditionally the serve the same basic purpose (to provide structural support to compromised blood vessels) there are many different types of

stents. The two major classifications of cardiovascular stents. The first includes bare metal stents, the original type of stent introduced in 1986. These are typically made of stainless steel but many other metals, alloys and polymers can be used such as gold, titanium, cobalt-chromium alloys and titanium alloys, to name a few. The second class includes drug eluting stents which are basically bare metal stents with a drug coating. The drug is released over the course of a few months in an effort to prevent the vessel from reclosing.

Although cardiovascular stents are typically only used in the treatment of atherosclerosis, they have potential for use in other applications requiring a similar type of support structure.

Chapter 3: Project Strategy

Initial Client Statement

Design a biologically inert device that restrains cells from moving from the implantation region in the heart while also allowing them to form cell to cell junctions.

Design Parameters

Objectives

- Scaffold should be permanent
- The total cost should not exceed $524.00
- Endovascular implantation

Functions

- Scaffold should prevent hMSC migration away from target location
- Scaffold should prevent hMSC migration out of the scaffold
- Scaffold should allow gap junction formation between the hMSCs and myocytes
- Scaffold should allow functional gap junction formation between hMSCs and myocytes
- Scaffold should not impede electrical activity of the native cells
- Scaffold should not impede electrical activity of hMSCs
- Implant without any damage to the scaffold
- Scaffold should minimize damage to the heart during implantation
- Scaffold can withstand contractile forces of the heart
- Scaffold should be able to withstand cyclic loading of the heart
- Scaffold should have attachment mechanisms
- Scaffold should not move except to comply with normal mechanical function
- Scaffold should be compliant enough to minimize decreases in regional mechanical function
- Scaffold size and shape should minimize interference with the mechanical function of the heart
- Scaffold size and shape should minimize interference with the electrical function of the heart

Specifications

- Pore size must be less than 3 μm

In order to determine an adequate pore size range for the electrospun polyurthenane fiber it is important to determine the typical size of a mesenchymal stem cell. Extensive literature searches yielded limited results with significant variation. The summarized results from the literature searches proceed. In a study titled 'T cell responses to allogeneic human mesenchymal stem cells: immunogenicity, tolerance, and suppression' the group stated the average size of the hMSCs they worked with were 30 μm in diameter.[41] However, beyond simply stating this figure the study provided no additional information. Another study by Toma et al. titled 'Fate Of Culture-Expanded Mesenchymal Stem Cells in The Microvasculature: In Vivo Observations of Cell Kinetics' stated that the average rat MSC size was around 23.6 ± .7 μm.[42] The study also noted that freshly isolated hMSCs had a smaller size than rat MSCs at around 10 μm. This figure was cited from a 2003 study titled 'Molecular and cellular characterisation of highly purified stromal stem cells derived from human bone marrow'. Interestingly a review of this cited article yielded no such specification of hMSC diameter.[43] The Toma et al. paper went on to state in the supplemental materials section that they used a polycarbonate filter in their study with a pore size of 10μM to be lower than the minimal cell size of the rat MSCs.
The previous MQP team referenced an article titled 'Parathyroid hormone improves contractile performance of adult rat ventricular cardiomyocytes at low concentrations in a non-acute way' where they concluded "a [MSC] cell has a length of 10.0μm and a thickness of 2.0μm". However, we are hesitant to use such a characterization because after review of the cited study no such figures were reported. In fact, the authors only provided raw data of a control group of cells (rat cardiomyocytes) where it can be estimated from the graphs that 'cell length' is around 100 μm and 'cell width' is around 26 μm.[44]

The most conclusive research found on the size of mesenchymal stem cells comes from Majore et. al in a paper titled "Identification of subpopulations in mesenchymal stem cell-like cultures from human umbilical cord". Based on this study of various subpopulation of cells it was concluded the average cell diameter was 15 ± .8 μm. However, this figure is based on the average of subpopulations which themselves had averages ranging from 11 to 19 μm.[45]
Based on the discussed results we speculate that a pore size of around 5 μm should contain the MSCs. However, this estimation is theoretical in nature. There are other factors that will determine the ultimate pore size such as ability for the cell to deform and squeeze through the pores, as well as deflection due to the contractile forces of the heart. Experimental data from the previous MQP team initial pilot studies to investigate cell migration through the scaffold. In a cell migration assay it was determined that a pore size of .4-3 μm would contain hMSCs. We have decided to use this specification.

- Scaffold should be able to withstand a minimal cyclic force of 32 kPa and resist fatigue for at least 3.7 x 10^8 cycles

The fatigue strength of the scaffold is related to the duration of the implant. Consider an implant that is to be implanted for at least 10 years. If the average heart rate of an adult is taken to be 70 beats per minute, this results in 36,792 ,000 beats.[46] For a 10 year period this yields nearly 3.7 x 10^8 cycles. Additionally consider, that the average systolic pressure of the heart is 120 mmHg or roughly 16 kPa.[47] If designed with a safety factor of 2, then the scaffold would need to withstand at least a force of 32 kPa under cyclical loading for at least 3.7 x 10^8 cycles.

- Gap junction formation should occur within a minimal time period

The previous MQP had stated that "gap junctions should be able to form within 48 hours". However, there was no justification or explanation for this specification.

Though gap junctions are integral membrane protein, they do not have a long half-life (>20 hours) as might be expected. Instead, research in many different cell lines has indicated the half-life for the connexin proteins, which form the gap junctions, to range from 1.5 to 4 hours.[48] What this indicates is a fast turnover speed, and that should the scaffold allow for cellular contact through the pores, gap junctions will be produced at a rate determined by the biochemical conditions. It is therefore difficult to establish a specification for a maximum time for gap junction formation. Such a time frame is not indicative of the capacity of the cells to form gap junctions but rather indicates if the scaffold itself physically allows gap junction formation. Perhaps, an experiment can be designed that can determine a time frame for expected gap junction formation, therefore if gap junctions have not formed after the allotted time it signifies failure of the scaffold to permit gap junction formation. For example, an experiment could be done using a porous scaffold with MSCs on one side and myocytes on the other. The experiment can be run in duplicates, and then stained at various time points (such as 30 min, 1 hr, 2hr, 4hr, etc.) to determine the time needed for gap junction formation. At this time there are no plans for such an experiment.

- Scaffold size and shape should allow for the seeding of at least 700,000 live hMSCs

Based on a previous interview with Dr. Ira Cohen of the Institute of Molecular Cardiology at SUNY Stony Brook he stated that his team estimates that 350,000 modified MSCs are needed to restore electrical function. However, with a transfection efficiency of 50% it is necessary to use 700,000 cells. Therefore the scaffold must allow the containment of this many cells.

Constraints

- Scaffold should not degrade within 10 years of implantation
- Scaffold should not detach from implant location
- Scaffold should not elicit an immune response

- Scaffold should not induce inflammation
- Scaffold should not cause scar tissue formation
- Scaffold must be compatible with life.
- Scaffold must be implantable via minimally invasive surgery (not requiring the opening of the thoracic cavity)
- Scaffold should be biologically inert
- Project must be completed by April 14, 2010

Assumptions

- Must be autonomically responsive to physiological changes
- hMSCs will not proliferate after transfection

Revised Client Statement

Design a permanent, biocompatible scaffold to contain HCN2 transfected human mesenchymal stem cells (hMSCs) that allows gap junction formation through the scaffold between hMSCs and the neighboring myocytes. Scaffold should not interfere with the normal functions of the heart. Implantation of the scaffold should be minimally invasive and minimize damage to the scaffold and the heart tissue. Ultimately, the device will act as a pacemaker to restore the natural electrical activity of the heart.

Project Approach

To begin the design and development process our team conducted extensive background research regarding all aspects relevant to the project including biological pacemakers, scaffold material and design. We then took a magnified look at the initial and revised client statements and detailed objectives, functions, specifications, constraints and assumptions of the project. Through evaluative measures such as pairwise comparison charts and client interviews, we determined which functions were more important to incorporate compared to others and starting creating conceptual designs.

From the conceptual design phase we completed further analysis of the logistics of each idea and narrowed our choices to a few preliminary designs. The specifications of these designs were then detailed and each design was compared to one another using additional evaluative tools. Using the synopsis of these analyses a final design was chosen and feasibility testing and research ensued. Further design verification was completed on the final design through various experiments that quantified the results. Cross-referencing the lists of objectives, functions and constraints also showed whether the ultimate design encompassed all the original goals.

All the while, we completed verification testing on other aspects of the design, not necessarily including the actual final design, in order to prove the validity of our theories. The ideas we were trying to prove include mechanical testing of the polyurethane to ensure that it was capable of withstanding the forces of the heart and migrational assays to ensure the hMSCs would remain contained within the polyurethane scaffold.

Chapter 4: Alternative Designs

The revised client statement (discussed in the previous chapter) states that the goal of this project is to create a permanent, biocompatible scaffold to contain hMSCs while allowing them to form gap junctions with myocytes on the other side of the scaffold. In order to design this device, the group needed to come up with some potential mechanisms to accomplish the functions and objectives that follow from this client statement. Once several conceptual designs were suggested, we needed to decide exactly what the requirements of the design we in terms of specific shape, size or manufacturing restrictions. These restrictions served as a metric used to determine whether we should continue investigating a conceptual design or not.

In addition to determining our needs, we also needed to conduct a feasibility study. This is incorporated in this chapter and includes a discussion of research and testing that would need to be done to ensure that each aspect of the design is conceptually sound and then to validate the design before it would be able to transition into the clinic. There are many limitations placed on a Major Qualifying Project including time constraints, budget constraints and limited resources, and as a result, there are many crucial steps in validating the design that cannot actually be taken given the scope of the project. Still, these aspects of the project are well-worth investigating and thoroughly discussing. Taking into account the feasibility study, three or four preliminary designs could be chosen from the conceptual designs. Finally, this chapter details all of the decision making and optimization processes that took place from the conceptual design phase through the final design phase.

Conceptual Designs

One of the most effective tools the team used in developing conceptual designs was a Morphological Chart. This approach allowed us to focus on brainstorming means to accomplish one function at a time, as opposed to trying to come up with an entire design that accomplishes all of our functions at the same time. This chart can be seen on the next page.

Table 1: Morphological Chart

Functions	Means								
Shape	"Football" shaped	Hollow/solid tube	Rolled up sheet into a "yoga mat"	"Donut" shaped	Coil or spiral	Crescent	Sandwich-something with multiple layers	Sphere	Pouch
Structural integrity (over time)	Ribs	Exoskeleton	Mesh material						
Structural integrity (during delivery)	Protective coating	Degradable capsule							
Closure method	Drawstring method	Bioglue	Sutures	Vacuum sealer (crimp)	Plug method				
Cell insertion	Injection	Seeding							
Attachment method	None	Staples	Sutures	Sewing cuff					
Post-delivery deployment	Umbrella deployment	Stent	Self-expanding stent	Inflatable					
Delivery route	Endovascular	Epicardial							

After several means were developed to address each function, these ideas were used to develop slightly more detailed conceptual designs. Some of these are depicted below.

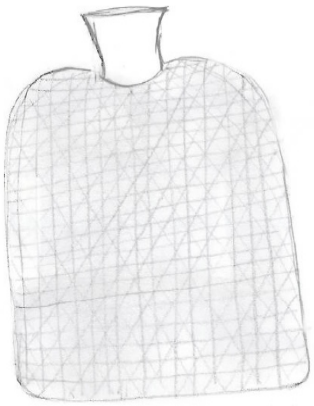


Figure 8: Water bag design
This water bag design incorporated the pouch idea, which would contain the cells. The main feature of this design was the injectable port for the cells. This allowed an interesting injection method that coupled as a way to close off the device after cell insertion.

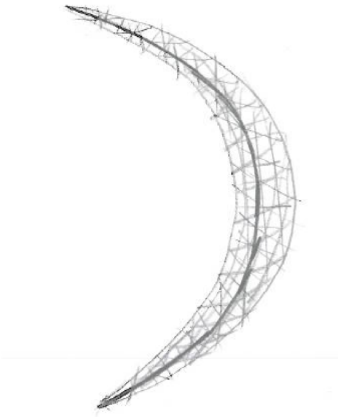


Figure 9: Crescent shaped design
This design took the crescent shape as the main part of the design for it's increased surface area. The main feature of this design was the support structure, which can be seen by the darkened line in the center of the device. This would allow for the device to have some sort of framework from a sturdier material. The polyurethane could then be spun onto the framework and the cells seeded on.

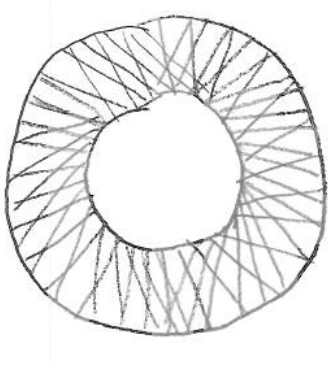


Figure 10: Donut design
This view of the design is from the top so it should be noted that the design is not flat but rather rounded on the sides, much like a donut is. The main feature of this design is the increase surface area due to the top, bottom, sides and middle being exposed to the implant location. Also, the device is meant to have a mesh layer that was a different, slightly more structural material for mechanical integrity.

Figure 11: Spiral shaped design
The image on the left is the top view of this design and the image on the right is the side view of one coil. This design was considered due to the increased surface contact of each coil with the implant location. This aspect of the design made it desirable due to the increase area for the cells to form gap junctions with the myocytes.

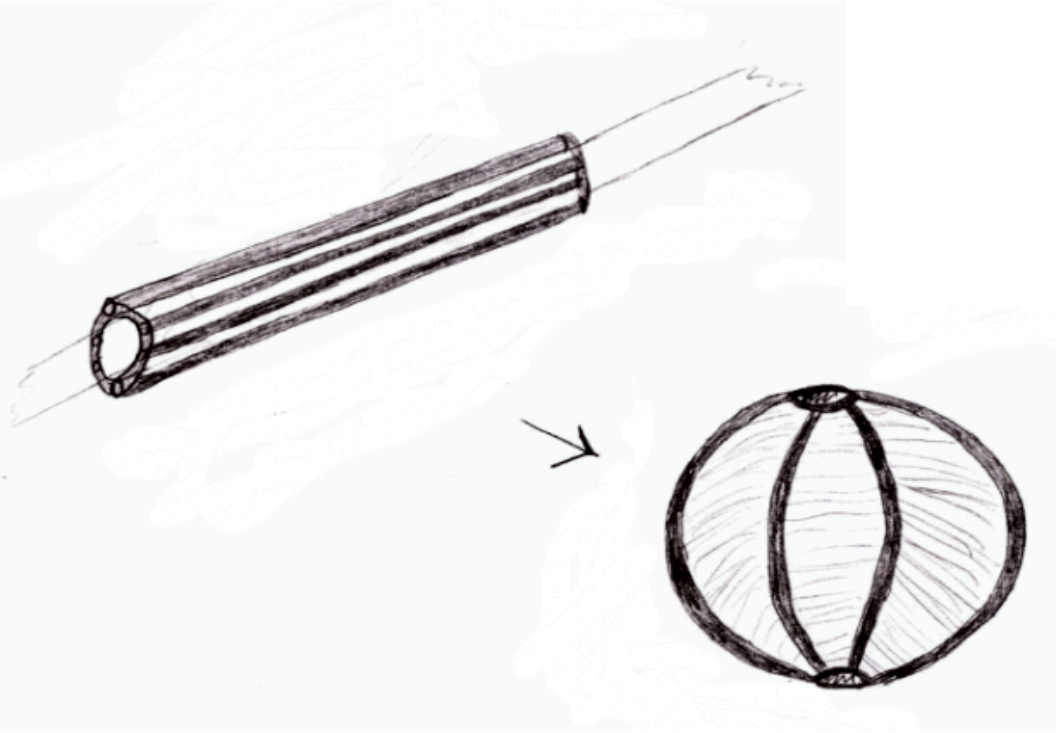


Figure 12: Chinese lantern design

The idea here would be to electrospin the polyurethane around a shape memory material in the form of a rod. Once it is implanted it would expand into a sphere, like a cage with the polyurethane stretched over it. The advantages of this design include the support structure and the increased surface area.

Figure 13: Tube design

This idea is very simple with polyurethane electrospin around a rod and taken off with the cells seeded on the inside. It could also have a solid center that the polyurethane would be wrapped around. The ends could be tied off in this design. The advantages of this design are the ease of manufacture and the increased contact with the implant location.

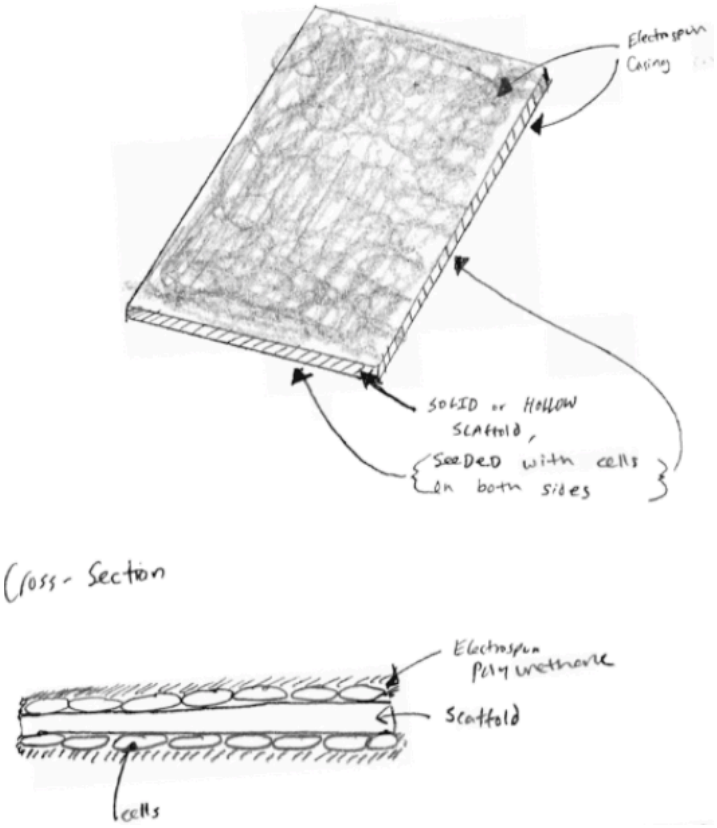


Figure 14: Double sandwich design
This design was made of many layers—the outside would be electrospun polyurethane and the middle layer would be a scaffold made with a sturdier material. The cells would be seeded in between the scaffold and the polyurethane. This design also provides and increased contact area for the cells as well as a central support structure for mechanical integrity.

Designs → / Function ↓	Water bag	Crescent	Donut	Spiral/Coil	Chinese lantern	Hollow/Solid Tube design	Sandwich
Prevent hMSC migration out of scaffold	Y	Y	N	Y	Y	Y	Y
Allow functional gap junction formation	Y	Y	Y	Y	Y	Y	Y
Not impede electrical activity	Y	Y	Y	Y	Y	Y	Y
Implantable without damage to scaffold	Y	N	Y	N	N	Y	Y
Minimize damage to heart	Y	N	Y	N	N	Y	Y
Withstand contractile forces of the heart	Y	N	Y	N	N	Y	Y
Withstand cyclic loading of the heart	Y	N	Y	N	N	Y	Y
Should not move except to comply with normal heart function	N	Y	N	Y	Y	Y	Y
Ease of manufacture	N	N	N	N	Y	Y	Y
Good potential for delivery	N	N	N	N	Y	Y	N

Table 2: Decision Matrix

We then took the conceptual designs and crossed them with main functions to see which designs matched our criteria the most. This information can be seen in the table above.

The functions we considered in the decision matrix were narrowed down to the major ones that affected our decision-making. We also added two criteria that were heavily considered when making our decision: the ease of manufacture and what the potential for delivery was like. When the functions were crossed with our conceptual designs, we were able to narrow our ideas to the "Chinese lantern design," the hollow or solid tube design, and the sandwich design. These choices were made based on the fact that these designs met most of the major criteria. Even at this point though, the conceptual designs were in no way finalized.

At this point we decided to use a pairwise comparison chart to further analyze our function to rank them according to level of importance. We also wanted to learn which functions were most important to the clients, Glenn and Matt. The completed pairwise comparison chart can be seen below.

Table 3: Completed Pairwise Comparison Chart

Functions	Glenn	Matt	Sam	Keeon	Kush	Bhavika	Client Avg.	Total Avg.
Prevent hMSC migration from scaffold	7	7	7	7	7	7	7	7
Minimize scar tissue	6	0	2	2	3	2	3	2.5
Minimize inflammatory response	5	4	2	1	3	5	4.5	3.3
Minimize mechanical interference	3	5	1	6	4	4	4	3.8
Minimize electrical interference	8	4	6	5	6	6	6	5.8
Removable	3	4	1	0	0	0	3.5	1.3
Withstand cyclic loading	3	1	4	4	2	1	2	2.5
Withstand contractile forces of heart	0	3	5	3	3	3	1.5	2.8

The rankings of the functions in order of importance with collective input can and client-only input can be seen below:

Collective Ranking

1. Prevent hMSC migration
2. Minimize electrical interference
3. Minimize mechanical interference
4. Minimize inflammatory response
5. Withstand contractile forces
6. Withstand cyclic loading
7. Minimize scar tissue formation
8. Removable

Client-Only Ranking

1. Prevent hMSC migration

2. Minimize electrical interference
3. Minimize inflammatory response
4. Minimize mechanical interference
5. Removable
6. Minimize scar tissue formation
7. Withstand cyclic loading
8. Withstand contractile force

The major difference was that the clients ranked removability higher than the collective ranking and withstanding contractile forces lower than the collective ranking. Most other rankings were within one place. We took this information into consideration when narrowing our conceptual designs down to preliminary designs. We also started making adjustments to our conceptual designs to add more details that would transfer to the final design, if that idea were to be selected.

Needs Analysis

In addition to the decision matrix and the pairwise comparison charts, there were also other requirements that we needed to consider as we began working to reduce and combine the conceptual designs into a handful of preliminary designs. In order to be able to do this, we needed to think about the clients and the users of our device and determine what the specific requirements are.

The device must be no wider than 3 mm.

Some of the first things to consider when we were narrowing down our potential designs were the size constraints. Ultimately, in order to be successful clinically, the device would need to be small enough to be implanted using minimally invasive techniques (i.e. through a catheter). Although there are currently no catheter systems that would be capable of delivering a device like this into the wall of the heart, we can conceptualize that the device could be housed inside a needle at the end of the catheter. The needle could then be inserted into the heart wall, leaving the device behind as the needle is removed. In order to accomplish this, the device must not only be small enough to fit inside a catheter, but also into a needle. In order to determine exactly what the size constraints were, we researched the diameters of standard needles. We decided that our device should be no wider than 3 mm, which could be accommodated by standard 11- or 10-gauge needles which have diameters of 3.048 mm and 3.404 mm respectively.

In addition to how the device was going to be implanted, we also needed to consider where the device was going to be implanted. The thickness of the average adult ventricular septum is about 8.8 mm. Thus, we decided that a size limit of 3 mm for the width was acceptable.

The surface area available for cell seeding must be at least 100 mm^2.

Based on the experience of personnel in Professor Gaudette's lab, we assumed a cell seeding density of 2,000 hMSCs per mm^2. Also based on the recommendations of Professor

Gaudette and his colleagues, we assumed that 200,000 cells will be required to create an adequate current to restore pacemaker function (this assumption will be discussed in more depth in the following section of this chapter). Based on these factors, we can calculate that the area required for gap junction formation across the surface of the scaffold must be at least 100 mm^2. The electrospun scaffold material should be around 40 μm thick.

The objectives and functions for the project specify that the scaffold must be capable of containing the hMSCs but thin enough to allow them to form gap junctions with myocytes on the other side of the scaffold. Based on the electrospinning experiences of BioSurfaces, Inc. and the previous work of Professor Gaudette, we hypothesize that the thickness of the scaffold will need to be around 40 μm thick. We planned to test this hypothesis through experiments to test different thicknesses of materials to determine the thinnest possible material that will still prevent migration of the hMSCs through the scaffold. Based on the results of this experiment, we planned to come up with a more precise determination of the necessary thickness by testing to see which thickness will allow gap junction formation. The protocols for these experiments can be found later on in this chapter, followed by results in the following chapter.

Feasibility Study

As with most engineering projects, there came a point where we needed to determine if the basic concepts upon which our device is based are actually valid and if each aspect of the device is feasible. A lot of this was addressed in the initial research that the project required and is included in the literature review. However, there are several aspects of the project that have yet to be addressed, several of which our group does not have the capabilities to directly investigate.

One of the primary constraints is budget. Our group is currently working within a budget of $1,024, and considering the fact that a single vial of hMSCs can cost upwards of $500, it's easy to see that such a limited budget doesn't go as far as we would like. In addition to monetary resources, we also don't have access to many of the resources necessary to validate several aspects of our project. For instance, we don't have access to an electrospinning machine so we are very limited in the amount of electrospun scaffold material that we have to run experiments on. On top of the limited resources available, time constraints are also a factor. In general, medical devices can take decades to develop from the time the idea is conceived to the time they are actually available to patients. In contrast, our project needs to take place within nine months. It is unreasonable to think that we would be able to address every single aspect of the project that will eventually need to be addressed before a device like this could transition to the clinic.

Still, even those parts of the project that we are unable to directly examine are crucial to the overall success of the project, and they are worth discussing in more depth.
It will be necessary to perform further characterization of the HCN modified hMSCs.

Much of what we know about the HCN modified cells is from unpublished data from Glenn Gaudette and his colleagues. While a lot of work has been done to research the properties of these cells, there is still a lot of speculation. For instance, the number of cells necessary to

create an adequate action potential is still a very theoretical number. It is based on even more unpublished data, personal experiences of Professor Gaudette and his colleagues, and mathematical models. Originally, our group was working under the assumption that it would be necessary for our device to contain 700,000 cells, a number used by the previous MQP team working on this project given to them via email by Dr. Ira Cohen. Recently, however, it has been suggested that as few as 10,000 cells are sufficient. Due to the fact that our group is unable to work with these modified cells, we decided to use 200,000 cells. However, further work must be done on the cells in the future if this device is ever going to make it market.

Furthermore, unpublished reports of researchers who have a lot of experience working with these cells report that the cells stop proliferating after they are transfected with the HCN gene. This is beneficial because once they are encased in our device it is crucial that they do not proliferate and over-crowd or even rupture the device. However, the transfection efficiency is currently another highly theoretical number. It has been reported to us that the transfection efficiency for these cells is around 50%, and although that number is getting better all the time, it still presents several problems. One of the major problems is the fact that we need to be able to separate the transfected cells from the non-transfected cells. This is necessary for two main reasons. First, we need to device to be as small as possible so we don't want non-transfected cells taking up any of the valuable scaffold because they are not functional. The minimum number of cells required to create an action potential only includes transfected cells. Thus, without a way to sort functional cells from non-functional cells the scaffold would need to be twice as big to account for the 50% transfection efficiency. Secondly, and more importantly, the cells that are not transfected do not stop proliferating. If these cells were seeded into the device and implanted into the body, they would likely continue to proliferate until the electrospun scaffold ruptures releasing all of the cells, transfected and non-transfected, which have the potential to create problems in other areas of the body.

In addition, while it's one thing to design a device, actually manufacturing the device is something else entirely. Particularly, the fact that we are using an electrospun scaffold presents some specific challenges. First of all, even if the parameters for electrospinning are kept the same, the thicknesses of the materials are often inconsistent. Since our device is dependent on a very precise thickness of the scaffold, this will need to be addressed before our device can be manufactured on a large scale. Also, we used polyurethane that had been electrospun into sheets for all of our experiments, however when our device is being manufactured the polyurethane will be spun into a tube-like shape. After we determine the thickness of the material necessary, we will need to adjust the electrospinning parameters to give us the same thickness of a very small tube as opposed to a large sheet. Since our group does not have access to an electrospinner, this is difficult for us to characterize at present.

Furthermore, if our design involves a shape other than a cylindrical tube or a sheet, previous experience tells us that this will exacerbate the unevenness of the electrospinning. This will also need to be addressed. It was recently brought to our attention that a polymer blend (such as PU/PET, perhaps) could create a more evenly distributed electrospun material. However,

since all of the materials we have worked with so far have been purely PU, our group decided to continue working with PU alone for the duration of this project. We would recommend that a polymer blend material be examined as an option in the future.

In addition to electrospinning, there are other aspects of manufacturing that complicate the process. After the device is electrospun, the cells must be seeded onto the device. Since the device is so small, this process will need to be developed and optimized. Finally, after the cells are inserted into the device, it will need to be closed off to ensure that the cells will not be able to escape the scaffold.

Antoher aspect of the design that was out of scope for the time period of this project was the incorporation of a drug into the material or as a coating that prevents or limits the foreign body response. Certain drugs could be cross-linked with the sheath material (polyurethane or a polyurethane blend) that could serve as protective measures against inflammation, scar tissue formation and immune responses to implantation. When considering this possibility, there are major barriers to consider. If the drug was going to encapsulate the whole device, an important feature to consider is how fast the drug degrades to ensure the hMSCs can receive nutrients from the surrounding area.

Implantation

Implanting the device is another component of the device that requires further research. There are limited studies available regarding the best location for this device, many simply focusing on ventricular pacing. Further research should be completed to understand which locations in the heart would be better to treat certain problems. The actual method of delivery is another aspect that requires a great deal of additional research. In the early design stages of this device, a catheter delivery system was conceptualized as the ultimate delivery vehicle. However, in order for this delivery system to function, it would be essential to consider whether the device could withstand the shear stresses of moving through a catheter.

Characterization of mechanical properties

When the details of the design start becoming more finalized, it is important to characterize the mechanical properties of the device itself as well as the environment it would be implanted in and delivered through. To complete thorough testing, scaled prototypes would be necessary which would be expensive. On the actual device, the properties of both the wire and the sheath would require characterization. Internally, the intramuscular forces of the septum would require characterization to understand the physical environment that the device would be placed into. Furthermore, the effect of cyclic loading over time would be an important factor to consider for this device to ensure long-term functionality.

Preliminary Designs

After considering all of the above, we began developing our preliminary designs and evaluating them against all the criteria. A description and list of advantages and disadvantages of each of the preliminary designs we chose can be seen below.

Chinese Lantern

This design entails polyurethane electrospun onto some sort of shape memory material in the form of a rod that would expand to a cage-like structure.

Advantages:

- Delivery could potentially be minimally invasive using an existing catheter based system
- May not require any attachment mechanism
- Good mechanical support
- Allows for a variety of closure mechanisms

Figure 15: Chinese Lantern Preliminary Design

Disadvantages:

- May be difficult to manufacture
- Shape may not be well suited for delivery into the septum
- May not be the optimal shape for the greatest surface area
- Removal may be complicated

Sandwich Design

The sandwich design would have a middle scaffold layer and electrospun polyurethane on the outsides with hMSCs seeded in between.

Advantages:

- Allow gap junction formation by having a layer on both sides of hMSCs
- Choice of middle material can be used to adjust strength and compliance of the scaffold
- Could be drug-coated to help reduce inflammation

Figure 16: Sandwich Preliminary Design

Disadvantages:

- Middle layer would have to be carefully chosen
- May need to be used as a patch because delivery of this shape into the septum may be difficult
- Device would be difficult to remove

Solid or Hollow Tube

Depending on whether the polyurethane tube would be spun onto a scaffold or left hollow, the tube idea consists of an outer layer of polyurethane.

Advantages:

- Shape would be flexible
- Solid design allows for structural support
- Scaffold shape and size minimizes scar tissue formation

Figure 17: Solid or Hollow Tube Preliminary Design

Disadvantages:

- Delivery may be difficult
- Hollow tube may not have enough mechanical strength and shear stress capacity

Stent Spun Design

This design was not one of the conceptual ideas but rather was a modification of the solid or hollow tube design The change here is that the polyurethane would be directly electrospun onto the stent with the cells seeded on the inside.

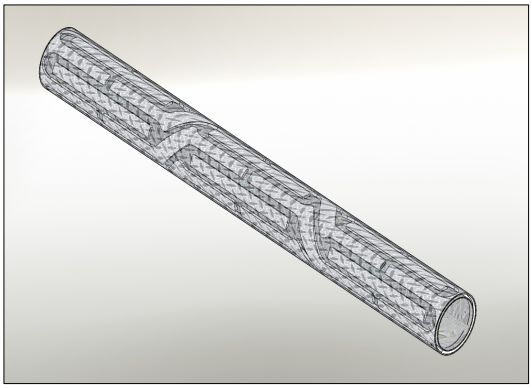


Figure 18: Stent Spun Preliminary Design

Advantages:

- Stent provides flexibility, support for the scaffold
- May have delivery mechanism through catheter based system
- Biocompatible (PU + existing stent material)

Disadvantages:

- Closing off the ends poses a challenge
- Catheter delivery may interfere with the seeded interior
- Sealing the ends would require delivery mechanism different from current stent delivery
- Amount of surface area for hMSCs in the unexpanded interior is limited

After further consideration, the stent spun design also gave way to a few more design alternatives. We originally chose this design as our "final design" but when it came time to perform

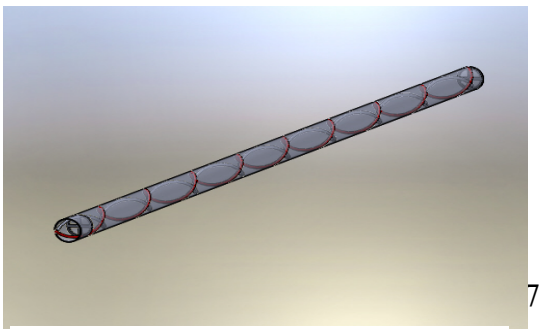


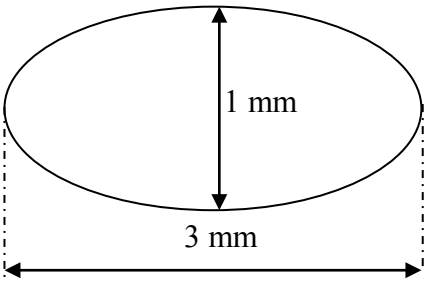
Figure 19: The "Double Helix" variation of the Stent Spun preliminary design.

validation experiments we had a very hard time acquiring a stent. This forced us to look for alternative methods of providing shape, support and flexibility to the scaffold. We examined many other forms of metal support structures including various meshes (such as a material resembling a window screen from the hardware store) and stent-like structures that we could potentially manufacture. We also came up with the idea to use a compression spring; it would be supportive yet flexible and have the capabilities of complying with the motions of the heart. However, we were worried that the spring would not be supportive enough to facilitate delivery of the device. We determined that a possible solution to this problem would be to use two springs, and we based our double helix variation of the tent spun design off of this concept.

Final Design and Design Calculations

After further consideration of the double helix design, it became obvious that a cylinder was not the optimum shape for something that was going to be implanted into the ventricular septum. To solve this problem, we wanted to do something along the lines of "squishing" the spring, so instead of being a cylindrical spring it would be more like an elliptical spring. This would allow the device to fit more easily into the septum.

Once we determined the shape of the device, we needed to determine the dimensions. As listed previously in the chapter, the device cannot be any wider than 3 mm, so we determined that the widest part of the ellipse must be 3 mm. We then chose the height of the ellipse to be 1 mm. With these dimensions set, we then needed to determine how long the elliptical spring must be to yield enough surface area for the 200,000 cells. These calculations are shown below:



Surface area required for 200,000 cells:

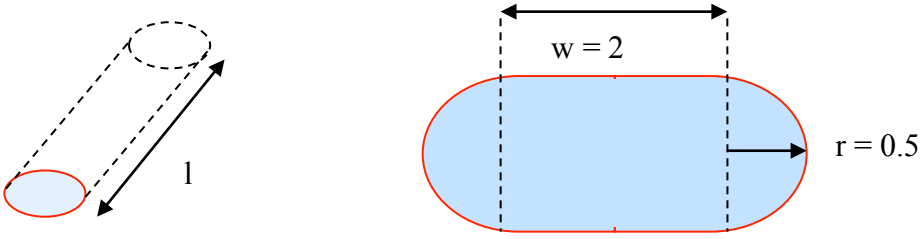
$$\frac{2{,}000\ cells}{1\ mm^2} = \frac{200{,}000\ cells}{x}$$

$$(2{,}000\ cells)(x) = (200{,}000\ cells)(1\ mm^2)$$

$$x = 100\ mm^2$$

We then used the surface area required of the cells as the surface area of the cylinder, taking into account the fact that the wires of the springs would take up roughly 20% of the surface area. This means that the actual outer surface area of the spring would need to be 120 mm^2. Using this number and the dimensions of the cross-section listed above, we worked in reverse to calculate the necessary length of the frame. It should also be noted that we used a model in SolidWorks that was in the shape of a slot instead of an ellipse to simplify the calculations. A slot essentially consists of a rectangle with a semicircle on either end (shown below with the calculations to determine the length needed).

Length of device to achieve a surface area of 120 mm^2:



$$l(2\pi r + 2w) = 120\ mm^2$$
$$l[2\pi(0.5) + 2(2)] = 120\ mm^2$$
$$l(7.14\ mm) = 120\ mm^2$$
$$l = 16.8\ mm = 1.68\ cm$$

To be conservative, our group decided on a length of 1.7 cm.

Once the size of the device was set, we needed to determine the materials. We already knew that the electrospun material on the outside of the double helix frame was going to be polyurethane, although we do recommend looking into using a polymer blend in the future. Next, we needed to decide on a material for the frame. In order to do that we started off by looking into materials that stents are most frequently made out of, and the two most popular and most successful materials were stainless steel and nitinol. The chart to the right highlights some of the properties of each material. This was one of the tools we used to decide which material was better suited to our application.

	316L Stainless Steel	Nitinol
Young's Modulus	Medium	Low
Radial Strength	High	Medium
UTS	Low	Medium
Flexibility	Low-Medium	High
Corrosiveness	High	Low
Biocompatibility	Low	High
Manufacturability	High	Medium
Cost Effective	High	Low

Nitinol's mechanical properties, (including a relatively low Young's modulus, moderate radial strength and ultimate tensile strength, and relatively high flexibility) make it an ideal material for our application. In addition, it is highly biocompatible and corrodes far less than stainless steel which is extremely important attributes of a material that will ideally be permanently implanted into the body. Although a nitinol frame may be more difficult to manufacture and more expensive, we believe that these are two of the less important considerations in choosing a material for a device. Thus, our group decided upon nitinol as the material for our double helix frame.

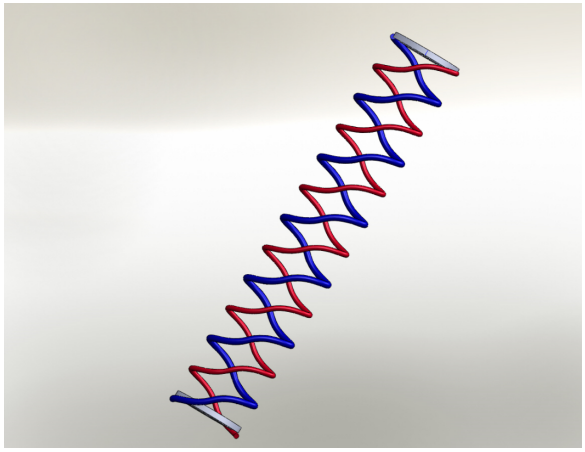


Figure 20: Double helix nitinol frame.

After the main aspects of the design were determined and the materials were chosen, there were some aspects of the manufacturing process that needed to be addressed. In order to actually produce this device, the nitinol frame must be able to be securely attached to the grounded mandrel for electrospinning. The frame must also be able to slide off the mandrel after electrospinning without damaging the polyurethane material it is now coated with. In order to address these problems, the group decided to design a new mandrel for electrospinning that would work with an end-cap on either end of the nitinol frame (see below). The crossbars on the end-caps line up with notches in the custom mandrel allowing the frame to slide partially down the length of the mandrel. A plug would be inserted in the notches after the frame to hold it in place during electrospinning. Once the electrospinning process was complete the plug could then be removed and the frame along with the polyurethane covering it could slide back off the mandrel.

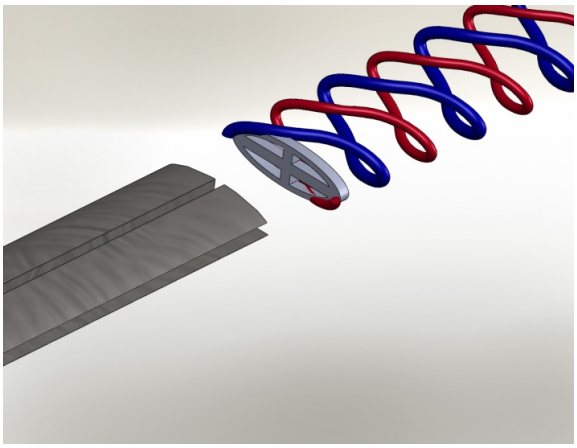


Figure 21: Representation of the end-cap on the nitinol frame and the notched mandrel.

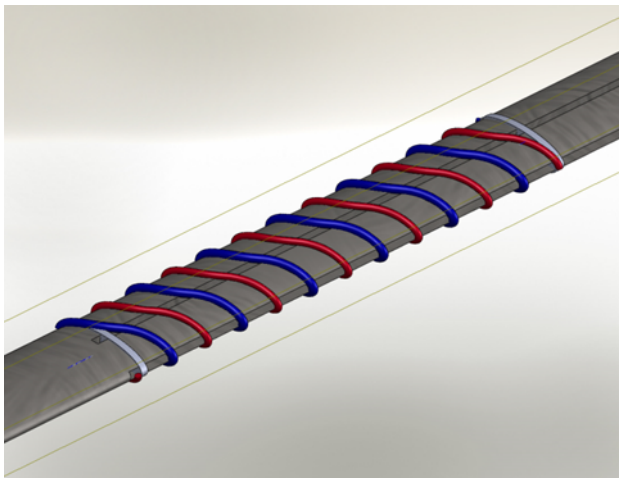


Figure 23: Nitinol double helix frame on the custom mandrel before electrospinning

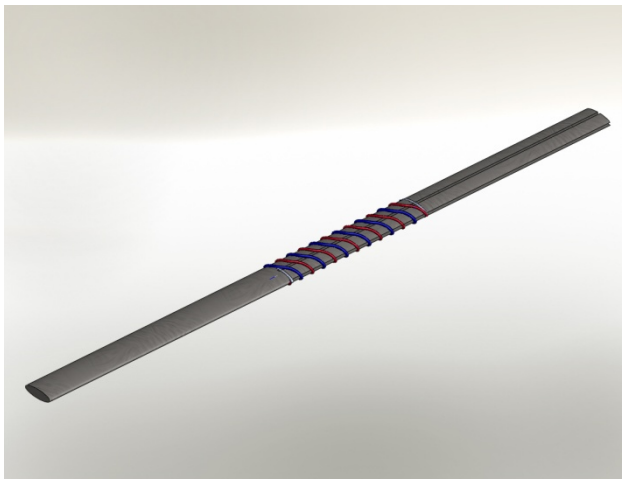


Figure 22: The frame would be positioned part of the way down the length of the mandrel to allow polyurethane distribution on the entire frame as well as on the mandrel on either side of the frame.

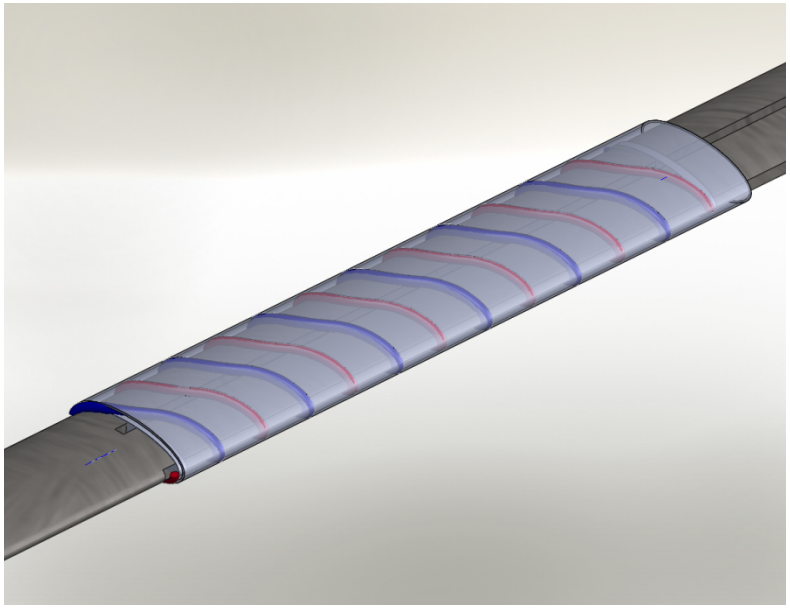


Figure 24: Double helix frame on the mandrel after electrospinning.

Optimization and Preliminary Data

Migration Assay

One of the primary functions of our scaffold is to contain the hMSCs and prevent them from migrating into the surrounding tissue. In order to characterize the proper pore size necessary to contain our cells and ensure that the cells are isolated within our scaffold a migration assay will be conducted. The test will be conducted on polyurethane samples of 3 different spin times (30, 45, and 60) minutes to optimize the required pore size. Prior to testing the polyurethane will be soaked in 70% Ethanol for 2 hours and followed by distilled water for 2 hours. After the sample is left to dry in a fume hood overnight the polyurethane will be sectioned into approximately 1cm x 1cm pieces. Following this procedure the sections will be placed into a device known as the Bio-Seeder. The Bio-Seeder is a device that will allow us to isolate the two sides of the polyurethane and create a proper environment to test whether the cells cross over from one side to other. The dimensions of the Bio-Seeder allow for it to be placed inside a well plate. The well plate can then be imaged through a confocal microscope to analyze the migration of the cells. Approximately 50,000 cells will be seeded onto one side of the polyurethane. There will be three groups of each spin time each of which will be incubated for different time periods (3, 7, and 14 days). The protocol that will be used to stain and test for migration is as follows:

1. Remove the well insert from the well and place into another sterile well. Rinse both in PBS solution for 5 minutes.
2. Remove the PBS solution and re-rinse both the well and the insert in PBS for another 5 minutes.
3. Remove the PBS solution. Place 4% paraformaldehyde at the bottom of the well and insert for a total of 10 minutes to fix the cells.
4. Remove the paraformaldehyde and rinse with PBS solution for 5 minutes.
5. Remove the PBS solution and re-rinse both the well and the insert in PBS for another 5minutes.
6. Remove the PBS solution. Place .25% Triton-X (in PBS) onto the well and insert for a total of 10 minutes.
7. Remove the Triton-X and rinse with PBS solution for 5 minutes.
8. Remove the PBS solution and rinse with 1% Bovine Serum Albumin (BSA) in PBS for 10 minutes.
9. Remove the PBS/BSA and place Alexa Fluor 488 Phalloidin stain (5μL Phalloidin per 200μL PBS) on the samples for 30 minutes.
10. Remove the stain and rinse with PBS/BSA for 10 minutes.
11. Remove the PBS/BSA and re-rinse with more PBS/BSA for 10 minutes.
12. Remove the PBS/BSA and re-rinse with more PBS/BSA for another 10 minutes.

13. Remove the PBS/BSA and place Hoechst 3342 Trihydrochloride Trihydrate stain (10μL/1mL Distilled Water) on the samples for 5 minutes.
14. Remove the stain and rinse with PBS/BSA for 5 minutes.
15. Remove the PBS/BSA and place pure PBS onto the samples.

The results of this experiment can be found in the Chapter 5.

Chapter 5: Results

Migration through Polyurethane

As mentioned earlier, migration assays were conducted across the polyurethane to characterize the appropriate thickness of the material that prevented migration of the hMSCs through the material. Specially manufactured wells were used to observe the migration of the cells. After the appropriate time periods had ended for the samples, (1, 3, and 7 days) the samples were removed from the wells and sandwiched between two slides. These slides were images using an inverted fluorescent microscope; the group flipped the slides to image both sides of the polyurethane.

The results of the test showed that through the 1-day time point no cells were able to penetrate the 30, 45, or 60-minute polyurethane sheets. At the 3-day time point it can be seen that cells are beginning to migrate through the 30 minutes sheets and still contained in the other two time segments. At the final 7-day time point the cells have migrated to the other side in the 30-minute sample. The 45-minute sample shows promising results as the cells have not migrated over, however it can be seen that they are at the edge of the polyurethane sheet which would allow them to come in contact with cardiac myocytes in order to make gap junctions. The 60-minute sample completely prevented migration, however the larger thickness also prevented cell integration into the polyurethane pores. This could hinder the formation of gap junctions across the polyurethane. Figure 26 shows the results from the 1, 3 and 7 day time points for the samples containing FGF in the cell media. The images of the Hoescht staining are not included due to the autofluorescence of the polyurethane which makes it nearly impossible to see the cells (Figure 25).

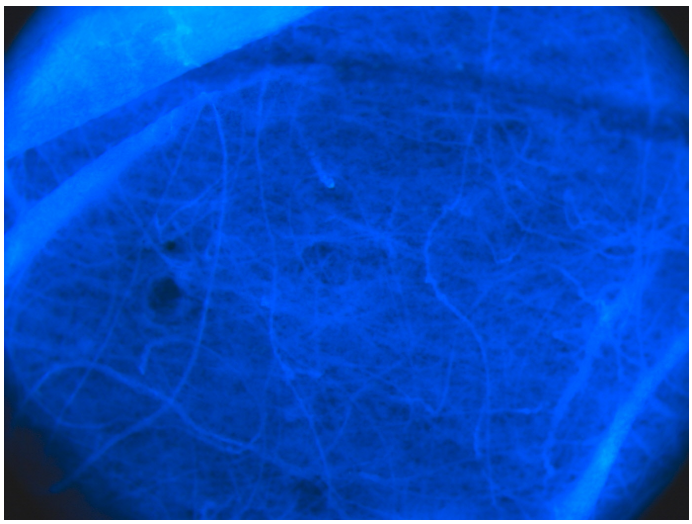


Figure 25: Polyurethane autofluorescence.

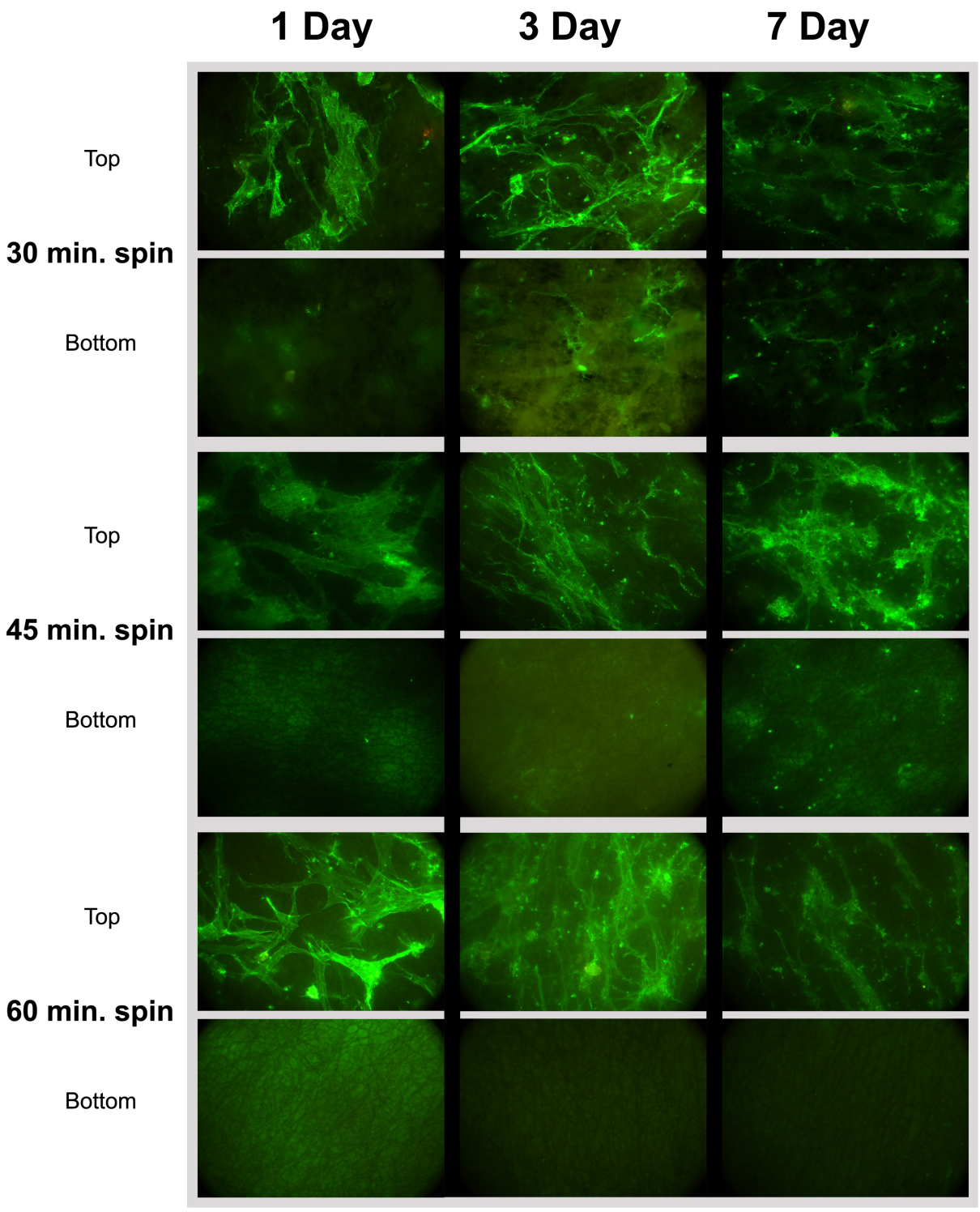


Figure 26: Results of the Migration Assay

Chapter 6: Discussion

The goal of this project was to design a biological pacemaker that could overcome the issues of electrical pacemakers. Challenges of autonomous biological pacing, such as cell migration and cell death, also had to be overcome in order to quicken the translation of this technology to the clinic. Our design, the BioPacer, contains genetically modified hMSCs within a polyurethane covered Nitinol frame. The BioPacer allows for the coupling of the stem cells with cardiac myocytes through carefully selected pore sizes that facilitate contact while inhibiting migration. The Nitinol framework of our design also gives the device both strength and flexibility during and after minimally invasive delivery. The biocompatibility of our materials, along with the size of the device and natural cardiac pacing via stem cells, allow us to meet our objective of creating an autonomous pacing unit that can be a permanent cure for cardiac patients.

Economic Influence

The results of this project would not drastically influence the economy of everyday living, however they would significantly reduce the money spent on pacemakers each year. A successful biological pacemaker is meant to last upwards of ten years and thus would not require repeat operations. Previous limitations of pacemakers increased the lifetime cost due to replacement and reimplantation procedures. The materials used in the biological pacemaker are also relatively inexpensive. Polyurethane and the electrospinning procedure are both cost effective while Nitinol for the metal frame is becoming an increasingly common biomaterial which will result in less expensive manufacturing procedures. Implantation of the device will be minimally invasive and the procedure will be similar to previous procedures, such as stent implantation, which will keep operating and training costs to a minimum. Thus although day-to-day costs would not be effected by our biological pacemaker, our product could significantly reduce the billions of dollars that are spent annually on heart related problems.

Environmental Impact

The current pacemaker industry uses metal, batteries, and polymeric coatings for the leads. Our biological pacemaker will be inserted into the septum and will be much smaller than current electrical pacemakers. The reduced size of our device will lead to a significant reduction in the amount of metal used. The polyurethane coating on the biological pacemaker will also be very small. All materials used in the scaffold will stay inside the body and will not produce any waste or have negative impact on the environment. Our biological pacemaker also does not have any electrical components, which will further reduce materials used, and also will result in no need to dispose of batteries. Although our project does not have direct environmental impacts the size and design of our product will not result in any adverse environmental effects and will create less waste that harms the environment compared to current pacemakers.

Societal Influence

Our product has the potential to be a breakthrough product in the medical device industry. In today's day an age the electronic pacemaker has become a very popular and familiar device. Across the globe people understand the significance and clinical importance of the device. It is easily considered the global gold standard to address many heart defects. Our device aims to eliminate the need and thus use of such a popular device. Such a drastic change has potential to impact people on a social level. People will need to be sold on the benefits of this device over electronic pacemakers. The biocompatibility, permanency, and efficiency of this device must be marketed to doctors, hospitals, and ordinary users for them to trust the product. The device's use of stem cells must also be clarified to avoid any ethical issues that could arise. Our product does not used embryonic stem cells, where much of the ethical controversy is focus. The use of adult stem cells does not harm any other people and can be taken from donors or from the client themselves.

Political Ramifications

As mentioned before the product we are designing has the potential to replace 600,000 pacemakers that are installed every year. The magnitude of such a device has great ramifications on the global market. Many companies, such as Boston Scientific, have a large return on investment on electronic pacemakers. A great deal of jobs and company finances rely on the manufacturing, marketing, and implantation of electronic pacemakers. If our product were to replace older pacemakers it would drastically influence the global market due to the 316.4 billion dollars that are spent around the world on heart disease treatments. The potential success of our product would not particularly change the culture of other countries. However, the technology used in our product could be adapted around the world and could change the gold standard for heart pacing related problems.

Ethical Concern

With many biomedical applications harnessing the regenerative potential of stem cells there is always ethical concern. However, the typical arguments against using stem cells are by and large addressed to (human) embryonic stem cells whose harvesting typically results in the destruction of day old embryos. The use of hMSCs does not have the associated ethical concerns as these stem cells are harvested from the bone marrow of a donor and have no repercussions on the outcome of an embryo. Thus, in terms of ethical concern related to our product, there is essentially little to none, as the products sole intent and purpose is to increase patient quality of life.

Health and Safety

As discussed, one of the key issues with conventional electronic pacemakers is the lack of an autonomic response to physiological changes that require increased or decreased cardiac

output. A biological pacemaker that can bring patients closer to the natural function of the heart is significantly positive influence on the health and personal safety of a person. One of the other concerns with using hMSCs in this application is the problems caused by uncontrolled proliferation and differentiation *in vivo*. It is a great concern to the health and safety of the patient that the contained hMSCs remain viable, non-proliferating, and undifferentiated. Based on unpublished research from Dr. Cohen it is clear that the transfected hMSCs lose their ability to proliferate. Researchers speculate that because the HCN channel is a key component in cardiac cells, hMSCs that express the protein exhibit other properties of cardiac derived cells such as non-proliferation. It is very important also that techniques be developed to separate non-transfected cells from transfected ones to ensure that only hMSCs with the HCN channel are incorporated into the device.

Manufacturability

The manufacturability of the device depends on several components. The first is a consistent input of transfected hMSCs separated from non-transfected cells. Secondly the production of the metal frame-architecture is small scale. Initially, it will be difficult and potentially expensive to produce Nitinol-based architectures. Lastly, the electrospun polyurethane must achieve an extremely high level of quality control. The density, thickness, and porosity of the electrospun material must be consistent for feasible manufacturing.

Sustainability

The design incorporates components such as polyurethane and nitinol that are used in many commercial applications. However, in terms of sustainability there are minimal effects. Sustainability is only a concern in the sense that the product outlasts conventional pacemakers that require component replacements.

Chapter 7: Final Design and Validation

This chapter describes the task-sequence the group completed to accomplish the goals of the project. The purpose to help future MQP groups working on a similar project be able to follow a logical plan and learn the shortcomings and highlights of this project plan to use towards their own project.

The first step in understanding the project goals is taking the client statement given by the advisor and any sponsors and breaking it down into smaller components. Each small part of the client statement can then be further assessed and developed into a more detailed need. An important part of this process is completing client and user interviews to understand which objectives are most important for your device. After thorough research, the initial client statement has then evolved to a much more comprehensive client statement that provides detailed objectives for the project.

Once a detailed client statement has been established and approved by the advisor and sponsors, the next step is to establish the goals of the project. Putting these goals in writing is an important part of the process so the group can revisit them to ensure they are staying on task. The goals of the project include the objectives, constraints, functions and specifications. The specifications in most cases will require research to provide numerical limits for some of the functions trying to be obtained. For example, if a function is to meet a certain size requirement, the specification would be stating the size and the justification. After these goals are established, the group should begin thinking of their design and the background of the project.

The background requires completing in depth research regarding the various facets of the project. If a particular material is being used, this would mean researching the material specifications, other uses, limitations and advantages amongst other things. The initial background will definitely change as features are added or removed from the design and new developments are made. The background is continuously worked on.

When beginning the initial design phase, one of the most useful tools is a function-means tree or table. This tool requires that for every function, the group brainstorm many possible means to attain that possible function. If one of the functions was to close off the device, gluing it shut or heating it shut might attain this. After brainstorming and filling out this chart, the next step would be to start developing conceptual designs.

When developing conceptual designs, using the function-means tree is a way to brainstorm many different possibilities to meet your project objectives. Conceptual designs do not require extreme detail or explanation of the logistics, such as how would the device be manufactured. Instead, conceptual designs are for general ideas that could meet the preset objectives. To further analyze these designs, other analysis tools should be used such as a pairwise comparison chart filled out by the group, clients and potential users. The rankings found from the completion of these charts can then be cross-referenced with each conceptual design to see which meets the functions and objectives best. A simple pro con chart can also be used as a way to decide which conceptual designs have potential for further development.

Once the conceptual designs are narrowed down to a few different options, experiments come in to play. Experiments are a way to validate the various parameters and prove that a certain material or design idea will in fact meet the design objective. For each experiment completed, the group should start by establishing the purpose of the experiment, making a list of the materials that will be required, actually designing the experiment and completing a schedule of experimental set up, execution and results analysis. These steps are essential for every experiment. Experiments also require anticipating things that might go wrong so that the group can be prepared in case something does not go as planned.

While completing experiments to validate various parts of the design, the group should also be analyzing each final design idea to start deciding which one will meet the most objectives and has potential for alterations and new features. Designs can be considered and altered infinitely so at one point, deciding on the most advantageous design is extremely important.

At this point in the project the experiments are being completed and the final design is chosen. From this point onwards, the project is about validity of the design and analysis of the results. Drawing conclusions from the experiments that help establish design limits is important as well as optimizing aspects of the design to best meet the goals initially set. All the while, it is important to think of limitations and recommendations for future projects to consider in the case that the project is continued in another year. This is one of the most important things a group can do because it allows for their unfinished worked to be continued without having to repeat experiments or certain research. Chapter 8 of this paper will describe the future recommendations and limitations of this project that should be addressed in future years.

Chapter 8: Conclusion and Future Recommendations

The results of the migration assay determined that a polyurethane scaffold electrospun for approximately 45 minutes would be effective at containing the hMSCs but still allowing gap junction formation. This scaffold allowed the hMSCs to migrate enough through the scaffold to make gap junctions connections with myocytes on the other side but prevent complete migration out of the scaffold. The design process the research team completed resulted in a detailed design that addressed many current limitations preventing biological pacemaker technology's advancement to the clinic.

For future work, the team recommends four major steps to be taken. The first experiment would be to test gap junction formation through the scaffold. This experiment would validate that the scaffold allows the gap junction formation necessary for the biological pacemaker to function. The next experiment would require completing a functional assay to test that the gap junctions actually work and the staining is not just showing the proteins for the gap junction channels. This assay would be vital in the advancement of the BioPacer to the clinic. The third step in the research process would be to complete mechanical testing on all components of the scaffold to make sure each component can withstand the cyclic loading of the heart. The final step the research team would recommend is creating a scaled prototype of the device for further testing, *in vivo.* The research team believes that these future recommendations are necessary to the successful advancement of the BioPacer to the clinic.

Appendix A: Gantt Chart

TASK NAME	DURATION	START	FINISH
Assess problem & need	6 days?	Tue 9/7/10	Tue 9/14/10
Conduct background research	16 days?	Wed 9/8/10	Tue 9/28/10
Write literature review	6 days?	Mon 9/27/10	Sun 10/3/10
Revise client statement	1 day?	Tue 10/12/10	Tue 10/12/10
Discuss Design Requirements	11 days?	Mon 9/13/10	Sun 9/26/10
Finalize Design Requirements	3 days?	Sun 9/26/10	Tue 9/28/10
Write project strategy chapter	6 days?	Sun 10/3/10	Fri 10/8/10
Generate conceptual designs	12 days?	Sun 10/3/10	Mon 10/18/10
Develop function-means tree	4 days?	Wed 10/6/10	Mon 10/11/10
Develop pairwise-comparison chart	4 days?	Wed 10/6/10	Mon 10/11/10
Develop morphological chart	4 days?	Wed 10/6/10	Mon 10/11/10
Fill out evaluative measures	7 days?	Fri 10/15/10	Mon 10/25/10
Send evaluative measures to clients	6 days	Mon 10/18/10	Mon 10/25/10
Narrow down designs by analysis	7 days?	Mon 10/25/10	Tue 11/2/10
Write Alternative Designs chapter	22 days?	Mon 11/1/10	Tue 11/30/10
Start electrospin characterization	11 days?	Mon 10/18/10	Mon 11/1/10
Mechanical testing of scaffold	22 days?	Mon 11/1/10	Tue 11/30/10
Migrational assay	22 days?	Mon 11/1/10	Tue 11/30/10
Connexin 43 staining	22 days?	Mon 11/1/10	Tue 11/30/10
Coculture on polyurethane	22 days?	Mon 11/1/10	Tue 11/30/10
Functional assays	28 days?	Mon 11/1/10	Wed 12/8/10

Develop preliminary designs	11 days?	Mon 11/1/10	Mon 11/15/10
Analyze preliminary design choices	20 days?	Tue 11/16/10	Mon 12/13/10
Develop prototype	15 days?	Thu 1/13/11	Wed 2/2/11
Design verification testing	11 days?	Thu 2/3/11	Thu 2/17/11
Write Design Verification chapter	15 days?	Tue 2/1/11	Mon 2/21/11
Refine final design	9 days?	Tue 2/22/11	Fri 3/4/11
Write Discussion chapter	6 days?	Tue 3/15/11	Tue 3/22/11
Write final design chapter	6 days?	Tue 3/22/11	Tue 3/29/11
Write introduction	6 days?	Tue 3/15/11	Tue 3/22/11
Write conclusion & recommendations	6 days?	Tue 3/29/11	Tue 4/5/11
Write abstract & executive summary	6 days?	Tue 3/29/11	Tue 4/5/11
Work on presentation	25 days?	Tue 3/1/11	Mon 4/4/11
Work on poster	25 days?	Tue 3/1/11	Mon 4/4/11
Finalize presentation	25 days?	Tue 3/1/11	Mon 4/4/11
Finalize poster	25 days?	Tue 3/1/11	Mon 4/4/11

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