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Design of a Novel Left Ventricular Restraint Device for the Treatment of Heart Failure

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Allen Lau

Alissa Marien

David Sansoucy

Katherine Soojian

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Approved by:

Glenn Gaudette

1. Heart failure
2. Left ventricular restraint
3. Microthreads

Abstract

Myocardial infarction is an event that causes the death of myocytes. In an effort to compensate for lost functional myocytes, left ventricular remodeling may occur, causing adverse geometric and functional changes. If untreated, this may lead to heart failure. The goal of this project was to develop a left ventricular restraint device that provides structural support and promotes angiogenesis to treat heart failure. Proof of concept testing indicated that the device would promote angiogenesis and reduce dilatation.

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Executive Summary

Cardiovascular disease is the leading cause of death in the United States. More deaths are caused by cardiovascular disease each year than cancer, respiratory diseases, accidents, and diabetes combined [1]. Myocardial infarction (MI) occurs when blood flow through the coronary arteries is reduced, which causes damage to the heart. Following MI, progressive left ventricular (LV) remodeling may occur, where the heart enlarges and expands along with increased thinning of the heart wall [2]. The process of LV remodeling may cause a decrease in global cardiac function which may eventually lead to heart failure [3, 4].

LV restraint devices provide structural support to prevent LV remodeling [5]. These devices have been shown to prevent further LV dilatation, or enlargement, and, in some cases, reverse remodeling has also been observed [6, 7]. Also, stem cells, growth factors, and biomolecules are currently being investigated for their use in the treatment of heart disease. Many different types of stem cells are currently being explored for their ability to regenerate myocardium, all with their own advantages and disadvantages [8]. Growth factors and biomolecules have been explored for their ability to induce angiogenesis and prevent left ventricular remodeling [9, 10, 11, 12]. Also, biomaterials that are being investigated for biological applications, such as contractile polymers and microthreads, could potentially be used in cardiac applications. Contractile polymers are polymers that can contract upon application of a stimulus [13]. Biologically-derived fibers called microthreads have been studied for their use in the repair and regeneration of tendons and ligaments. These threads, made of collagen or fibrin, are useful for these applications because of their chemical stability, biocompatibility, and structural similarity to native tendon or ligament [14].

Despite the fact that there have been advances made in the treatment of heart failure there are currently no devices on the market that simultaneously reverse the effects of left ventricular (LV) remodeling and promote regeneration of the myocardium. The initial goal of this project was to design, build, and test a device that improves the function of an infarcted heart by providing structural support, providing contractile assistance, and delivering a therapeutic agent. The device would be intended for patients with late stage III and early stage IV heart failure.

A restraint device was designed that incorporated polyaniline (PANI) as the major component. Various protocols for manufacturing PANI were performed in order to obtain a method that produced uniform films of PANI with the appropriate mechanical and contractile properties. Unfortunately, after utilizing many manufacturing protocols, uniform films of PANI that contracted with the application of an electrical stimulus could not be obtained. Due to these results, PANI did not appear to be a viable material for use in a contractile LV restraint device. Thus, a consensus was reached to re-evaluate the preliminary designs in order to select a more feasible design.

A passive wrap restraint design that incorporates a therapeutic agent was selected. Collagen microthreads were chosen as the major component of the device because they are highly biocompatible, can be manufactured to hold proteins and/or growth factors, have good mechanical properties, and were readily available. In the selected design, the microthreads were configured in a mesh that wrapped around the ventricles of the heart. Additionally, platelet-derived growth factor (PDGF) was selected as the therapeutic agent because it promotes angiogenesis, or blood vessel formation, which would provide an environment conducive to myocardial regeneration.

In order to perform preliminary testing of the design, proof of concept tests were conducted to confirm the device's ability to promote angiogenesis and provide structural support. First order approximations were used to determine the number of microthreads required to produce the mesh restraint. A cell migration assay using smooth muscle cells (SMCs) was performed to determine the ability of the device to promote angiogenesis with three groups tested – PDGF coated microthreads, control microthreads, and control petri dishes with no microthreads. The extent of migration and proliferation of the SMCs in each plate was assessed by observing specific regions of the plate using an inverted light microscope.

As a first approximation, it was found that 34 microthreads would be required to produce a mesh that provides the appropriate amount of structural support. A basic mechanics equation demonstrated that the mesh would reduce the dilatation of the heart. With the cell migration assay, a plethora of cells was observed surrounding the PDGF coated microthreads, while few cells were observed surrounding the control samples.

The results presented demonstrate that PDGF coated microthreads could be used to develop a LV restraint that promotes angiogenesis. Since only proof of concept testing was performed, future testing and development of this device is necessary before it can be used in a clinical setting. It is recommended that the PDGF concentration on the microthreads be optimized and that mechanical testing be performed on the microthread mesh. Also, it is necessary to test the device in small and large animal models to confirm its ability to promote angiogenesis and possibly deliver regenerative agents *in vivo*. Although all the desired testing could not be accomplished due to time constraints, this design provides a strong foundation for future development in creating a left ventricular restraint device that promotes angiogenesis for patients that suffer from heart failure.

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Authorship

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1 Introduction

Cardiovascular disease is the leading cause of death in the United States. More deaths are caused by cardiovascular disease each year than cancer, respiratory diseases, accidents, and diabetes combined. Approximately 1 in 3 American adults have cardiovascular disease, which corresponds to an estimated 79 million people, about 8 million of which have suffered a myocardial infarction. In 2007 alone, the estimated cost of cardiovascular disease was \$431.8 billion USD [1].

Myocardial infarction occurs when blood flow through the coronary arteries is reduced. With a prolonged reduction in blood supply, the cells within the myocardium may begin to die [15]. When myocyte necrosis occurs, the function of the heart declines because some contractile ability is lost [3, 16]. Myocyte necrosis may induce progressive left ventricular (LV) remodeling, where the heart enlarges and expands along with increased thinning of the heart wall [2]. The process of LV remodeling eventually may lead to a decrease in global cardiac function and an increase in mortality [3, 4].

Clinical treatment following MI involves a regimen of beta-blockers, ACE inhibitors, and nitric oxide administration. However, the aim of these treatments is to increase the patient's cardiac output by providing symptomatic relief, and therefore delaying disease progression. Current treatments do not prevent future cardiac events, including further LV remodeling, from occurring [17, 18]. Treatments and therapies are currently being developed to prevent LV remodeling and regenerate myocardium. LV restraint devices provide structural support to prevent LV remodeling [5]. These devices have been shown to prevent further LV dilatation and reverse remodeling has also been observed [6, 7].

Stem cells, growth factors, and biomolecules are currently being investigated for use in the treatment of heart disease. Many different types of stem cells are currently being explored for their ability to regenerate myocardium, all with their own advantages and disadvantages [8]. Growth factors and biomolecules are being examined for their ability to induce angiogenesis and prevent further remodeling following myocardial infarction [11, 12].

Biomaterials that are being investigated for biological applications, such as contractile polymers and microthreads, could potentially be used in cardiac applications.

Contractile polymers are polymers that can contract upon application of a stimulus [13]. Biologically-derived fibers called microthreads have been studied for use in the repair and regeneration of tendons and ligaments. These threads, made of collagen or fibrin, are useful for these applications because of their chemical stability, biocompatibility, and structural similarity to native tendon or ligament [14].

Despite the fact that there have been advances made in the treatment of heart failure, there are currently no devices on the market that simultaneously reverse the effects of LV remodeling and aid in the regeneration of the myocardium. The initial goal of this project was to design, build, and test a device that improves the function of the failing heart by providing structural support, providing contractile assistance, and delivering a therapeutic agent. However, a device that provided contractile assistance could not be developed within the given time and budget constraints. Therefore, the designs were re-evaluated to select a design that did not provide contractile assistance. The revised goal of this project was to design, build, and test a device that improves the function of the failing heart by providing structural support and simultaneously delivering a therapeutic agent.

Collagen microthreads were selected as the major component of the device due to their ability to hold proteins and/or growth factors and their mechanical properties [19]. Platelet-derived growth factor (PDGF) was selected as the therapeutic agent because it promotes angiogenesis, or blood vessel formation, which would provide an environment conducive to myocardial regeneration [10, 20]. To test the device's ability to promote angiogenesis, a cell migration assay was performed in order to observe the migration and proliferation of smooth muscle cells with PDGF coated microthreads in comparison to control samples. Basic mechanics calculations were used to verify that the mesh would reduce dilatation of the heart wall. Preliminary testing suggested that the device would promote angiogenesis and reduce dilatation of the heart wall. The results obtained demonstrated a proof of concept of the device design.

2 Background

2.1 *The Heart and Myocardial Infarction*

The cardiovascular system is essential to the proper functioning of the body. Blood transports nearly everything within the body, including oxygen, nutrients, waste products, and hormones. The heart is of vital importance within the cardiovascular system because its pumping action drives the circulation of the blood [21].

2.1.1 Mechanisms of the Heart

The heart wall consists of three main layers – the epicardium, myocardium, and endocardium. The epicardium is the outermost layer of the heart, which also contains the pericardium – a double layer membrane filled with fluid that allows the heart to contract in a relatively friction-free environment. The endocardium is the innermost layer of the heart that interfaces with the blood. This layer consists of endothelial cells that are continuous with the lining of the blood vessels. The myocardium is located in between the epicardium and endocardium. This layer contains the cardiac muscle cells, or cardiomyocytes, that perform contraction. The myocytes contract upon stimulation from the heart's pacemakers – the sinoatrial node and atrioventricular node. This causes contraction of the right and left ventricles and subsequently leads to blood flow through the systemic and pulmonary circulatory systems [21].

The heart itself is supplied with nutrients and oxygen by the surrounding coronary arteries. Blood flow through these arteries occurs during ventricular diastole, or filling, because when the ventricles are contracting the coronary vessels are compressed. When stenosis, or blockage, of a coronary artery occurs, blood flow to the heart is reduced causing a myocardial infarction [21].

2.1.2 Myocardial Infarction (MI)

Myocardial infarction (MI) is caused by ischemia of the myocardium as a result of reduced blood flow through the coronary arteries. Ischemia occurs when there is an imbalance between the nutrient and oxygen supply to the tissue and the demands of the tissue [15]. Immediately following ischemia, myocardial stunning occurs. The myocytes

enter a state of “hibernation,” where the cells continue to survive but are unable to contract because of deficient arterial flow. When myocardial stunning occurs, the myocytes appear to down-regulate their metabolism, allowing them to survive with insufficient nutrients. The cells may recover their contractile function if blood flow is restored via reperfusion [22]. Cell death, or necrosis, begins to occur in as little as 15 minutes, with complete necrosis occurring in 4-6 hours if blood flow is not restored or therapeutic measures are not taken [15]. When necrosis occurs after MI, the process of LV remodeling may begin in order to normalize the function of the left ventricle.

2.1.3 Process of LV Remodeling

LV remodeling is the process by which the size, shape, and function of the left ventricle are altered by mechanical, neurohormonal, and other factors [2]. Remodeling can occur due to pressure overload, which is caused by stenosis, hypertension, or dilated cardiomyopathy, or volume overload, which is caused by valve regurgitation or myocardial infarction [18]. After myocardial infarction, the process of remodeling is stimulated by increased loading conditions due to a loss of functional myocardium [2].

Ventricular remodeling occurs most frequently after transmural infarctions that span the thickness of the ventricular wall and with infarctions in the anterior apical region of the heart because this region has the thinnest wall and greatest curvature. Some factors that affect the extent of remodeling are infarct size, patency of the arteries, and ventricular loading conditions [3, 4, 23]. Among studies, there is a general consensus that remodeling begins to occur within the first 24 hours following MI [2, 18, 24]. However, LV remodeling does not always occur after MI – one study noted that only 46% of patients who suffered myocardial infarction experienced remodeling and 20% of the total number of patients experienced progressive remodeling [4]. Progressive remodeling is the term used to describe cases where remodeling continues to occur longer than three to six months post-infarction [3, 4]. LV remodeling can result in a vicious cycle that puts patients at risk for congestive heart failure, myocardial rupture, aneurysm, and ultimately leads to death [23]. The process detailed below describes the events that occur during progressive remodeling.

Changes in Infarct Zone

Immediately following myocardial infarction, necrotic tissue within the infarct zone is absorbed by macrophages. The infarcted region is most vulnerable to forces in the time after necrotic tissue is absorbed, but before collagen reinforcement [23]. It is during this time period that the infarcted region can expand and enlarge, leading to thinning and dilatation [2, 3, 23]. Infarct expansion is due mainly to the rearrangement and stretching of myocytes. Rearrangement of myocytes occurs as adjacent muscle bundles slip due to the degradation of intracellular collagen struts as a consequence of a reduced number of myocytes. Cell stretch occurs due to an increase in sarcomere length [2, 23, 24].

Once collagen deposition occurs and scar tissue begins to form, the infarcted region becomes more resistant to deformation [23]. This allows the infarct to better resist stresses, which eventually halts infarct expansion [3].

Changes in non-infarcted myocardium

In addition to infarct expansion, the non-infarcted myocardium also undergoes structural changes post-infarction in response to different loading patterns [2, 4, 16, 23]. After infarction, stroke volume is decreased due to a reduced number of functional myocytes in the infarcted region. This places a burden on the functional myocardium in the non-infarcted region [3, 16]. As a compensatory mechanism, the non-infarcted myocardium undergoes hypertrophy, or enlargement, to maintain stroke volume and stabilize contractile function [16, 23]. Enlargement of the non-infarcted region is a cyclic process – the process begins in order to maintain stroke volume, but enlargement causes increased wall stresses, which cause further enlargement [23].

The mechanism of hypertrophy depends on the type of overload that occurs. With volume overload, which is a result of increased diastolic pressure, hypertrophy occurs by the addition of new sarcomeres and fiber elongation. With pressure overload, which is a result of increased systolic wall stresses, hypertrophy occurs due to the addition of parallel myofibrils. Regardless of the type of hypertrophy, the result is the enlargement of the non-infarcted region [3].

Numerous studies note that compensatory hypertrophy causes an initial improvement in ventricular function, but with further expansion, function declines [3, 4,

25]. It has been noted that within approximately two to four weeks, stroke volume returns to normal due to hypertrophy of non-infarcted myocardium, however function begins to decline thereafter and deterioration is evident approximately six months post-infarction [4, 25].

Changes in LV Performance

Although progressive remodeling initially results in an improvement of hemodynamics, such as increased stroke volume, the long-term effects of remodeling result in decreased regional and global performance [3, 4]. The heart undergoes geometrical changes including increases in volume, mass, and changes in shape, where the heart becomes more spherical and less elliptical [18]. Along with the alterations in ventricular geometry, expansion produces elevated left ventricular end-diastolic pressure, increased end-diastolic volume, increased end systolic volume, reduced ejection fraction, increased systemic vascular resistance, increased right atrial pressure, and decreased stroke volume [4, 16, 25]. A summary of the mechanisms and results of progressive LV remodeling can be seen in Figure 1 below. These hemodynamic changes increase mortality by increasing the potential for heart failure [25].

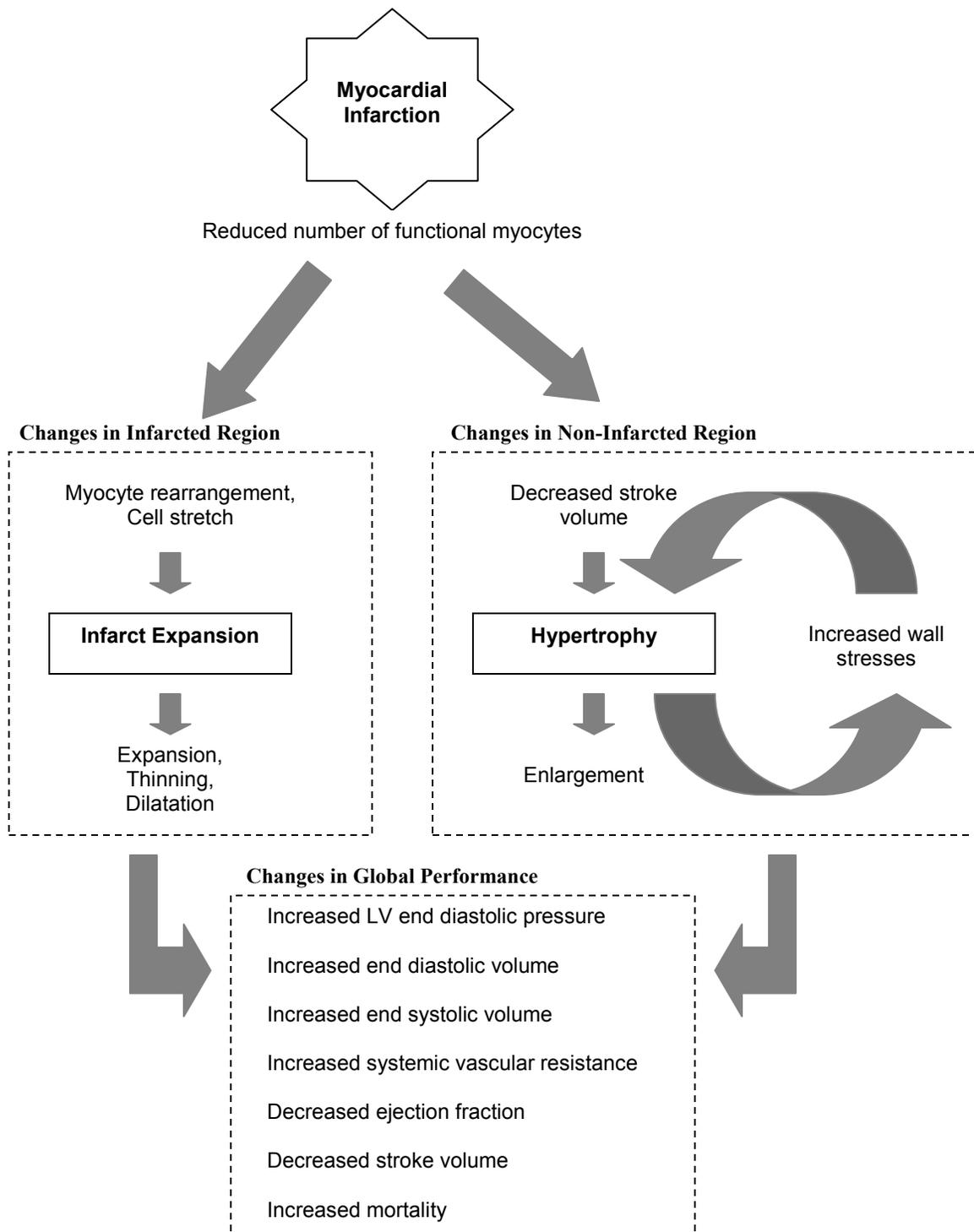


Figure 1: Overview of LV Remodeling

2.1.4 Heart Failure Classifications

The New York Heart Association (NYHA) has developed a classification system for the stages of heart failure. This classification system describes four major stages. In classes I and II, the patient is said to have mild heart failure. Patients in class I do not have limitations in physical activity and do not experience fatigue or shortness of breath during normal activity. Patients in class II experience slight limitations in physical activity and ordinary physical activity may cause fatigue or shortness of breath. In class III, patients experience marked limitations in activity and ordinary activity causes fatigue. Class IV patients have severe heart failure and are unable to carry out any physical activity without discomfort [26]. These classifications established by the NYHA will be referred to throughout this paper.

2.1.5 Current Treatments for LV Remodeling and MI

Reperfusion, thrombolysis, angioplasty, and stenting are treatments typically performed after MI to salvage myocardium, improve patency of arteries, and restore blood flow to the infarcted area [2]. Following these therapies, patients are normally treated with ACE inhibitors, beta-blockers, and/or nitroglycerin.

Current treatments and therapies prescribed to patients to treat MI and LV remodeling are aimed at increasing cardiac output by providing symptomatic relief [18]. The drugs and therapies described in this section simply delay disease progression, but do not prevent future events from occurring [17].

ACE Inhibitors

Angiotensin converting enzyme inhibitors (ACE inhibitors) have been shown to increase stroke volume, increase ejection fraction, and reduce LV volumes in patients following MI compared to patients treated with placebos [2, 17, 27]. ACE inhibitors act by preventing the conversion of angiotensin I to angiotensin II. Angiotensin II is a vasoconstrictor that raises arterial blood pressure. Thus by preventing production of angiotensin II ACE inhibitors have vasodilating effects and result in lower blood pressure [21]. ACE inhibitors attenuate ventricular enlargement, dilatation, and thinning by ventricular unloading and have been shown to offer long-term survival benefits to

patients following MI [2, 16, 17, 28]. Many experts agree that for ACE inhibitors to provide the best benefit, they should be administered very early following infarction (possibly as early as one week) [2, 27].

Although ACE inhibitors offer beneficial results, they can also cause harmful effects. Since ACE inhibitors are vasodilators they cause hypotension, which may decrease myocardial perfusion resulting in additional myocardial apoptosis [2].

Beta-Blockers

Beta-adrenergic receptor blockers, commonly known as beta-blockers, are often used in conjunction with ACE inhibitors to provide complimentary effects [2]. Beta-blockers have been shown to improve ejection fraction, reduce left ventricular end-systolic volume, and subsequently reduce mortality when used concomitantly with ACE inhibitors [17, 27]. A disadvantage of beta-blockers is that they promote hypotension, which may reduce myocardial perfusion, similar to ACE inhibitors [2].

Nitric Oxide

A current treatment available for myocardial infarction is nitric oxide. At first nitric oxide was solely used to increase vasodilatation. However, it was later found to improve the function of the myocardium [29]. It has also been shown by Liu et al. that nitric oxide decreases infarction size [30]. Tests of the effects of nitric oxide on dogs found that it preserved coronary vasodilatation, inhibited neutrophil accumulation, and reduced myocardial necrosis. This treatment has been proven most effective when administered immediately post-infarction [30].

2.2 Left Ventricular (LV) Restraints

Myocardial infarction may initiate the cyclic processes of progressive ventricular remodeling which may eventually result in chronic heart failure. Left ventricular remodeling attempts to preserve the cardiac output in an infarcted heart. Early on the remodeling process is beneficial, but over time can become detrimental. The cessation of adverse geometrical changes may help preserve the function of the heart. LV restraints

are devices that are designed to prevent further enlargement of a patient's heart and thus improve the patient's overall cardiac health.

2.2.1 Biologically-derived Restraints

One of the first types of LV restraints was a biological restraint comprised of a patient's own skeletal muscle. These early biologically-derived restraints were successful in that the materials needed were taken from the patient's body so that a foreign body response was avoided.

Dynamic Cardiomyoplasty

Dynamic cardiomyoplasty was first introduced by Carpentier and Chachques in 1985. It is a surgical technique that was developed as a treatment for dilated cardiomyopathies and late-stage heart failure. In the procedure, a skeletal muscle flap, typically taken from the latissimus dorsi, is removed from the patient and then secured around the heart. A few weeks after the surgery, the muscle flap undergoes a conditioning regimen using specialized stimulator devices. The reported benefits of the procedure include increased ejection fraction, improved LV stroke work index, and decreased New York Heart Association (NYHA) functional class. Furthermore, 6 months after the operation, more than 75% of the surviving patients had improved by one or more NYHA class [31].

Despite the seemingly positive results of the surgery, the technique has not become widely used due to negative postoperative and long-term effects. Immediate and long-term deaths following the procedure have been attributed to ventricular failure and arrhythmia. According to a Medtronic database, procedural deaths occurred in 36% of patients in NYHA class IV and in 15% of those in class III. Another potential short-term complication is that the ischemic skeletal muscle flap fails to contract upon electrical stimulation [31]. Other long-term concerns involve the duration of the muscle flap's functioning. Similarly, it has been stated that the short-term clinical improvements of dynamic cardiomyoplasty were followed by a gradual decrease in cardiac performance because over time the muscle flap atrophies and is replaced by fat [32].

Even though the procedure's long-term efficacy may be questionable, it nonetheless has served as an important learning tool. Aside from the obvious support of LV systolic function from the muscle's contraction, the muscle wrap acts as an elastic restraint. The elastic restraint limited cardiac dilatation, which aided reverse remodeling and prevented further enlargement [31]. In a canine study it was observed that a non-stimulated latissimus dorsi muscle preserved heart function compared to controls [33]. Such studies encouraged investigation into passive cardiomyoplasty devices made of biomaterials that would attenuate LV dilatation.

2.2.2 Passive Biomaterial Restraints

Despite the advances that have been made with biologically-derived restraints, they do have limitations, which prompted the development of biomaterial based restraints. Currently, these restraints are being evaluated for prevention of cardiac dilatation. There are several different types of biomaterial based restraints that are being developed.

CorCap™ Cardiac Support Device (CSD)

The Acorn Cardiovascular CorCap™ CSD is a polyethylene terephthalate (PET-polyester) mesh that encircles the heart from the atrioventricular groove to its apex as shown in Figure 2. PET-polyester provides the necessary mechanical properties, is resistant to biodegradation, interfaces easily with the heart's surface and its physical and mechanical characteristics can be controlled. The CorCap™ is designed to provide passive pressure on the LV. This prevents further dilatation and adverse remodeling by reducing chronic dilatation of end-diastolic volume, ventricular wall stress, myocardial stretch, and eventually promotes reverse remodeling. The device provides a slightly greater amount of pressure on the heart at the end of diastole than at the end of systole and it is not specifically intended to provide assistance during systolic contraction. Furthermore, CorCap's design allows for bidirectional compliance, with more compliance available in the longitudinal direction (apex to base) than in the circumferential direction. This facilitates the gradual reshaping of the heart from

spherical to its natural ellipsoid shape [34]. It is available in six standard sizes and the final fitting is done by the surgeon [35].

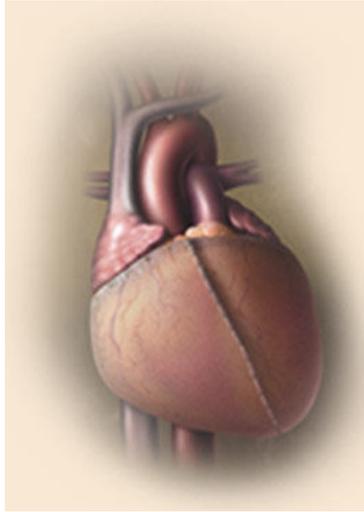


Figure 2: CorCap™ CSD (with permission from Acorn Cardiovascular) [36]

The implantation involves a sternotomy and typically requires the patient to be on full cardiopulmonary bypass (CPB). The device is adjusted to fit snugly around the ventricles and reduce the diameter of the heart by 5-10% as measured by transesophageal echocardiography. Due to the invasiveness of the surgery, the implantation of the device is carried out mainly in patients with NYHA chronic heart failure class from late II to early IV. Patients in class IV heart failure that receive continuous medication or mechanical support, as well as those with other medical conditions, may be at too high of a risk to undergo the procedure and may not fully benefit from the CSD [35]. Additional cardiac procedures may be performed concurrently with CorCap™ implantation.

Functional mitral regurgitation (MR) is a typical result of the increased spherical shape of the LV and it results in further mechanical burden on the LV [37]. Consequently, surgical implantation of the CorCap™ is usually accompanied by mitral valve (MV) surgery.

Coronary artery bypass graft (CABG) is another procedure that is typically carried out in patients receiving CorCap™ [35]. Average total operative times reported for the CorCap™ initial safety studies include: 2.3 hours \pm 0.6 hours for CorCap™ only and 3.5 hours \pm 1.2 hours for concomitant cardiac procedures [38].

The efficacy of the CorCap™ has been assessed by many different methods in the months and years of postoperative patient monitoring. In the initial safety studies it was

observed that many patients experienced significant decreases in LV end-diastolic dimension (LVEDD) and an increased LV ejection fraction (LVEF) with changes observed as early as 3 months post-infarction. The safety studies also reported a decrease in the MR classification, even in patients who did not undergo concurrent mitral valve surgery. More holistic long-term improvements included a decrease in NYHA heart failure class and a better quality of life as indicated by questionnaires [38]. Although long-term data from the Acorn Trial, a large randomized multicentered trial of 300 patients, is still being collected and evaluated, the initial results support many of the above-mentioned cardiac changes. However, it was reported that there was no significant survival difference at the one-year follow-up between the Cardiac Support Device and control groups, even though the CSD treatment group was significantly less likely to require a major cardiac procedure [6].

In addition to changes in global heart function and patients' well being, several animal studies have reported changes at the cellular and biomolecular levels as a result of long-term CSD therapy. In a canine model of heart failure, it was demonstrated that long-term CSD therapy was associated with improved cardiomyocyte contraction and relaxation, down regulation of stretch response proteins, and attenuation of cardiomyocyte hypertrophy compared to the controls [7]. Additionally, other studies have shown that CorCap™ treatment leads to less interstitial fibrosis, greater myocardial capillary density, and reduced myocyte apoptosis [38]. The above-mentioned studies provided insight into the mechanisms of improvement that may be responsible for the reverse remodeling observed in clinical trials.

Among the LV restraints investigated thus far, the CorCap™ appears to be the furthest along in terms of clinical trials. The CorCap™ received CE mark approval in Europe in 2001 (the equivalent to the US's FDA) and has been available to a small number of hospitals in a Limited Market Release Surveillance Study (LMRSS) [38]. The device is currently not FDA approved and is limited to investigational use in the US [39, 40].

HeartNet™ Ventricular Support System

The Paracor Medical HeartNet™ is a passive circumferential restraint made from a superelastic nitinol mesh shown in Figure 3. It is similar to the CorCap™ in that it surrounds both ventricles, but it does not cover the apex of the heart. The nitinol mesh exerts a small but continuous force on the heart to prevent adverse LV remodeling. Nitinol is used because of its physical properties, its proven biocompatibility in human implants, and its superelastic properties which allow it to exert a nearly constant restraining force over a large range of strain. The above-mentioned properties reduce the possibility of constricted coronary vessels and ensure that force is still applied even if the heart decreases in size [41].

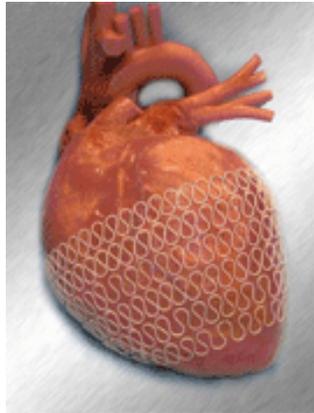


Figure 3: Paracor HeartNet™ (with permission from Paracor Medical, Inc.) [42]

The implantation is minimally invasive, involves general anesthesia, and operative times have averaged under 60 minutes with many as low as 30 minutes. A small incision is typically made in the 6th or 7th intercostal space of the thoracic cavity and the device is delivered in compressed form by a proprietary delivery tool. The mesh is then expanded and elastically conforms to the heart without the need for sutures. Imaging is then utilized to ensure proper placement. Since the surgery is perceived to be low risk, the patients selected for the HeartNet™ trials generally had a slightly better initial NYHA functional status than the CorCap™ patients [41].

The HeartNet™ is not FDA approved and is in early clinical trials. Early canine and bovine experiments have established the safety and feasibility of the device. An initial clinical trial involved 20 patients, 10 in the US and 10 in Europe, and decreases in dilatation, functional status, and exercise capacity were observed. Importantly, the initial

human trial reported no signs or symptoms of constricted coronary vessels. There were no operative or late deaths in the study and the results were favorable, but have not been presented in detail [41].

Myosplint®

The Myocor™ Myosplint® is a passive LV restraint of the “nut and bolt” type as shown in Figure 4. It is designed to correct the LV spherical shape that is characteristic of patients with advanced heart failure. It consists of an implantable transventricular splint and two epicardial pads on each end of the splint. The splint bisects the left ventricle and upon applying tension, it applies forces such that the LV takes on a more normal shape. The splint is made from 1.4mm diameter braided polyethylene and is coated with expanded polytetrafluoroethylene (ePTFE). The epicardial pads are covered with a polyester fabric [5].

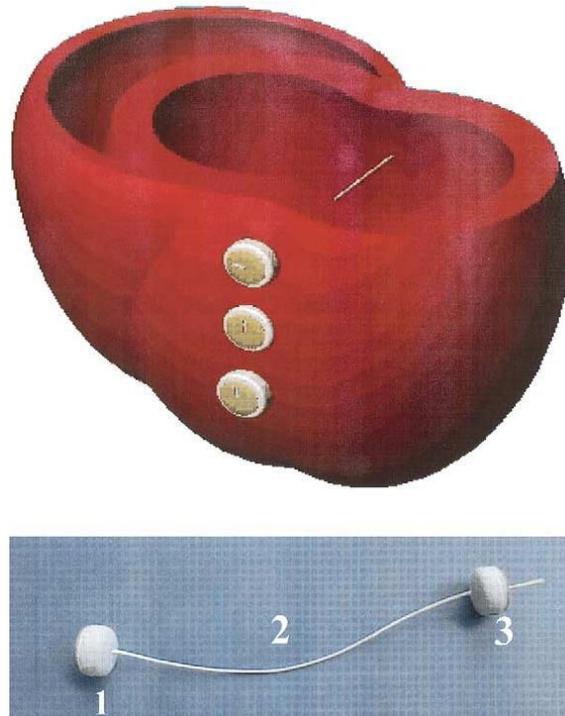


Figure 4: Myosplint® Components and Cardiac Configuration (with permission from Elsevier) [43]

Typically, three splints are implanted in an open-chest procedure by a proprietary delivery tool. The three splints are equally spaced between the apex and the base of the LV. Coronary vasculature is identified during implantation in order to avoid damage or

restriction of coronary vessels. Epicardial echocardiography is used to place the splints and ensure the avoidance of papillary muscles. A measurement and tensioning instrument adjusts the device to produce a 20% reduction in wall stress. In a non-randomized clinical safety trial, it was reported that implantation typically took from 40-50 minutes [44].

Preclinical canine studies established the safety of the device at 180 days. No pad piercing of the ventricular wall, device migration, or thrombus formation was observed [5]. The significant results of one human clinical study included improvements in NYHA heart failure classification, significant reductions in LVEDV and LVESV, and increased LVEF [44].

The study of the Myosplint led to the development of the Myocor™ Coapsys® device to treat functional MR in a minimally invasive procedure [44]. The aforementioned study did not indicate whether more trials were planned or are being conducted with the Myosplint®.

Coapsys® Annuloplasty System

The Myocor™ Coapsys® device is designed to treat mitral annular dilatation and papillary muscle displacement. Its mechanism of action can be seen in Figure 5. Ventricular dilatation in dysfunctional LVs results in remodeling of the mitral valve, which causes mitral regurgitation (MR). The subsequent MR is commonly considered to be one of the initial causes of heart failure and is an ongoing condition during the disease [45].

The Coapsys® device is similar to the Myosplint® in terms of the polymers used. It is a passive device that contains two epicardial pads connected by a transventricular polymer splint. The posterior side of the device consists of a rig, which holds two posterior epicardial pads: a superior and inferior one. The superior pad creates a shape change at the MV level to correct the valve's poor closure and the inferior pad creates a change at the papillary muscle level. The placement ensures that the splint passes between the papillary muscles and below the valve leaflets. Once in place, the device is then tightened by drawing the anterior and posterior pads together and then fixing the anterior pad to the polymer splint [45].

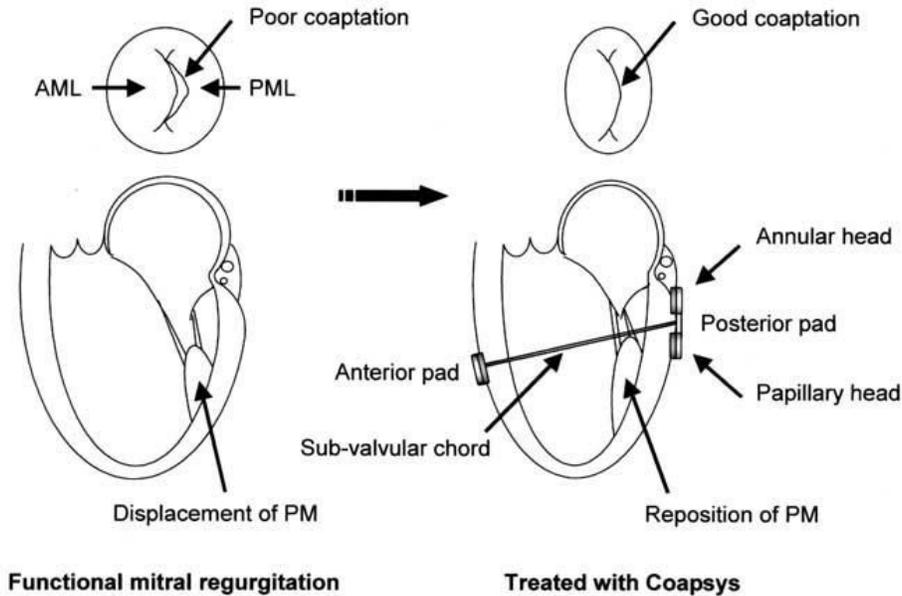


Figure 5: Coapsys® Mechanism of Action (with permission from Elsevier) [46]

The surgical procedure for the implantation of the Coapsys device does not require CPB [47]. An incision is typically made along the sternum and the device is positioned by hand with a proprietary delivery device and vacuum assistance. Verification of the placement is done with handheld echocardiography [45]. The sizing of the device is determined by real-time color flow Doppler imaging to quantitatively assess the MR and a proprietary device is used to shorten the splint until MR is eliminated or a maximum shortening of 35% has been reached [47].

The results of the few clinical safety and feasibility studies performed were promising. In one study, 19 patients with functional MR and coronary artery disease were selected to undergo CABG and Coapsys® device implantation. Mean LV chamber diameter, sphericity index (ratio of LV short-axis to long-axis dimensions), and MR grade were significantly reduced [47]. Another study also reported decreased NYHA classification. Importantly, the aforementioned results were sustained through 1-year follow-up [45]. At this point randomized clinical trials of Coapsys® are ongoing.

2.3 Left Ventricular Assist Device (LVAD) and LV Unloading

Unloading the dysfunctional LV can often lead to some improvement in function. Left ventricular assist devices (LVADs) typically consist of electrically powered pumps

that serve to transport blood from the LV to the ascending aorta, thereby reducing the workload of the dysfunctional LV [48]. LVADs have become a mainstream bridge to cardiac transplant [49]. However, in a small subset of LVAD recipients, about 5%, it was observed that the patients could be weaned off the device with nearly normal cardiac function. Consequently, LVADs have recently been used as bridges to recovery to restore basic cardiac function without subsequent transplantation [48].

LVADs provide a significant volume and pressure unloading of the LV and therefore normalizations of neurohormonal and cytokine activities have been reported. Several reports have further documented the recovery of native LV function after long-term LVAD support [48]. The reported results of long-term LVAD support were significant normalization of cardiomyocyte geometry, cellular level changes that were associated with reductions in LV dilatation and mass, and the normalization of expression and function of proteins involved in calcium handling, cellular hypertrophy, cell cycling, and apoptosis [50]. However, a number of properties including abnormal extracellular matrix metabolism, increased tissue angiotensin levels, and myocardial stiffening did not improve with LVAD support [51].

2.4 Biomaterials for Myocardial Infarction

Researchers have been exploring the possibilities of using biomaterials for the treatment of heart failure for over ten years [52]. Different biomaterials, including contractile polymers, have been investigated to create heart patches and LV restraints. A biomaterial must exhibit appropriate characteristics for its intended use since it is ultimately going to be implanted in humans. Biomaterials must be biocompatible, wear resistant, non-thrombogenic, and possess appropriate mechanical strength.

2.4.1 Heart Patches

One type of treatment that is currently being developed is the use of heart patches or grafts to provide structural support and deliver treatments. The foundation of the heart patch or graft is used for mechanical strength. The porous nature of the scaffold makes it possible for a therapeutic agent, such as stem cells, drugs, proteins, or growth hormones, to be seeded onto it for various applications. In other cases, the scaffold can be implanted

without a therapeutic agent and used to promote cellular interactions. Some scaffolds that have been researched include extra cellular matrix (ECM), fibrin glue, polyester urethane urea (PEUU), and poly-glycolide-co-caprolactone (PGCL) [53, 54, 55, 56, 57, 58, 59, 60].

Extracellular Matrix (ECM)

ECM is a biomaterial proven to be useful in many kinds of biological applications. ECM is made out of animal tissue, which consists of collagens, elastins, proteoglycans, and adhesive proteins such as fibronectins and laminins. Due to the fact that ECM is taken from animal tissue it is highly biocompatibility and has a mechanical strength similar to that of human tissue. Because ECM has properties similar to native tissue it can also regulate cell interactions and act as a local reservoir for developing cells due to its porosity [53].

Recent studies using ECM as a heart patch for the treatment of heart failure have shown promising results. In a canine study, a heart patch derived from ECM was shown to significantly improve heart function in infarcted regions. This study also explored the possibility of replacing the traditionally used Dacron heart patch with the biological ECM patch. Dacron is a FDA approved biomaterial that exhibits some biocompatibility and provides appropriate mechanical properties [61]. Similar to that of Dacron, ECM provides appropriate mechanical support. ECM also performs additional functions such as serving as a place for cardiomyocytes to anchor to and contracting in sync with the native myocardium. It is also important to note that ECM has been approved by the Food and Drug Association (FDA) [61].

Fibrin Glue

Another biomaterial that has been used as a heart patch is fibrin glue. Fibrin glue has received a lot of attention in biological applications due to its superior cell retention and cell transport survival abilities. There are many scaffolds that possess appropriate mechanical properties but may not be able to house or transport cells efficiently. Fibrin glue has both of these traits and has been shown in recent studies to be useful in treating cardiac diseases. By placing the fibrin glue scaffold on an infarcted area, cells seeded on

the scaffold will have a higher chance of staying attached to the infarcted region. Another attractive trait of fibrin glue is that it can be injected as a liquid and will form a scaffold *in vivo*. Therefore, the fibrin glue scaffold can be used in minimally invasive procedures [54].

The use of fibrin glue as an injectable scaffold to treat heart failure has been investigated in rats. Fibrin glue scaffolds seeded with skeletal myoblasts were tested to determine if the cells could survive on the scaffold. Skeletal myoblasts were able to adhere to the scaffold, while surviving and proliferating. When the fibrin glue scaffolds were implanted onto the infarcted myocardium of rats the scaffolds improved heart function. However, the scaffold did not retain the skeletal myoblasts upon injection. Without cells seeded on the scaffold, it still was still able to enhance myocyte growth possibly due to increased neovascularization promoted by the fibrin glue, which may have promoted cell migration and proliferation. Therefore, it seemed that when the fibrin glue turned into a semi-solid, it acted as a temporary extracellular matrix for cellular proliferation [62].

Based on this article and other literature, fibrin glue has shown potential to serve as a scaffold for cellular treatment of heart failure. Similar to extracellular matrix, fibrin glue is also FDA approved. Additionally, it is biocompatible and does not elicit an inflammatory response, foreign body reaction, necrosis, or extensive fibrosis.

Polyester Urethane Urea (PEUU)

Polyester urethane urea (PEUU) is another promising biomaterial that may be used as a heart patch. Unlike the other scaffolds previously described, PEUU is a biodegradable polymer. In theory, a biodegradable patch will allow the heart to gradually repair itself so that it will no longer require a supporting patch. As the heart patch degrades it will be replaced with healthy myocardium.

There have been several studies that have demonstrated use of this patch *in vivo*. Heart patches tested in rats preserved LV geometry, demonstrated growth factor expression, and improved contractile function relative to non-treated rats. The PEUU patch was found to degrade over a period of eight weeks and was replaced with smooth

muscle bundles, which increased wall thickness and compliance. The results of this study showed that the patch increased temporary mechanical support while healthy tissue was regenerated in the infarcted region [55].

Other studies have also confirmed that a biodegradable PEUU patch can improve heart function, preserve LV geometry, and provide temporary mechanical support to the infarcted heart. Additionally, it has been shown that PEUU is biocompatible, permits cellular interaction, and promotes endothelialization with minimal inflammation [56, 57].

Composite Scaffolds

Many studies have explored the possibilities of combining multiple biomaterials to make a composite scaffold. By doing so, researchers can combine the advantages of multiple biomaterials to create a scaffold that best fits their needs. One example of a composite scaffold was made by Ameer and Langer by combining fibrin gel and polyglycolic acid (PGA) seeded with freshly isolated pig chondrocytes. This scaffold was made for targeted cell therapy and therefore required appropriate biodegradation, mechanical strength, and the ability to hold cells. In this case, the fibrin gel was used for its ability to anchor cells while the PGA acted as the drug-delivering unit by releasing the cells upon degradation [63].

Biodegradable scaffolds are an area of research that has been heavily investigated. PGA, PLA (polylactic acid), and the copolymer of the two (poly(DL-lactic-co-glycolic acid)), have been used to make biodegradable heart patches. Both polymers are biocompatible, possess good mechanical properties, and are easy to handle. However, PLA and PGA are highly hydrophobic and in turn, do not demonstrate good cell-seeding properties. On the other hand, other biomaterials, such as collagen, have good cell interactions but poor mechanical strength. Consequently, PLGA and collagen sponges have been combined to produce novel scaffolds that possess the advantageous properties of both biomaterials [58].

Another polymer that has received a lot of attention is poly-glycolide-co-caprolactone (PGCL). PGCL is biodegradable and has an extremely low immunogenic response in humans [59]. Piao et al. have combined gelatin mesh, collagen gel, alginate, PGA, poly-L-lactide fabric, and PGCL to make a composite scaffold as part of a heart

patch for the treatment of heart failure. In addition, they seeded the scaffold with bone marrow derived mononuclear cells (BMMNC). By combining these biomaterials, a scaffold was created that has good elastic properties, good mechanical properties, and promotes cellular interaction.

2.4.2 Contractile Polymers

Contractile polymers are relatively new areas of research and therefore there is limited information regarding their use in heart patches in biological applications. Generally, contractile polymers are used in commercial applications such as light emitting diodes (LEDs), battery electrodes, and sensors [60]. However, the possibility of using these polymers to treat heart failure has been explored in a small number of studies. Contractile polymers may be able to aid in heart contraction, which would reduce the heart's workload and provide an opportunity for regeneration, similar to a LVAD.

Polyaniline (PANI)

One of the most promising contractile polymers is polyaniline (PANI) because of its unique properties such as controllable electrical conductivity, environmental stability, and rich redox chemistry [64]. Nevertheless, there are many obstacles to overcome in order to use this polymer *in vivo* including addressing the issues of biocompatibility, solubility, and processibility. Recent studies have investigated the possibility of using PANI for biological applications.

Huang et al. have synthesized a tri-block copolymer of PANI and PLA (PLA-PANI-PLA), which retains the contractile properties of PANI, but also incorporates the biodegradable properties of PLA. Making PANI biodegradable is very beneficial for biological applications because this would help eliminate potential long-term biocompatibility issues. This copolymer has the potential to promote cell attachment and proliferation, although *in vivo* experiments have not yet confirmed the effects of the polymer on living systems [60].

Other Contractile Polymers

Polythiophene, polypyrrole, and polyfuran are three other contractile polymers used in commercial applications similar to PANI. However, there has been limited research done with these polymers for cardiac applications. On the other hand, research has been conducted to determine their biocompatibility and potential use in stimulating the brain in neurodegenerative diseases [13].

Polypyrrole has been researched for use in applications dealing with neural disorders. The biocompatibility and cellular interaction of polypyrrole have been tested with neural tissue. Effective electrical stimulation of polypyrrole can help repair damaged neural tissue, by causing a chain of chemical signals to induce tissue regeneration [13].

Polythiophene has shown to be biocompatible, non-toxic and easily fabricated. Due to its biocompatibility, polythiophene has been shown to retain endothelial cells. When polythiophene is immersed in a solution of endothelial cells it still exhibits its electrocontractile characteristics [65].

2.4.3 Microthreads

Microthreads are biologically-derived thin fibers, generally made of either fibrin or collagen. Over the last few years, microthreads have been investigated for use in the repair and regeneration of tendons and ligaments. These threads are useful for these applications because they are chemically stable, biocompatible, and are structurally similar to native tendon and ligament. Additionally, these threads can be bundled together to improve their mechanical properties [14].

Collagen Microthreads

Collagen microthreads can be derived from a number of biological sources including rat tails and bovine tendons. They can be made from either soluble or insoluble type I collagen. Generally, they have very good mechanical properties when bundled, braided, or cross-linked together. However, collagen microthreads do not sufficiently promote cell migration, which leads to inadequate tissue in-growth from the wound site. Research has been focused on improving cell migration onto microthreads using growth factors, proteins, and cells. Depending on how the microthreads are cross-linked and

bundled they can have ultimate tensile strength (UTS) values ranging from ~1MPa to about 40MPa, elastic modulus values ranging from <10MPa to 250MPa, and strain values ranging from 0.1 to 0.4 [66]. Biodegradation rates can also vary from weeks to multiple years depending on the chemical structure of the microthreads.

Fibrin Microthreads

Fibrin microthreads are highly biocompatible with properties similar to native tendon and ligament. However, the mechanical strengths of fibrin microthreads are lower than those made of collagen. Depending on how they are cross-linked, fibrin microthreads have UTS values ranging from 1MPa to 8MPa, elastic modulus values ranging from 25MPa to 110MPa, and strain values from 0.2 to 0.3. Fibrin microthreads have a biodegradation rate of about a week and are being studied for their superior cell migration properties [19].

A summary of the advantages and disadvantages of the biomaterials discussed in this section can be seen in Table 1.

Table 1: Summary of Biomaterials

	PROS	CONS
Heart Patch Scaffold		
ECM	<ul style="list-style-type: none"> • High biocompatibility • Appropriate mechanical strength • Regulates cell interactions • FDA approved 	<ul style="list-style-type: none"> • Could provoke immunogenic response • Non-biodegradable
Fibrin Glue	<ul style="list-style-type: none"> • Superior cell retention • Minimally invasive implantation procedure • Provides structural support • FDA approved • Biocompatible 	<ul style="list-style-type: none"> • Non-biodegradable
PEUU	<ul style="list-style-type: none"> • Biodegradable • Provides appropriate mechanical support • Promotes cellular interactions 	<ul style="list-style-type: none"> • Could provoke immunogenic response • Not FDA approved
Composite Scaffolds	<ul style="list-style-type: none"> • Combines advantages of different biomaterials 	<ul style="list-style-type: none"> • Limited research
Contractile Polymers		
PANI	<ul style="list-style-type: none"> • Can be biocompatible • Can be biodegradable • Controllable electrical conductivity 	<ul style="list-style-type: none"> • No long term studies
Polypyrrole	<ul style="list-style-type: none"> • Studies have been done <i>in vivo</i> • Can be biocompatible • Can promote cellular interaction 	<ul style="list-style-type: none"> • No long term studies
Polythiophene	<ul style="list-style-type: none"> • Can be biocompatible • Able to anchor cells 	<ul style="list-style-type: none"> • No long term studies
Microthreads		
Fibrin Microthreads	<ul style="list-style-type: none"> • Variable mechanical properties (UTS ranging from 1MPa to 8MPa, elastic modulus ranging from 25MPa to 110MPa, strain ranging from 0.2 to 0.3) 	<ul style="list-style-type: none"> • No long term studies

	<ul style="list-style-type: none"> • Biodegradable within the time frame of weeks to years • Properties similar to native tendon and ligament • Low cost • High cell migration rates • Easily produced 	
Collagen Microthreads	<ul style="list-style-type: none"> • Variable mechanical properties (UTS ranging from 1MPa to 40MPa, elastic modulus ranging from 10MPa to 250 MPa, strain ranging from 0.2 to 0.4) • Biodegradable within the time frame of 1 week • Properties similar to native tendon and ligament • Easily produced 	<ul style="list-style-type: none"> • No long term studies • Higher cost than fibrin microthreads • Cell migration may be difficult

2.5 Regenerative Therapies for Infarcted Myocardium

Regenerative medicine is currently a hot topic in the medical world, which could revolutionize medicine. However, because regenerative medicine is still undergoing vast research and scrutiny there are many different methods for regenerative therapy that are being explored for treatment of heart failure.

2.5.1 Cell based therapies

The therapies and drugs most commonly used to treat heart failure have only been shown to maintain the damaged state of the heart or keep it from progressively worsening. In the last several years however, the possibility of stimulating regeneration of damaged tissue has been at the forefront of research. This is due to the fact that triggering the body to heal itself could still allow for beneficial treatment while avoiding some of the greatest barriers facing the field of biomaterials today. The research that has

been done over the past several years in the field of regenerative medicine and in particular cardiovascular regeneration has been rather promising. The ability to implant cells of various types into the damaged region and have them promote myocardial regeneration as well as improve cardiac function has been demonstrated in both pre-clinical and clinical studies. However, it is crucial to note that all of the various cell types that can be used to regenerate the myocardium are not without complications. Technical worries, as well as ethical issues are different and vary in degree for the different types of cells explored for therapeutic purposes. For these reasons it is important to explore each of the different researched cell lines and their individual pros and cons, a summary of which is provided in Table 2.

Embryonic Stem Cells

One type of cell that is being explored for use as a cell-based therapy is the stem cell. Stem cells are characterized by their ability to be capable of self-renewal, differentiate into one or more types of mature cells, and generate colonies of differentiated cells. There are two major types of stem cells – adult stem cells and embryonic stem cells (ESCs). The difference between these two types of stem cells is that while adult stem cells have the ability to differentiate into several different types cells, embryonic stem cells have the ability to differentiate into any type of cell. Specifically, embryonic stem cells have the ability to differentiate into cardiomyocytes with the capacity to propagate action potentials, making them a focus in the field of cardiac regeneration [96]. Studies have shown that when human ESCs are cultivated in suspension they form contracting areas known as embryoid bodies. These embryoid bodies contain components such as a heavy myosin chain, α -actinin, desmin, and troponin I which indicate cardiomyocyte formation [67].

Despite all of these positive attributes, embryonic stem cells are one of the most controversial cell types being explored for cardiac regeneration. This is due mostly in part to controversy surrounding the ethical questions raised by their use. However, things such as the formation of masses of tissue cells known as a teratomas and negative immune response have also raised question as to whether the ideal integration of these cells is worth the potential risk [8].

Skeletal Myoblasts

To eliminate some of the controversy associated with the use of embryonic stem cells, research has been focused on finding a cell-based therapy that is derived from adult cells. One such type of cells that have undergone significant research is skeletal myoblasts. Skeletal myoblasts are acquired via skeletal muscle biopsy. The fact that these cells are derived directly from the patient avoids obstacles such as immunosuppression and finding a consistent source of cells. Additionally, the risk of teratoma formation is minimized due to the fact that these are myogenic cells [8].

Despite the fact that studies examining the administration of these cells have demonstrated a functional improvement when engrafted post myocardial infarction, the skeletal myoblasts have not been proven to differentiate into cardiomyocytes [68, 69]. They do, however, secrete various angiogenic and anti-apoptotic factors that are believed to be responsible for the beneficial effects observed in these studies [70]. This lack of differentiation is one of the major disadvantages of using skeletal myoblasts for cardiac regeneration as this means that they do not form electrical junctions and therefore the patient may exhibit tachycardia [68].

Bone Marrow Derived Mononuclear Cells

Bone marrow contains hematopoietic stem cells as well as other stem cells that are believed to have the plasticity necessary to form cardiomyocytes. For this reason, studies have explored the use of bone marrow mononuclear cells for cardiac regeneration [8]. These cells, stimulated by granulocyte colony-stimulating factor (G-CSF) and stem cell factor improved ventricular function and regenerated cardiomyocytes [71]. Several other studies, however, showed an accelerated recovery but not a continued improvement in the ventricular function. Additionally, some studies showed only neutral results. For this reason, clinical applications of the bone marrow derived mononuclear cells have received much scrutiny [8].

Endothelial Progenitor Cells

Endothelial progenitor cells are the precursors to endothelial cells. Studies using various methods to deliver endothelial progenitor cells in a rat model of myocardial infarction have been executed over the last several years. These studies highlighted the fact that different methods of delivery can produce different results. For example, neovascularization was exhibited when endothelial progenitor cells were administered intravenously. However, when injected locally an increased proliferation of both myocytes and vascular structures was observed. The primary mechanism of action of these cells is based on neovascularization, which helps to protect the damaged cardiomyocytes from apoptosis. This method does not, however, proliferate cardiomyocytes, which puts it behind some of the more preferred cell types [8].

Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) were first found in bone marrow. They can also be found in umbilical chord blood, adipose tissue and the heart itself. This fact makes it one of the easiest cell types to obtain. These non-hematopoietic cells have exhibited the ability to differentiate into various types of mesodermal cells [8]. Studies using MSCs in both rat and porcine models showed that the introduction of MSCs after myocardial infarction reduced infarct size and improved remodeling [72, 73]. Studies suggest that because of the reduction in infarct size and improved remodeling, as well as an increase in myocardial tissue and left ventricular function, that the MSCs stimulate a repair mechanism [8].

Unfortunately, the mesenchymal stem cells studied have not shown any evidence to suggest that they are able to differentiate into cardiomyocytes. Additionally, these studies also show a decline in the presence of the mesenchymal cells over time. This leads to the belief that if one could maintain the presence of the mesenchymal stem cells in the infarcted area for a longer period of time, there would be even better repair of the infarct. It is this belief that has led studies to suggest the use of growth factors or other stimuli to improve the length of cell survival [8].

Cardiac Stem Cells

When cell-based therapies began to be explored for use in the heart, it was only a matter of time before the idea of using a cell-therapy that was derived directly from the heart would be explored. Cardiac stem cells are cells that are found in the heart and are part of the cardiomyocyte lineage. This indicates the potential ability of these cells to differentiate into cardiomyocytes. Additionally, because cardiac stem cells can be grown from samples taken from endocardial biopsy, these cells boast the possibility of being used for autologous therapy. This would greatly minimize the chance of autoimmune response upon injection of the therapy. Despite the fact that cardiac stem cell therapy has been shown to stimulate myocardial and vascular regeneration, there has not been substantial evidence that these cells differentiate into cardiomyocytes when introduced into the heart post-infarction [8].

Table 2: Summary of Stem Cells Therapies

Types of Stem Cells	PROS	CONS
Embryonic Stem Cells (ESC)	<ul style="list-style-type: none"> • Can differentiate into cardiomyocytes with the ability to propagate action potentials • Can become endothelial and smooth muscle cells 	<ul style="list-style-type: none"> • Teratoma formation • Ethical issues
Skeletal Myoblasts	<ul style="list-style-type: none"> • Derived from autologous cells, no need for immune suppression • Resistant to ischemia • Less teratogenic than ESCs 	<ul style="list-style-type: none"> • Have not been proven to differentiate into cardiomyocytes
Bone Marrow Derived Mononuclear Cells (BMNC)	<ul style="list-style-type: none"> • Can improve LV Function • Regenerates cardiomyocytes, endothelial cells, and smooth muscle cells 	<ul style="list-style-type: none"> • Neutral study results add to controversy surrounding BMNCs
Endothelial Progenitor Cells (EPC)	<p>Intracoronary applications showed-</p> <ul style="list-style-type: none"> • Improved LV function • Improved myocardial perfusion • Reduction in infarct size 	<ul style="list-style-type: none"> • Not a lot of work has been done with these types of cells in comparison
Mesenchymal Stem Cells (MSC)	<p>Animal studies showed-</p> <ul style="list-style-type: none"> • Improved LV Function • Reduction in infarct size • Easy to harvest for use 	<ul style="list-style-type: none"> • MSC retention declines over time • Not sufficient evidence that MSCs differentiate into cardiomyocytes
Cardiac Stem Cells (CSC)	<ul style="list-style-type: none"> • Exhibit myocardial and vascular regeneration • Relatively easy to obtain • Are derived from patient and can be used for autologous therapy 	<ul style="list-style-type: none"> • A lot is unknown about CSCs and what the best ways to use them are • Have not been proven to differentiate into cardiomyocytes

2.6 Other Therapies for MI Treatment

The controversy surrounding cell-based therapy has made its integration into the medical world a difficult one. Other novel treatments for heart failure are also being explored that may be less controversial and equally or more effective. Although these therapies do not regenerate the myocardium, they may be able to prevent further dilatation and remodeling.

2.6.1 Growth Hormone

Animal studies have shown that growth hormone (GH) may improve myocardial function and attenuate LV remodeling following myocardial infarction. GH treatment acts by reducing apoptosis, enhancing angiogenesis, and inducing cardiomyocyte hypertrophy, which prevents wall thinning and prevents increases in wall stresses. Although studies in animals have shown positive results, human studies have not had such positive outcomes [74, 75, 76]. This may be due to the fact that patients simply did not respond to GH treatment, sample sizes may have been too low to detect significant differences, doses may have been too low, or it may be due to the fact that the treatment takes a long period of time before results can be detected [74, 75]. One clinical study reported that patients treated with GH reported a feeling of “well being,” although no significant changes in LV function were observed [77].

The main drawback of growth hormone treatment is that it may prevent LV remodeling, but it will not be able to reverse the effects of remodeling [11]. Also, high doses of GH administered for treatment may be unsafe because systemic exposure can cause hyperglycemia, diabetes, and hypertension [74].

2.6.2 Atrial Natriuretic Peptide (ANP)

Atrial Natriuretic Peptide (ANP) is produced primarily in the heart’s atria. ANP increases venous capacitance and promotes the excretion of sodium, thereby reducing extracellular fluid volume. It further reduces peripheral vascular resistance and lowers blood pressure. Several hormones and neurotransmitters including endothelin, arginine vasopressin, and catecholamines directly stimulate the release of ANP. In the context of heart failure, cardiomyocyte hypertrophy leads to elevated ventricular production of ANP and increased plasma levels of angiotensin II and endothelin-1 further initiate its release into circulation. In NYHA class IV heart failure patients, the concentration of ANP can be as high as 30 times the normal level. Animal heart failure studies have demonstrated that ANP serves to inhibit the production of molecules such as angiotensin II and endothelin-1, inhibit the growth of cardiac fibroblasts and induce cardiomyocyte apoptosis. The mechanisms of ANP may serve to attenuate the early hypertrophic cardiomyocyte response to an event such as myocardial infarction [78].

ANP release is seemingly a mechanism utilized by the heart to limit remodeling following an event such as myocardial infarction. However, high doses of ANP increase peripheral vascular resistance even though low doses initially decrease blood pressure [78]. In chronic heart failure patients, there is a decrease in the responsiveness of ANP's receptor. Treatment with a LVAD or CSD has been shown to reestablish the sensitivity of the ANP receptor and to decrease mRNA expression of ANP to near normal levels [48, 79]. One study observed that ANP infusion immediately after percutaneous transluminal coronary angioplasty (PTCA) can prevent LV dilatation and improve left ventricular ejection fraction in patients with a first anterior acute myocardial infarction. The suggested mechanism of action included suppression of circulating levels of angiotensin II and endothelin-1 [12].

2.6.3 Granulocyte Colony-Stimulating Factor (G-CSF)

G-CSF is one of the most thoroughly studied growth factors in the context of myocardial infarction. It has been reported to perform a number of functions including the mobilization of hematopoietic precursor cells from the bone marrow and the stimulation of endothelial cell migration. Other studies with G-CSF delivery as a therapy following MI have shown overall improvements in cardiac function and reduction in LV remodeling. Recent clinical trials have also demonstrated that G-CSF is safe and effective in improving LV functions in patients post-infarction. However, the employment of G-CSF as a treatment has not been without negative results. In one study, G-CSF was associated with three patient deaths, while in another it was shown to be ineffective at improving LV function [80].

In an animal study, it was observed that the timing of the start of G-CSF treatment was important. The beneficial effects of the treatment were larger when the treatment was started as soon after MI as possible. Early clinical safety and feasibility studies reported mixed results and the efficacy of G-CSF treatment was inconsistent, especially because the inclusion criteria for patients differed markedly from trial to trial. Furthermore, the effective dosage, administration rates, and time period to start the treatment have not been established [81].

2.6.4 Matrix Metalloproteinase (MMP) Inhibitors

Another promising therapy after myocardial infarction is the delivery of matrix metalloproteinase (MMP) inhibitors. MMP is an enzyme that has multiple functions, most importantly degradation of ECM. MMPs function at neutral pH level can be controlled by use of specific tissue inhibitors of metalloproteinases (TIMPs). MMPs usually are activated during embryonic development, bone remodeling, and wound healing. They destroy the damaged tissue including the ECM, which is eventually replaced with scar tissue. The use of MMP inhibitors may prevent further remodeling after myocardial infarction. MMP inhibitors are most effective when used immediately following myocardial infarction (less than one day) in order to prevent MMPs from destroying the ECM [82].

It has been shown in many animal studies that MMP inhibitors are able to improve heart function by reducing infarct zones and improving ejection fraction. However, these positive outcomes have not been shown during human clinical studies [83]. Clearly, there is a need for further investigation on MMP inhibitors for the treatment of infarcted tissue in humans.

2.6.5 Vascular Endothelial Growth Factor (VEGF)

VEGF is a vital promoter of angiogenesis – the process of forming new blood vessels from existing vessels through the recruitment of associated supporting cells. The rate of angiogenesis is determined by the levels of both angiogenesis inhibitors and promoters. After ischemia, hypertrophy increases the heart's demand for oxygen and nutrients. However, hypertrophy causes a reduced number of capillaries to supply a larger volume of cardiac tissue and thus coronary flow is greatly impaired. The decrease in perfusion results in a reduced supply of nutrients and oxygen, which may be a contributing factor to the decline of contractile function [9].

VEGF stimulates the formation of capillaries by increasing the proliferation and migration of endothelial cells, the remodeling of the extracellular matrix, and the formation of capillary tubules. VEGF production may be stimulated by interleukin 1- β , platelet-derived growth factor (PDGF), and transforming growth factor β . Additionally, the absence of VEGF has been linked to a degeneration of vasculature [9, 84].

The administration of VEGF either via direct administration or genetic transfer using plasmids or adenoviruses is currently being investigated as a therapy post-infarction. Early VEGF administration post-infarction reduced ventricular enlargement, increased capillary density, and reduced the size of the infarcted region [10, 85]. In addition, early administration decreased the rate of cardiomyocyte apoptosis in the border zone of the infarcted region [85]. In later phases of hypertrophy VEGF administration has been shown to aid in the recovery of contractile function, slow the process of ventricular dilatation, and reestablish near normal levels of developed pressure [10, 85].

2.6.6 Platelet-Derived Growth Factor (PDGF)

PDGF is an angiogenesis promoter that stimulates the formation of arterioles by promoting smooth muscle cell recruitment. PDGF is also partially responsible for the maturation of blood vessels because it stimulates smooth muscle cell recruitment to newly formed vessels. Early administration of PDGF using an injectable, biodegradable hydrogel increased the density of vessels containing smooth muscle cells, but did not increase capillary density. Also, ejection fraction was sustained and a smaller degree of LV dilatation was noted, demonstrating an improvement in left ventricular function [85, 86].

The use of PDGF and VEGF in combination has also been investigated because it is hypothesized that simultaneous treatment with these two proteins will offer the benefits of both proteins by stimulating vessel formation and subsequently enhancing vessel maturation. Hao et al. have shown that treatment with PDGF and VEGF increased capillary density, increased the density of smooth muscle containing vessels, and improved left ventricular function [85, 86].

3 Project Approach

A thorough literature review provided the team with the necessary information to begin formulating design solutions to achieve the project objectives.

3.1 Initial Hypothesis

The main objective of this project was to design a left ventricular (LV) restraint device that improves LV function through a combination of contractile support and delivery of a therapeutic agent. Current post-infarction drug therapies serve mainly to prevent further worsening of LV function. Furthermore, many of the researched LV restraint devices are passive devices that serve to limit LV enlargement, but they do not actively assist in contraction. Some regenerative agents are currently being investigated which may hold promise as therapies that can aid in the regeneration of functional myocardium. We hypothesized that a LV restraint device can be made of a contractile material that can contract 75 beats/min with a contraction duration of 0.3 seconds [21]. In addition, we further hypothesized that a regenerative agent can be incorporated into the device.

3.2 Revised Hypothesis

The main objective of this project was to design a left ventricular (LV) restraint device that improves LV function by providing structural support and delivering a therapeutic agent. Current post-infarction drug therapies serve mainly to prevent further worsening of LV function. Furthermore, many of the researched LV restraint devices serve to limit LV enlargement, but do not serve as a treatment for heart failure. We hypothesized that a LV restraint that incorporates growth factors, biomolecules, or stem cells and provides structural support can serve as a treatment for heart failure.

3.3 Assumptions

To simplify the scope of the project and provide for its feasibility, the following assumptions were made:

- Appropriate therapeutic agents delivered locally to the infarct can aid in the repair of myocardium.

- The above-mentioned therapeutic agents will not interfere with the functioning of the non-infarcted myocardium.
- Contractile assistance to the dysfunctional LV will result in beneficial unloading of the LV.

3.4 Specific Aims

The specific aims of the project that will achieve the above-mentioned objectives were the following:

- Characterize and test a suitable material to serve as the contractile component of the device.
- Select a suitable therapeutic agent based on the literature that will aid in the repair of the myocardium.
- Perform testing on the biomaterial to ensure that the therapeutic agent has been incorporated into the device.

4 Design

At the beginning of the project, the team received the following initial client statement: “Design and build a left ventricular restraint device that also serves as a therapeutic delivery system for patients suffering from advanced heart failure.”

In order to further develop this client statement, an engineering design process was followed. First, functions, objectives, and constraints were developed for the device based on input from both the team and the stakeholders. Based on the identified functions and objectives, a revised client statement was developed that clearly reflects the design problem being assessed. Next, design specifications were developed to identify performance levels for the design and establish specific values for attributes of the device. Finally, a list of design alternatives was generated and evaluated to select a final design for the device.

4.1 Stakeholders

With any design process, there are three main stakeholders involved – the designers, the users, and the clients. In the context of this project, the designers were the members of the MQP team and the client was the project advisor, Professor Glenn Gaudette. The designers and the client had all the input in defining the objectives and functions, generating design alternatives, and selecting a final design.

The users of the device will be cardiac surgeons. In addition to considering input from the client, the needs of the users were considered because if the device did not meet the users’ needs, the device would not be marketable.

4.2 Objectives, Constraints, and Functions

Based on input from the client as well as a thorough review of the literature, the desired and required objectives of the device were identified. The primary desired and required objectives with sub-objectives can be seen below, where required objectives are shown in bold and desired objectives are italicized:

OBJECTIVES (**Required**, *Desired*)

- 1. Have appropriate physical properties**
 - i. Have appropriate mechanical properties**
 - **Have the necessary tensile strength**
 - **Be fatigue resistant**
 - **Be wear resistant**
 - **Have the necessary elastic properties**
 - ii. Have appropriate contractile properties*
- 2. Clinically appropriate**
 - i. Require a simple implantation procedure**
 - *Be compatible with existing surgical techniques*
 - **Require a short recovery time for the patient**
- 3. Marketable**
 - i. Be minimal cost**
 - ii. Use available materials**
 - **Use materials that are easily accessible**
 - **Use economical materials**
 - iii. Be easily produced**

The first primary objective was for the device to have the appropriate physical properties, which included both mechanical properties and contractile properties. Without the necessary physical properties, the device will be unable to perform its intended functions and may undergo failure upon implantation. It was important for the device to have the necessary tensile strength because it must be able to withstand the ventricular pressure during systole without failing. Similarly, the device must also be elastic in order to withstand the strains that occur during diastolic filling. Fatigue and wear resistance were also important since the device will undergo cyclic loading as the heart beats. In addition to mechanical properties, if the device is intended to contract, it should have contractile properties similar to that of the native heart. Without the appropriate contractile properties, the device may induce arrhythmias, which could be fatal for the patient.

Along with having the appropriate physical properties, the device must also be clinically applicable. The device should be designed such that a simple implantation procedure is required with a short recovery time for the patient. It was desirable that the implantation procedure be compatible with current surgical techniques because this will not require cardiac surgeons to learn new surgical techniques or purchase new surgical tools, making it more likely that they will use the device.

The last primary objective was that the device be marketable. In order for it to be marketable, it should be of minimal cost and use available materials that are both economical and easily accessible. It was desirable that the device be easily produced because this will minimize the cost of manufacturing as well as the time it takes to manufacture.

In order to better understand the relative relationships of each objective and sub-objective, an objective tree was developed. A weighted objective tree was then created using only the first and second level objectives to understand the relative importance of each objective. In order to define the weight of each objective, a pairwise comparison chart was completed by the design team which can be found in Appendix B. The weighted objective tree that was developed can be seen below in Figure 6, where the number under each objective corresponds to the weight of the objective out of 100%.

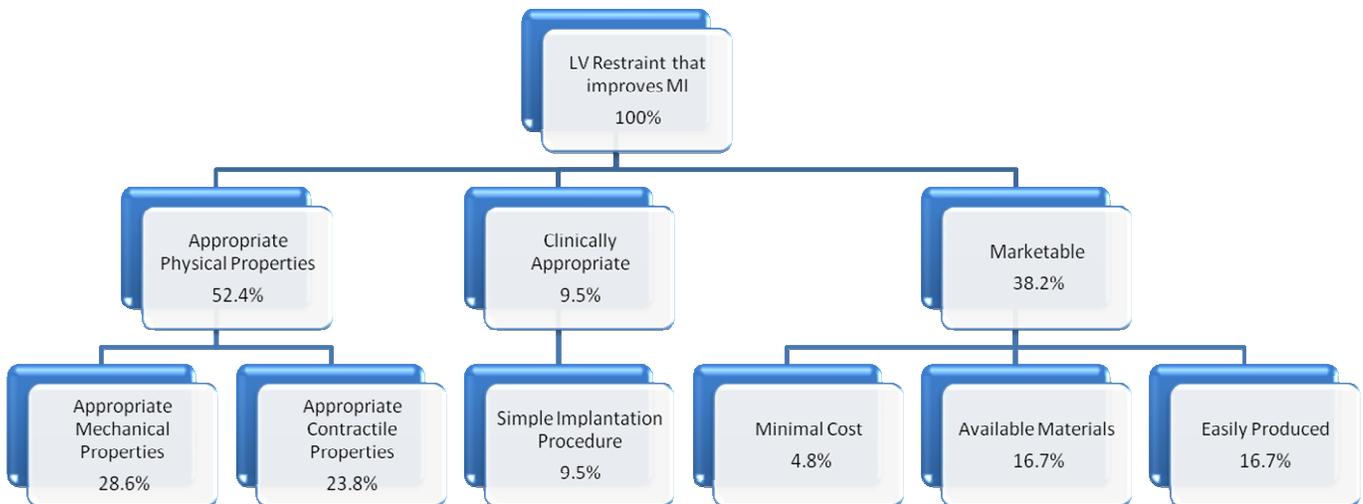


Figure 6: Weighted Objective Tree

In addition to objectives, the following constraints were developed in order to limit the design space:

CONSTRAINTS

1. Must be biocompatible
 - i. Must be non-toxic
 - ii. Must not induce an inflammatory response
 - iii. Must be hemocompatible
2. Design cost cannot exceed \$624
3. Must be completed by April 2008

It was imperative that the device be biocompatible so that it would not induce an undesirable immune or inflammatory response. Since the device may be in contact with blood, it should not induce thrombus formation or promote the adherence of proteins on the surface. Also, the design must be developed within the team's budget and time frame.

Based on the objectives and constraints identified, the team then developed a list of functions that the device must perform in order to meet the established requirements. The following primary functions were developed:

PRIMARY FUNCTIONS (**Required**, *Desired*)

- 1. Provide structural/mechanical support to prevent LV remodeling**
- 2. Provide contractile support to increase cardiac output*
- 3. Incorporate a therapeutic agent**

To improve the function of the infarcted area, the device should provide support to the left ventricle to prevent further remodeling. It was desirable that the device provide contractile support to the heart during contraction, thus increasing cardiac output and unloading the left ventricle. Finally, the device should incorporate a therapeutic agent that will aid in regeneration of the myocardium.

In addition to the list of primary functions described above, the following desired and undesired secondary functions were also identified:

DESIRED SECONDARY FUNCTIONS

1. Increase tissue perfusion
2. Decrease wall stresses
3. Decrease LV dilatation
4. Differentiation of cells to cardiomyocytes
5. Attachment of the device to the heart
6. Synchronous contraction with the heart
7. Increase ejection fraction

UNDESIRED SECONDARY FUNCTIONS

1. Produce degradation products
2. Obstruction of blood vessels
3. Induce changes in pH
4. Induce changes in action potential
5. Induce arrhythmias
6. Induce clotting
7. Induce foreign body response
8. Systemic delivery of therapeutic treatment
9. Weakening of existing myocardium

The first desired secondary function was that the device increase tissue perfusion as a result of unloading the left ventricle. The device should decrease wall stresses and LV dilatation as it prevents further LV remodeling. Should stem cells be incorporated into the device as a therapeutic agent, the cells must differentiate into cardiomyocytes. The device should be able to be attached directly to the heart. In the case of a contractile restraint, it was important that the device contract synchronously with the heart in order to prevent arrhythmias. Additionally, as the device provides contractile support to the heart, it should subsequently increase ejection fraction.

Along with the primary functions and desired secondary functions came a set of undesired secondary functions. The device should not produce harmful degradation

products that can damage the surrounding tissue or can travel through the blood stream thereby damaging other tissues. The placement or function of the device cannot obstruct the surrounding coronary arteries and other vessels because this will limit blood flow and decrease tissue perfusion. Changes in pH should not be induced by the device as this may cause changes in the performance of the heart. The device should not induce arrhythmias or changes in action potential because this will disrupt the natural contraction of the heart. The device should not induce blood clotting or a foreign body response. The therapeutic agent incorporated into the device should not be delivered systemically because this may interfere with the functions of the body's vital organs. Finally, the device should not cause a weakening of the existing myocardium since this will cause a decrease in cardiac output and ejection fraction because this will lead to a decrease in global heart function.

4.3 Revised Client Statement

With the objectives, constraints, and functions of the device defined, the following revised client statement was developed that clearly reflects the design problem:

“Myocardial infarction can lead to LV remodeling characterized by dilatation and wall thinning which can cause end-stage heart failure and eventually death. Despite the fact that there have been advances made in the treatment of heart failure, there are currently no devices on the market that simultaneously reverse the effects of LV remodeling and regenerate myocardium. Design, build, and test a device that will improve the function of the infarcted area by providing structural support to the failing heart while also incorporating a therapeutic agent. It is also desirable that the device provide contractile assistance to the heart to aid in the recovery of global heart function. The device would be intended for patients with late stage III and early stage IV heart failure.”

4.4 Design Specifications

Once the objectives and functions of the device were identified, a set of design specifications was developed to identify performance levels for the design. The design

specifications for the device are listed below in Table 3 and calculations are further explained in Appendices C-D.

Table 3: Design Specifications

Design Specification	Description
Wall tension	The device must be able to withstand a wall tension of 1.37 N/mm [87]
Strain	The device must be able to withstand a strain of 15-20% [88]
Fatigue	The device must have a wall tension and strain that is not statistically significant after 1,000 cycles [87, 89]
Contraction rate (if applicable)	The device should be able to contract 75 times/min [21]
Contraction duration (if applicable)	The duration of contraction should not be more than 0.3 sec [21]

To ensure that the device has the appropriate mechanical properties, it should be able to withstand the specified wall tension, strain, and fatigue strength. The calculated wall tension was based on the wall stress of patients with high ventricular pressure and incorporated a safety factor of 5. The wall tension is a normalized function of wall stress because it is divided by the thickness of the wall. Since the device would be intended for patients with class III or IV heart failure, it should withstand cyclic loading for a minimum of 2 years, which corresponds to 78,840,000 cycles based on an average heart beat of 75 beats/min [21, 87, 89]. Since this number of cycles was not reasonable to test, the wall tension and strain after 1,000 cycles should be tested.

In addition to mechanical properties, a contractile device must also have the appropriate contractile properties to ensure that it does not induce arrhythmias. If the device was designed to contract, it should contract at a rate equivalent to a normal heart rate, which is 75 beats/min, and the contraction duration should be equivalent to the duration of ventricular contraction, which is 0.3 sec [21].

4.5 Developing Design Alternatives

In order to begin identifying potential designs, a morphological chart, which can be found in Table 4, was created to effectively visualize the size of the design space.

Table 4: Morphological Chart

<u>FUNCTIONS</u>	<u>MEANS</u>			
<i>Provide structural/mechanical support</i>	Cardiac patch	Wrap restraint	Nut & bolt restraint	Band
<i>Incorporate a therapeutic agent</i>	Cells	Drugs	Growth factors	Proteins
<i>Provide contractile support (desired)</i>	Contractile biomaterial	LVAD	-----	-----

Subsequently, a functions-means tree was created to graphically represent the means identified for each function in the morphological chart. The functions-means tree can be seen in Figure 7 on the next page.

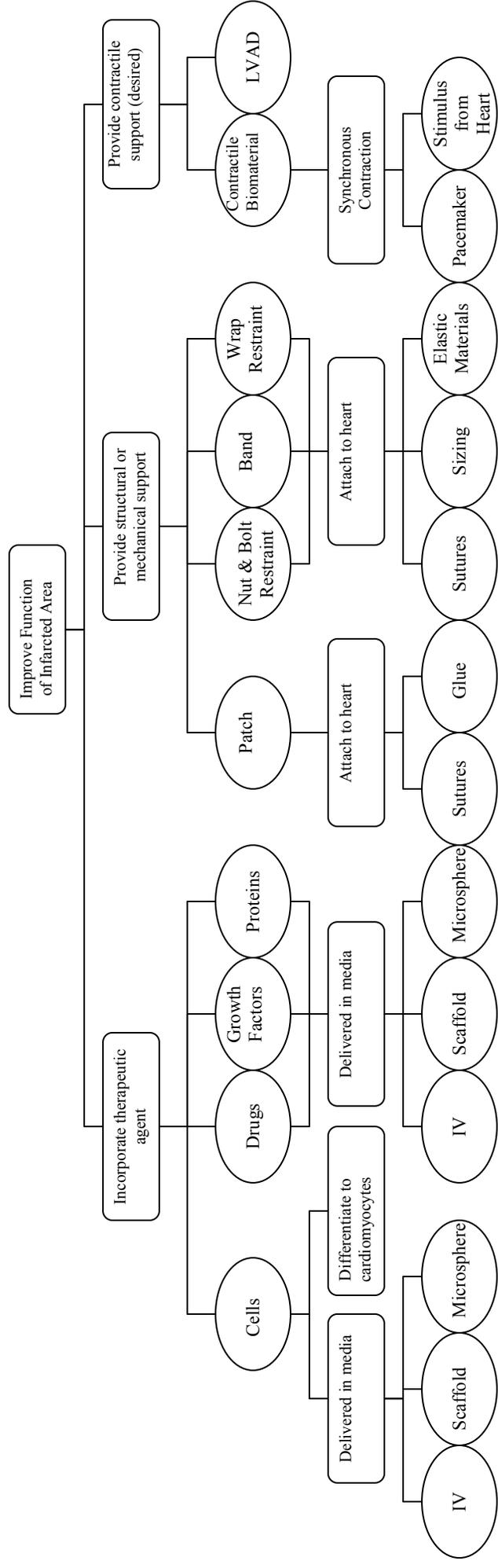


Figure 7: Functions-Means Tree

The means identified for each function were combined to create a number of different design alternatives. For example, the following design alternatives were generated:

1. Cardiac patch, contractile biomaterial, cells
2. Cardiac patch, contractile biomaterial, growth factors
3. Wrap restraint, contractile biomaterial, drugs
4. Band restraint, contractile biomaterial, growth factors
5. Nut & bolt restraint, contractile biomaterial, proteins
6. Nut & bolt restraint, LVAD, proteins

The design alternatives that were generated can be found in Appendix E.

4.6 Evaluating Design Alternatives

The design alternatives developed in the previous section were evaluated using an evaluation matrix. The designs were evaluated based on the constraints to ensure that the design met the specified constraint. The design alternatives were evaluated to determine how well each design satisfied the established objectives. The designs were rated on a scale of 0 to 5, where 0 indicated that the design would not meet the objective at all and 5 indicated the design would completely meet the objective. The weight of each objective was obtained from the pairwise comparison chart found in Appendix B. To determine the total score of each design, the weight for each objective was multiplied by the score for the objective, then each rating was added to obtain the combined total. The evaluation matrix can be found in Appendix F along with an explanation of the basis for comparison that was used and the scores assigned to each design. The total score for each design can be seen in Table 5 below.

Table 5: Evaluation of Design Alternatives

Design	Total score
Contractile biomaterial, wrap restraint, and cells or growth factors	2.803
Contractile biomaterial, nut & bolt restraint, and cells or growth factors on end caps	2.979
Contractile biomaterial, hoops or strips, cells or growth factors	2.479
Contractile biomaterial, patch, cells or growth factors	2.717
Passive wrap restraint with cells or growth factors	2.575
Passive band restraint with cells or growth factors	2.432
Passive patch with cells or growth factors	2.099

The design alternative with the highest score was the nut and bolt restraint using a contractile biomaterial with cells or growth factors on the end caps. The major advantage of this design was that the propagation of the contraction would not need to be precisely controlled because the contractile biomaterial would not be wrapped around a large area of the heart. This design could also be manufactured more easily due to its relatively simple design, in comparison to the wrap restraint, which would be more difficult to manufacture. Additionally, the delivery of cells or growth factors could be localized to the infarct by placing them only on the end pads of the device. This design alternative also has some disadvantages including the difficulty of implantation as the device must penetrate the ventricular wall and the limited mechanical support.

4.7 Final Design with Polyaniline

With the nut and bolt restraint design, the device consisted of two parts – the splint and the end pads. Polyaniline (PANI) was chosen for the contractile biomaterial

component because it was the most extensively researched contractile biomaterial and the most promising for biological applications. Studies have demonstrated that PANI can be biocompatible and potentially biodegradable. Also, the properties of PANI including the electrical conductivity and stability can be controlled by altering the manufacturing processes used. PANI can be used for the splint as well as the end caps, if it is able to sufficiently hold growth factors and/or cells. If the entire device was made of PANI, this would minimize any stress concentrations and mismatch of mechanical properties at the interface between the splint and end pads. However, if PANI is unable to hold growth factors or cells, other materials such as collagen sponges, PEUU, ECM, and fibrin glue, which can hold and deliver the therapeutic agent, could possibly be used for the end pads.

A detailed drawing of the device can be seen below in Figure 8. The splint would penetrate through the myocardium without passing through the septum. The epicardial pads would be located on the epicardium and would attach to the ends of the splint. The contractile biomaterial could be stimulated by a stimulus unit, such as a pacemaker, which would ensure that the device contracted simultaneously with the native heart to prevent arrhythmias.

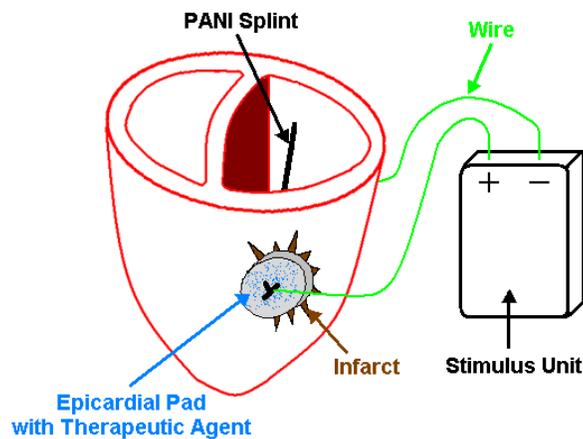


Figure 8: Final Design with Polyaniline

4.8 Re-evaluating Design Alternatives

As described in the Results section (Sections 6.1 and 6.2), PANI proved to be very difficult to manufacture and could not be electrically stimulated. It was unlikely that PANI would meet the design objectives, specifically because it could not be easily produced and would likely not have the appropriate mechanical or contractile properties. Thus, the designs were re-evaluated to select a design that did not incorporate a

contractile biomaterial. From Appendix F the design alternative that scored the highest and did not incorporate a contractile biomaterial was the passive wrap restraint that incorporated cells or growth factors. The major advantage of this design was that it can provide the largest degree of mechanical support because it would enclose the entire heart, preventing hypertrophy in all areas. Also, it would require a relatively simple implantation procedure because it would be able to be secured by wrapping it around the heart. The major disadvantage of the design was that it may be difficult to produce due to the intricacies of the complex mesh design. Also, it was not as cost efficient as other designs because it required the use of more material.

4.9 Final Design with Microthreads

For the final design, soluble collagen microthreads were selected as the major component of the device. As described in Section 2.4.3, collagen microthreads offer the advantage of high mechanical strength and can be manufactured to hold proteins, growth factors, or cells. Collagen microthreads are biocompatible and have been investigated extensively for use in repair and regeneration of ligaments and tendons [19]. In addition a therapeutic agent was incorporated into the microthreads to promote angiogenesis around the infarcted region. Platelet-derived growth factor (PDGF) was selected due to its availability because the group was unable to purchase other growth factors, proteins, or cells due to budget constraints.

A detailed drawing of the final design can be seen in Figure 9. In order to ensure that the device is as cost effective as possible, PDGF could only be incorporated into the microthreads placed over the infarcted region. The surgeon would be able to position the wrap such that the area with PDGF is placed over the desired region. Additionally, local delivery of growth factors would prevent any adverse consequences that may occur in other areas of the heart.

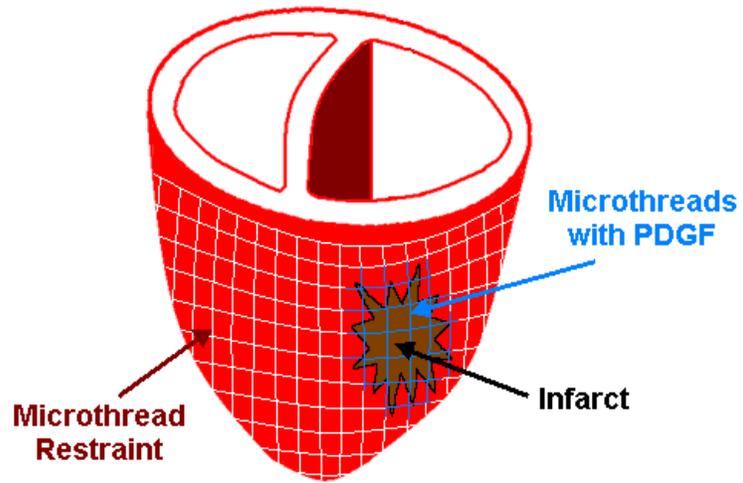


Figure 9: Final Design with Microthreads

5 Methodology

This section describes the methodology used to manufacture and test PANI and collagen microthreads. These methods were used to determine and test a suitable material for use in a LV restraint device.

5.1 Cell Culture

Human mesenchymal stem cells (HMSCs) and rat aortic smooth muscle cells (SMCs) were grown using cell culture techniques. Biocompatibility testing of polyaniline was planned using HMSCs and cell migration assays on microthreads were performed using SMCs. HMSCs were between passages 9 and 14. SMCs were obtained from Professor Rolle's lab and the passages were unknown. Both types of cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS). The cells were stored in T-75 culture flasks in a 37°C, 5% CO₂ incubator. The cell media was replaced every 3 to 4 days for HMSCs and every 2 days for SMCs.

When splitting cells due to over confluence 0.25% trypsin was used to detach cells. In order to trypsinize the cells the media was first aspirated. A rinse of 10mL Hank's Balance or DMEM solution was used to wash the flask. Afterwards, 1mL of 0.25% trypsin was added and the flask was put under a microscope. The cells were then watched until they were visibly detached from the bottom of the flask (approximately 2-3 minutes for HMSCs and approximately 7 minutes for SMCs). 10mL of media was then added and pipetted into a 20mL centrifuge tube. The cells were then spun down at 600RPMs for 3 minutes. The media was aspirated off and fresh media was added while agitating the cells. The desired amount of cells was then put into T-75 culture flasks and additional media was added to a total volume of 10mL.

5.2 Manufacturing Polyaniline Films

PANI films were produced by solvent casting solutions of PANI dissolved in NMP onto glass surfaces. Protocols for producing PANI films were developed based on literature and information gathered by material science specialists, the best of which was determined through trial and error. Detailed protocols for all methods used to produce

PANI films can be found in Appendix H, including the methods used to produce optimal films.

To produce optimal films, 4% wt/vol of PANI was mixed in NMP solvent overnight. The solution was then cast on to glass tissue culture discs or glass slides by placing 35 μ L on each disc or 300 μ L on each slide. The solutions must be cast onto a glass surface because NMP, which is an organic solvent, will dissolve plastic surfaces. In order to ensure that the NMP solvent diffused uniformly out of the solution, the slides and/or discs were placed in a vacuum chamber overnight with a light vacuum applied. The chamber was cracked open slightly to allow airflow to circulate throughout, as shown in Figure 10 below, where the red arrow points to a slide of PANI solution. When the films were dry, they were washed in phosphate buffer solution (PBS) three times for 5 minutes and doped in 1M HCl for 15 minutes.



Figure 10: Set Up for Manufacturing PANI Films

5.3 Preliminary Testing of Contractile Properties of Polyaniline

Once electroactive PANI films were produced, preliminary testing was conducted to determine their contractile properties, as detailed in Appendix I. The experimental setup for field stimulation is shown in Figure 11, where a PANI strip (about 1 mm wide by 1 cm long) was placed in PBS solution in line with the wires as shown in Figure 12.

Three PANI strips were tested. Using field stimulation, a single pulse was applied at 1V and the voltage was increased in increments of 0.5V until the voltage reached 10V. As described in Section 6.2, no visual contraction was observed. Point stimulation of PANI strips was also tested, where the electrodes were directly connected to each end of the PANI strip. Although further testing of PANI was not performed, Appendix I contains protocols for testing the contraction length, contraction duration, and contraction force.

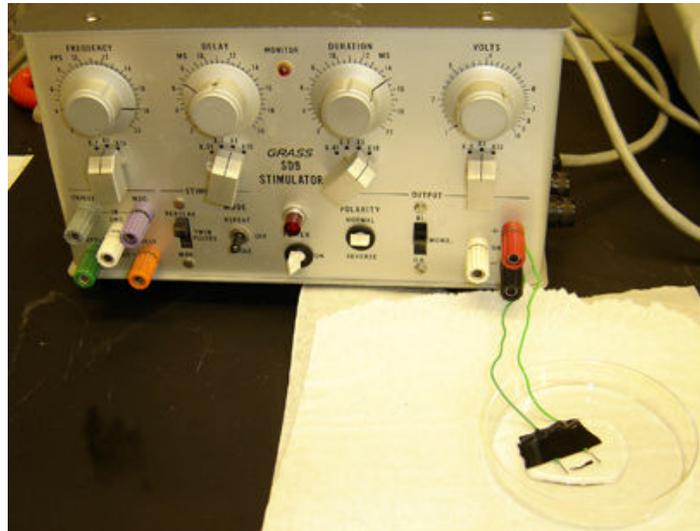


Figure 11: Contractile Testing Set Up

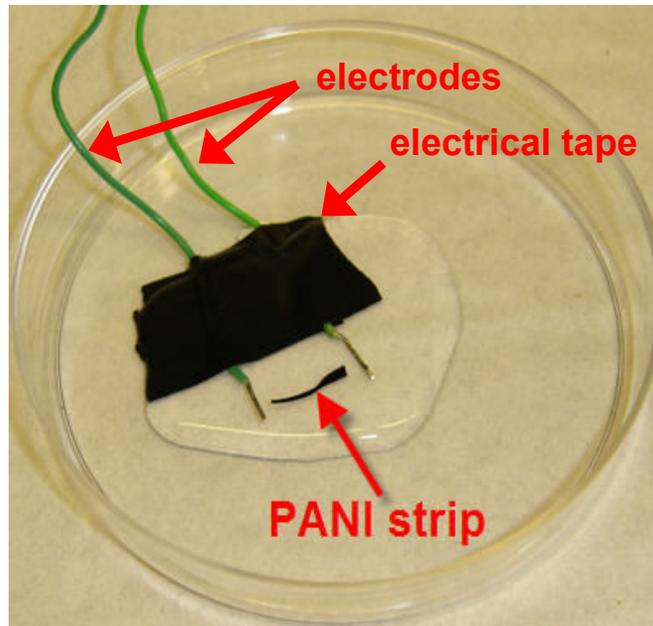


Figure 12: Close-up of PANI Strip During Contractile Testing

5.4 Manufacturing Microthreads

Soluble type I collagen fibers were obtained from rat tails and were processed to produce a collagen solution. Prior to extrusion, the collagen solution was dissolved in a rotating vessel in 5mM HCl at 4°C at a final concentration of 10mg/mL. The solution was then spun in a centrifuge to remove any air bubbles [19].

The water bath was set to 37°C and a glass tray filled with 500mL of fiber formation buffer (FFB) was placed into the water bath. A syringe was used to take up 3mL of the collagen solution and polyethylene (PE) tubing with an inner diameter of 0.86 mm was attached to the syringe. The extrusion pump was then set to extrude the collagen at a rate of 0.3mL/min. The syringe was connected to the pump, the pump was turned on, and the collagen was carefully watched as it passed through the tubing, as shown in Figure 13.

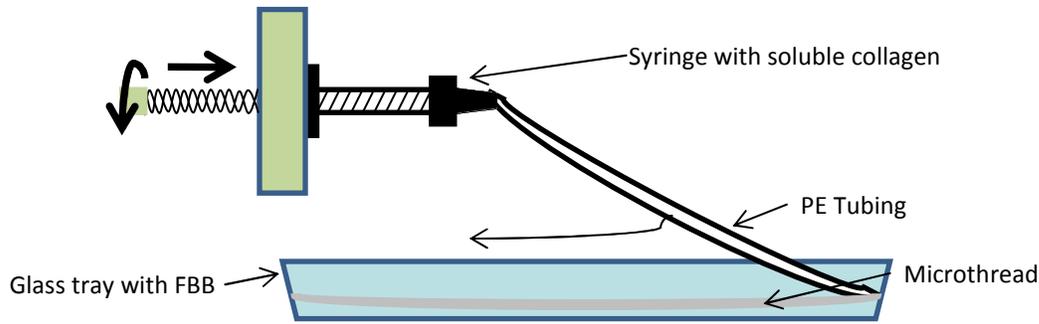


Figure 13: Set Up for Manufacturing Microthreads

When the collagen came through the end of the tubing, the tubing was slowly dragged along the bottom of the glass tray. After the desired number of microthreads was extruded, saran wrap was placed over the glass tray and the microthreads were left in FIB for 24 hours. After 24 hours, the threads were moved to a bath of fiber incubation buffer (FIB) for 24 hours. Afterwards, the microthreads were taken out of the FIB bath and hung on a cardboard box using forceps to dry overnight. Multiple soluble collagen microthreads were obtained that were about 20cm in length and about 50 μ m in diameter [19].

5.5 Mechanical Properties of Microthread Mesh

The microthread mesh will be placed over the heart wall to provide structural support. As a result, a composite material is created which consists of the heart wall and the microthread mesh. In order to provide structural support, the resulting composite material should have a higher stiffness than the native heart wall. Mechanics equations for composite materials were used to determine the number of microthreads that would be required for the stiffness of the composite to be 10% greater than the stiffness of a hypertrophied right ventricular heart wall. The stiffness of the composite material was taken to be 10% greater than the native heart wall because it is expected that a small increase in stiffness would provide structural support to prevent dilatation, but at the same time would not interfere greatly with the contraction of the healthy myocardium. A mechanics of materials approach was used with the assumption that the microthreads and the heart wall undergo the same strain [90]. This approach is referred to as a constant strain model and can be viewed as a model with two springs in parallel, as seen in Figure 14.

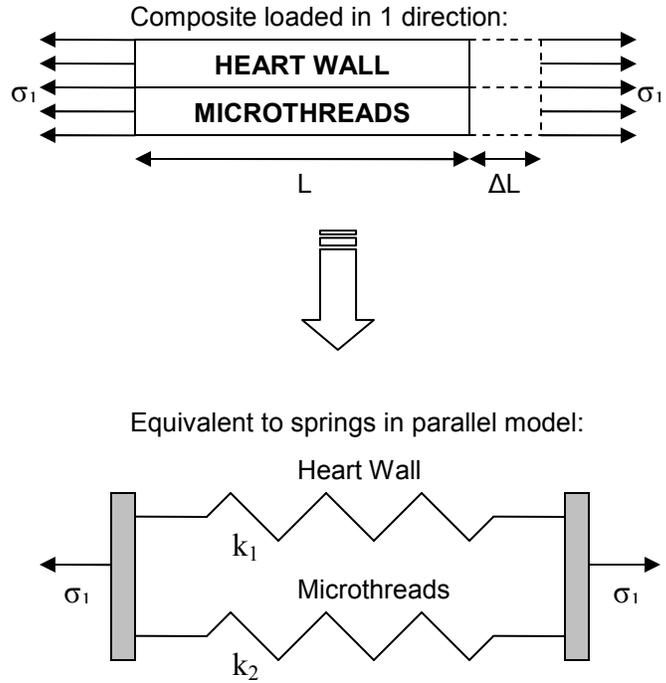


Figure 14: Mechanical Model of Microthreads-Heart Wall Composite

In order to determine the number of microthreads required to increase the stiffness of the composite material the rule of mixtures for composite materials was applied. The rule of mixtures equation relates each individual material's contribution to the total composite stiffness:

$$E_C = E_1 \bar{V}_1 + E_2 \bar{V}_2 + E_3 \bar{V}_3 + \dots$$

In this equation, E_C is the stiffness of the composite material, $\bar{V}_1, \bar{V}_2, \bar{V}_3, \dots$ are the volume fractions of each material, and E_1, E_2, E_3, \dots are the stiffnesses of each material. Each material's contribution to the composite stiffness is dependent upon the stiffness of the material and the fraction of the composite volume that the material occupies [90].

The following assumptions were made to simplify the calculations:

- The heart is a hollow sphere
- All microthreads have the same properties (length, cross-sectional area, and stiffness)
- The stiffness of the right and left ventricles is the same

- The heart wall is linear elastic
- The stiffness of the heart wall is constant in the direction of the fibers and is constant in the direction perpendicular to the fibers.
- Fibers in the heart wall run vertically from apex to base

By using the rule of mixtures constant strain model equation, the number of microthreads required to increase the composite stiffness by 10% was calculated.

Detailed calculations can be found in Appendix K.

Once the number of microthreads was calculated, basic mechanics equations were used to determine if the structural support provided by the mesh would decrease the dilatation of the heart wall. The following formula was used to calculate the strain, or dilatation, of the heart wall:

$$\varepsilon = \frac{T \times t}{E \times A}$$

In this formula, ε = strain, T = wall tension, t = wall thickness, E = stiffness, and A = cross-sectional area. In order for the mesh restraint to decrease the dilatation of the heart wall, the stiffness multiplied by the area of the heart wall-microthread composite (product of EA) should be higher than that of the heart wall alone. This quantity was calculated for both the composite and heart wall alone in the fiber and cross fiber directions. Detailed calculations can be found in Appendix K.

5.6 Cell Migration Assay

A cell migration assay was developed in order to determine whether platelet-derived growth factor (PDGF) would affect the migration rate of cells to the microthread. As described in Section 2.6.6, PDGF promotes smooth muscle cell (SMC) recruitment to newly formed blood vessels, which is an important part of angiogenesis [9, 86]. Therefore the migration rate of SMCs should be greater with microthreads coated in PDGF.

Polydimethylsiloxane (PDMS) was used as a blocker between the microthread and SMCs. SMCs were plated on half of the petri dish and the microthread was placed on the other side with the PDMS block in between. Once the cells had plated, the PDMS block was removed and the cells were allowed to migrate to the other half of the petri

dish. The migration and proliferation of SMCs was observed with PDGF coated microthreads and control samples (microthreads not coated with PDGF and petri dishes without microthreads). The setup for the cell migration assay can be seen in Figure 15.

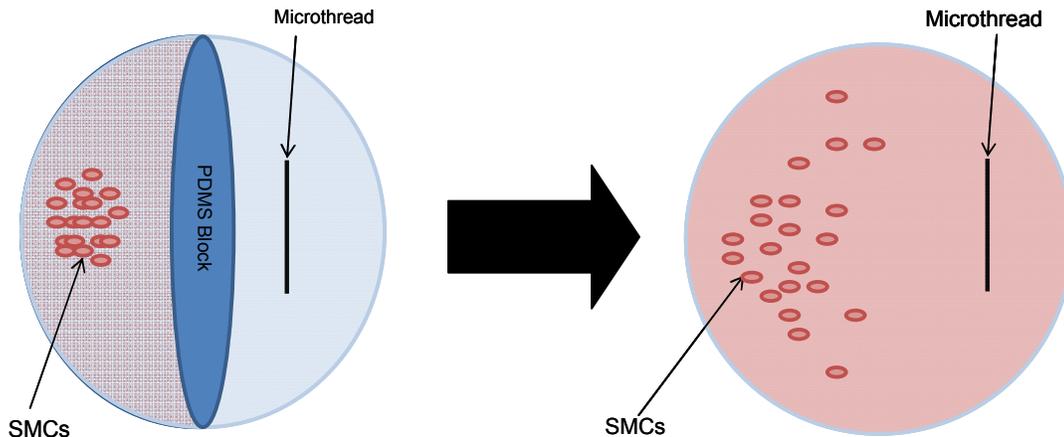


Figure 15: Set Up for Cell Migration Assay

Liquid polydimethylsiloxane (PDMS) was made by mixing silicone elastomer and PDMS curing agent in a 10:1 ratio and pouring it into tissue culture plates. The PDMS filled plates were placed in a vacuum chamber to remove air bubbles that may have formed and was then placed in an oven set to 50°C for approximately 2 hours. Upon hardening, the PDMS was removed from the petri dish. The samples were cut in half with a scalpel. Subsequently the ends of the half circles were removed to allow for placement of the microthreads on the other end of the petri dish. The PDMS blocks were then sterilized in an autoclave.

All microthreads were sterilized in 70% ethanol for 24 hours and were then washed in PBS three times for 5 minutes per wash. PDGF coated microthreads were placed in a PDGF solution (10ng/mL PDGF- $\beta\beta$ in DMEM +20%FBS + 1% pen/strep) for 24 hours and subsequently washed in PBS three times for 5 minutes per wash. The microthreads were attached to one side of the petri dish (diameter = 100mm) using sterile silicone glue.

Under the sterile hood, the sterile PDMS blocks were placed in the petri dishes. Rat aortic smooth muscle cells (SMCs) were trypsinized and counted using a

hemocytometer. SMCs were cultured on the side of the plate opposite the microthread, with 50,000 cells cultured on each plate. The cells were allowed to plate down for 24 hours and the media was aspirated to remove any floating cells. The PDMS block was removed and new media was added to the dish. The plates were visualized and photographed at regular intervals under an inverted light microscope to observe the differences in migration and proliferation of cells for each sample. Grid lines were drawn on the petri dish cover as shown in Figure 16. Pictures were taken at the intersections of the grid lines to produce a total of 21 pictures per dish. The pictures were then stitched together using Adobe Photoshop CS2.

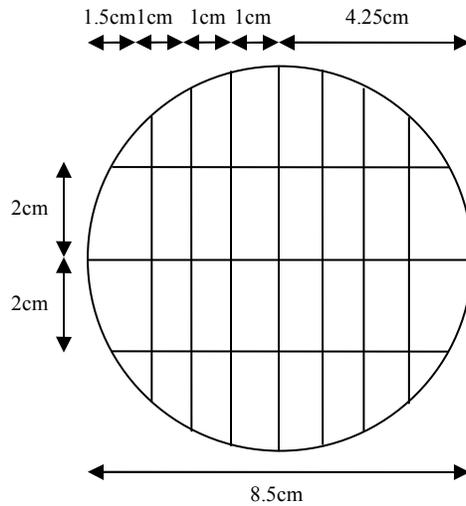


Figure 16: Cell Migration Assay Photograph Grid Lines

6 Results

This section describes the results obtained from the procedures in the Methodology section.

6.1 Manufacturing Polyaniline Films

The initial protocol for producing PANI films (obtained from Dr. Gouma's laboratory at Stony Brook University, found in Appendix A) involved making a solution of 12% wt/vol PANI in NMP and then casting this solution onto glass slides. The resulting films were cracked in certain places, while other areas were very thin and stuck to the slides, as seen in Figure 17 below. The slivers of PANI could easily be removed from the slide simply by tipping the slide, allowing the pieces to fall off. This type of film was not desirable because it was not a continuous, uniform film.



Figure 17: 12% wt/vol PANI Film That is Highly Cracked

After reviewing the literature, a solution of 4% wt/vol of PANI in NMP was made and compared to the films obtained using a 12 % wt/vol solution [91, 92]. Furthermore, the films were cast on glass tissue culture discs with 35 μ L of solution on each disc. The slides obtained using a 4% wt/vol solution of PANI were more continuous, more uniform, and were not cracked as seen in Figure 18. However these PANI films were very thin and could not be removed from the slide.

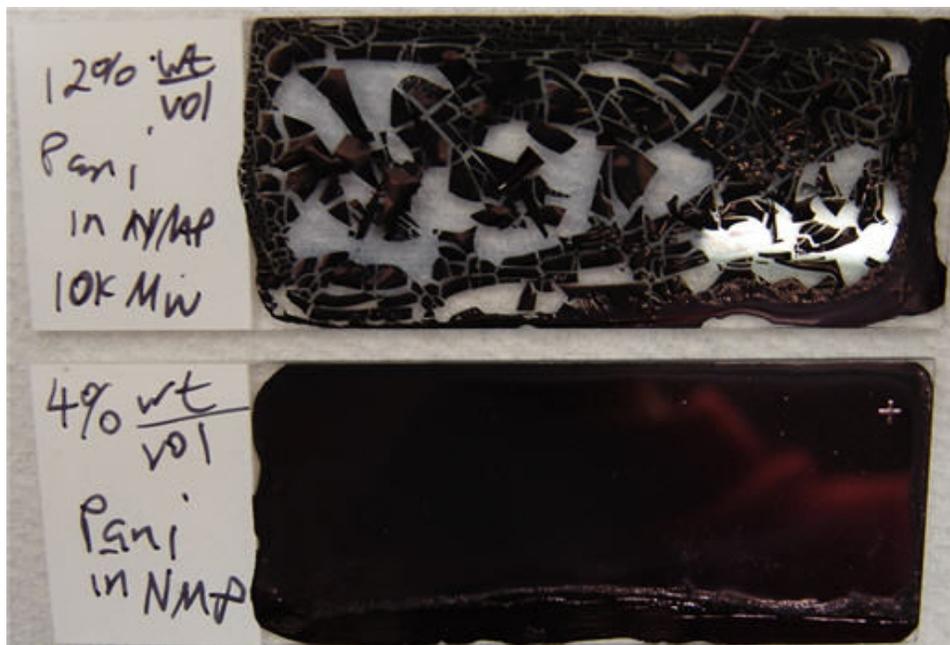


Figure 18: Slide Obtained Using 12% wt/vol Solution of PANI (top) and Slide Obtained Using 4% wt/vol Solution of PANI (bottom)

After many iterations it was determined that placing 300 μL on each glass slide produced the most uniform and minimally cracked film. A vacuum chamber was used as a means of obtaining a more uniform film because it allowed for more even diffusion of NMP. Overall, the films dried in the vacuum chamber were more uniform and less cracked than those that dried in the fume hood.

The uniform PANI films that were obtained on the discs and slides were initially doped in 1 M HCl for 15 minutes. Within 1 minute of doping, the films were observed to crack, split, and fall off the slide as shown in Figure 19. The top slide in Figure 19 is from the same batch of PANI solution but was not doped in HCl. However, when slivers of PANI were doped (obtained from batches of cracked PANI films when solvent casting 12% wt/vol solutions), the slivers were able to withstand the doping process.

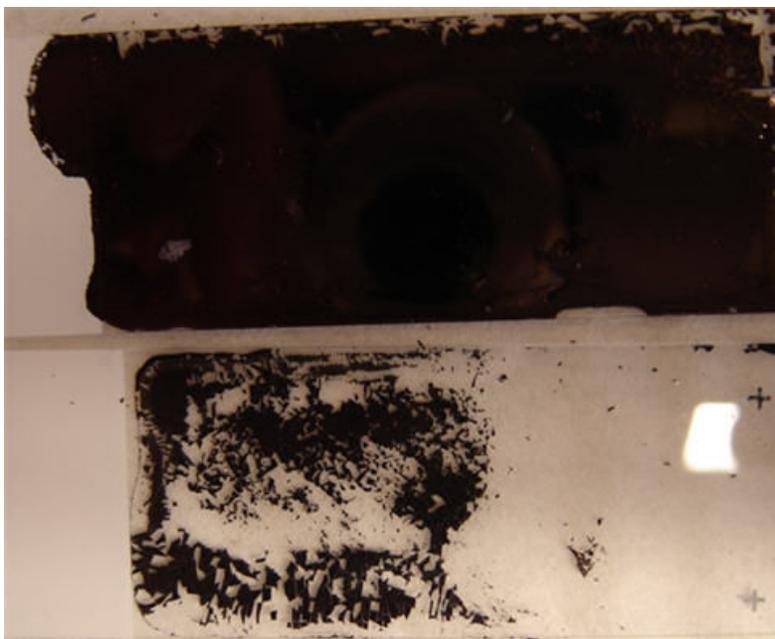


Figure 19: PANI Film Exposed to HCl (bottom) Compared to Control (top)

In addition to doping in HCl, PANI coated tissue culture discs were washed in PBS to see if the films would crack when exposed to PBS. The discs were found to crack upon washing in PBS and with additional washes in PBS, the amount of cracking increased. Three glass discs of 4 % wt/vol PANI that were washed in PBS for 5 minutes are shown in Figure 20. The disc in well 1 was not washed in PBS, the disc in well 3 was washed once in PBS, and the disc in well 2 was washed twice in PBS.

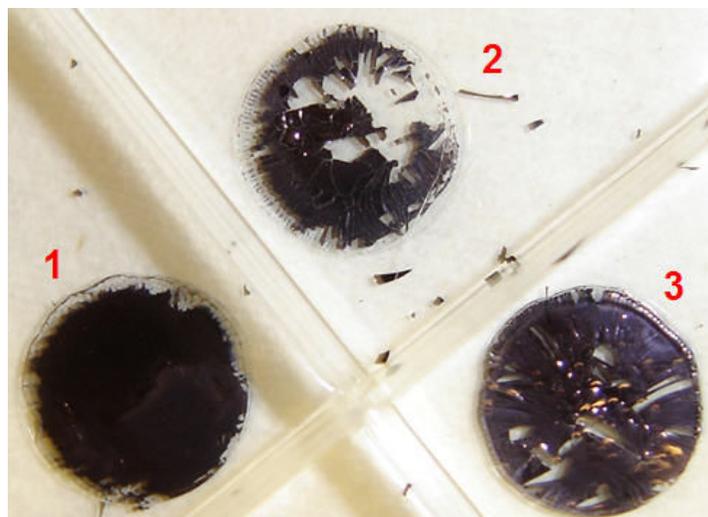


Figure 20: PANI Films Exposed to PBS Washes. (Disc 1 was not washed, disc 3 was exposed to one wash in PBS, and disc 2 was exposed to two washes.)

In order to potentially prevent the cracking that was observed when doping the PANI films, a higher molecular weight polymer was used (65 kD, in comparison to 10kD) to make 4 % wt/vol PANI solutions. Upon mixing the PANI in NMP, it was observed that the PANI did not dissolve completely in the solvent. Subsequently, the dissolved portion of the solution was pipetted off and used to cast PANI films. However, the first and only batch of high molecular weight PANI did not form well. As can be seen in Figure 21, both PANI films have surface irregularities and roughness in comparison to the more uniform films of the lower molecular weight (10kD) PANI.

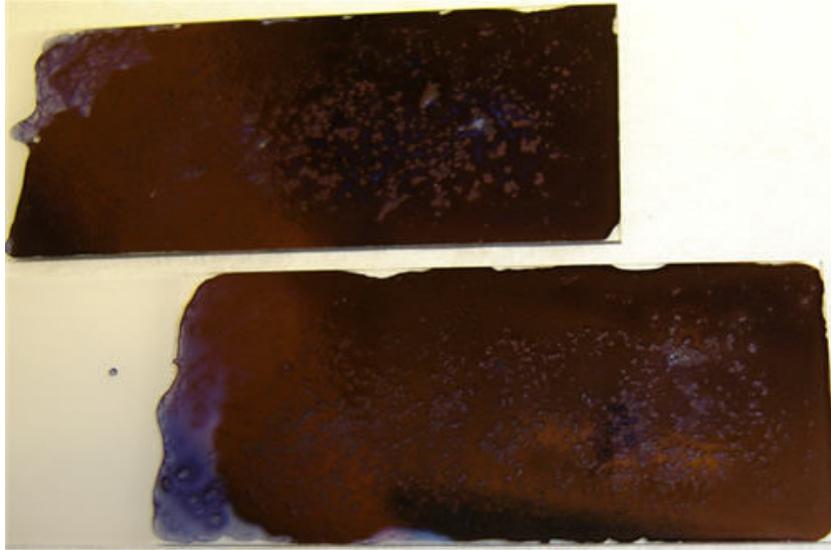


Figure 21: PANI Films of High Molecular Weight Polymer

6.2 Preliminary Testing of Contractile Properties of Polyaniline

No visible contraction was observed in any of the three PANI strips that were tested. Neither field nor point stimulation produced any macroscopic contraction of the doped slivers from a voltage range of 0 to 10 V.

6.3 Mechanical Properties of Microthread Mesh

To create a composite material with a stiffness 10% greater than the heart wall, the number of microthreads was determined in both the fiber and cross fiber directions using the rule of mixtures calculations described in Section 5.5. In the direction of the fibers, the number of microthreads required was found to be 21.8 and in the cross fiber direction, the number of microthreads required was found to be 11.5.

In Section 5.5, basic mechanics equations were used to show that the microthread mesh can prevent dilatation of the heart. As the product of the elastic modulus and cross-sectional area (EA) increased, the dilatation of the heart wall would be reduced. In the fiber direction, the product of EA for the heart wall was 495.1 N and with the mesh restraint, the product was 544.6 N. In the cross fiber direction, the product of EA for the heart wall was 137.5 N and with the mesh restraint, the product was 151.3 N. Since the product of EA increased with the addition of the mesh restraint, the basic mechanics

equation indicated that the mesh would reduce the dilatation of the heart wall. The results for these calculations are summarized in Table 6 below.

Table 6: Summary of Calculations of Mechanical Properties

	Number of microthreads	EA product for heart wall [N]	EA for heart wall with mesh restraint [N]
Fiber direction	21.8	495.1	544.6
Cross fiber direction	11.5	137.5	151.3

6.4 Cell Migration Assay

As described in Section 5.6, a cell migration assay was performed to observe the migration and proliferation of smooth muscle cells (SMCs) with PDGF coated microthreads, control microthreads, and control petri dishes without microthreads. SMCs were cultured on one side of the petri dish and the microthread was attached to the other side, with a PDMS block separating the two sides. After allowing the cells to plate for 24 hours, the PDMS block was removed. The experiment was repeated three times to verify the reproducibility of the results.

On the first trial, the cells were not counted properly prior to plating the cells on the dish. Seven days after being plated, SMCs were found surrounding the PDGF coated microthread, as seen in Figure 22. Cells were seen directly next to the microthread, with a few cells branching out toward the microthread. With the control microthread, very few cells were seen near the microthread. With the control petri dish (no microthread), a few cells were scattered around the black marker, where the microthread would have been. Overall, there were many more cells surrounding the PDGF coated microthread in comparison to the control samples. Eight days after being plated, sheets of cells began lifting off the petri dish and folding over because the cells had overgrown the dish. At this point, further testing could not be performed with these samples.

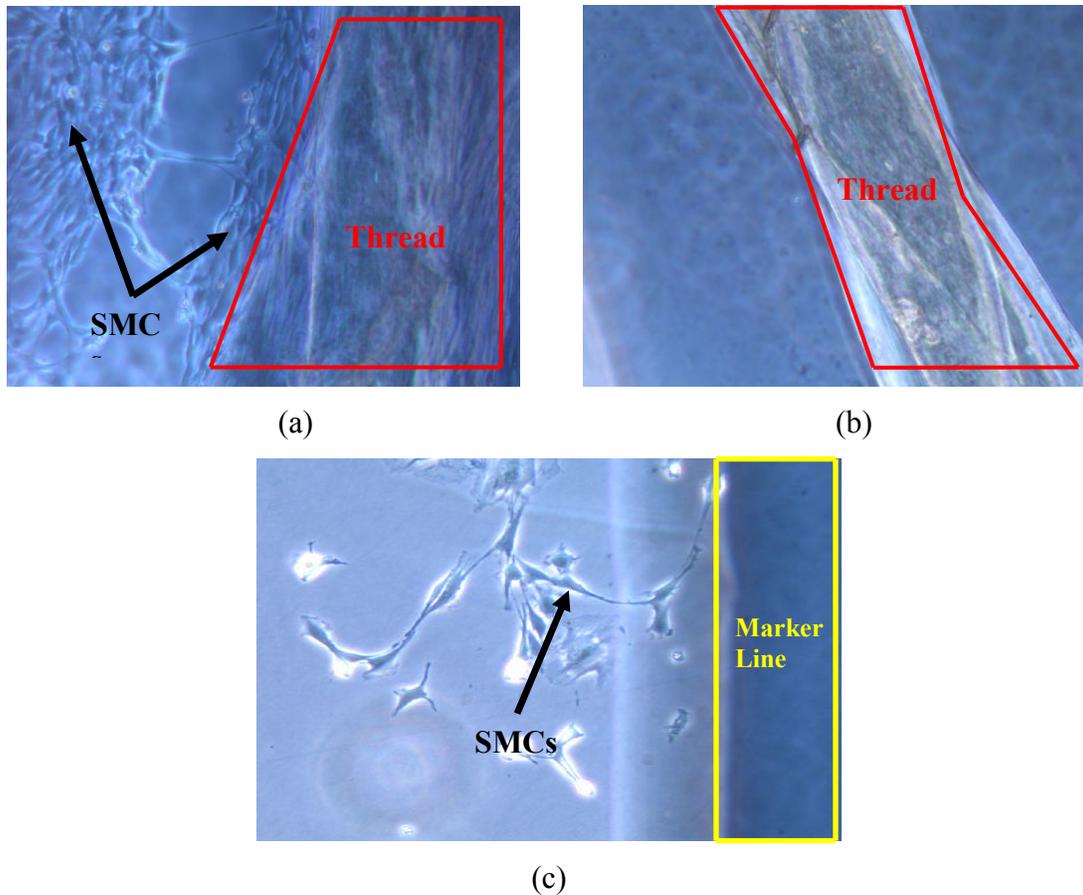


Figure 22: Microthreads Seven Days After Plating Taken at 200X Magnification (a) PDGF Microthread – Note many smooth muscle cells (SMCs) near microthread (b) Control Microthread – Note no SMCs near microthread (c) Control Dish with No Microthread – Note some SMCs near marker line

In the second trial, 50,000 SMCs were plated per dish. Three days after being plated, cells were observed around the PDGF coated microthread. However, four days after being plated, the cells had overgrown the dish. This trial was discontinued because many of the cells were aspirated from the dish when the media was changed.

In the third trial, two samples were used per group and were labeled as A and B. A smaller number of cells were plated on each petri dish (4,700 cells per dish) due to the low number of viable SMCs that were available. After being plated, the PDMS block was removed after 48 hours, allowing the cells to proliferate in order to increase the cell count on each dish. Grid lines were marked on each dish and 21 pictures were taken each day for all dishes, as described in Appendix L. The 21 pictures were then stitched together using Adobe Photoshop CS2. A characteristic picture of the dishes at the initial time

point can be seen in Figure 23, where the halfway point marks where the PDMS block was located.

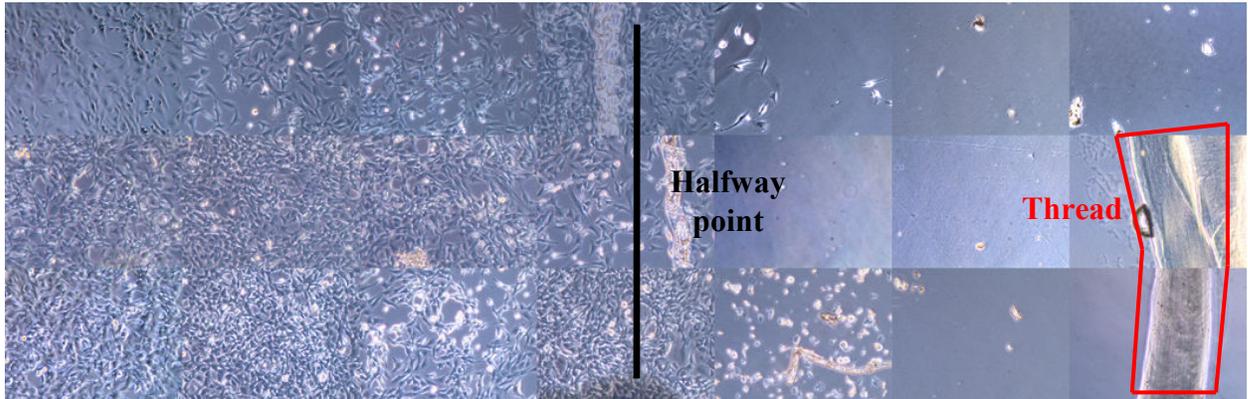


Figure 23: Stitched Picture of Petri Dish at Initial Time Point (Control thread A). 21 individual pictures from various points on the petri dish were stitched together using Adobe Photoshop CS2.

Seven days after plating, SMCs were observed surrounding the PDGF coated microthread A. However, PDGF coated microthread B had very few cells surrounding it in comparison to PDGF coated microthread A and the control dishes without microthreads. The control microthread dishes had very few cells surrounding the microthreads, while the control dishes without microthreads had some cells scattered around the black marker where the microthread would have been. These results can be seen in Figure 24 below.

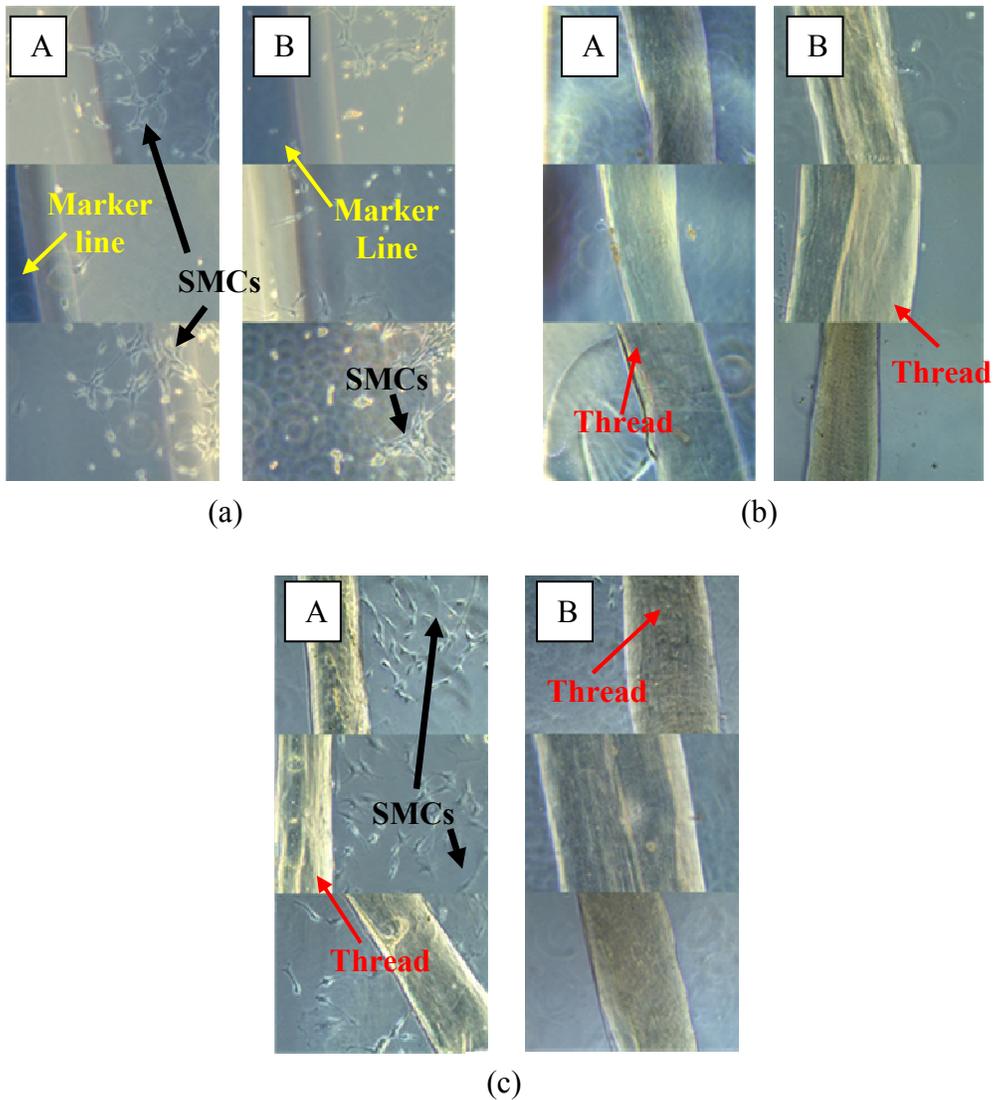


Figure 24: Microthreads Seven Days After Plating (a) Control Dish with No Microthread (b) Control Microthread (c) PDGF Coated Microthread. Individual pictures from various points on the petri dish were stitched together using Adobe Photoshop CS2.

Although cells were found surrounding PDGF coated microthread A, the cells did not migrate significantly past the halfway point, as seen in Figure 25. Similar to previous trials, after eight days the cells overgrew the dish and began folding over, preventing any further testing.

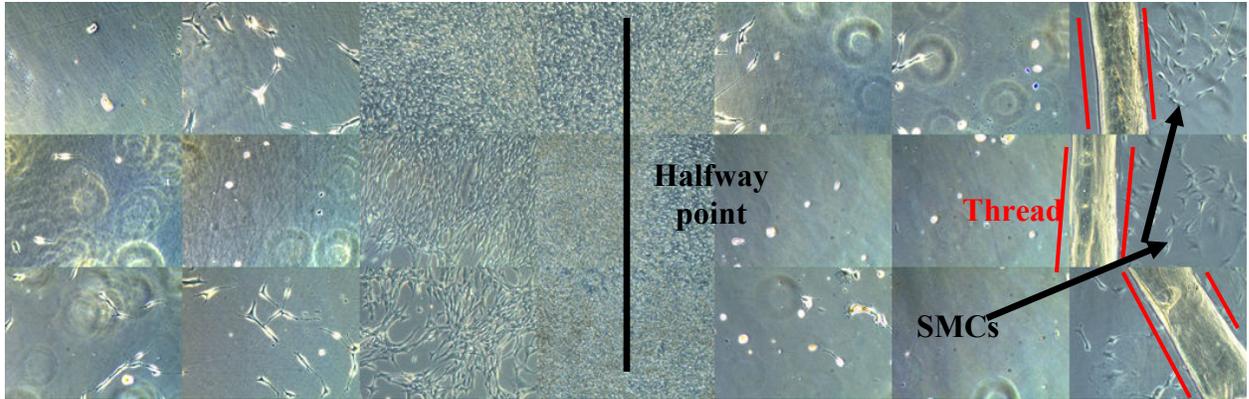


Figure 25: PDGF Coated Microthread A Seven Days After Plating. 21 individual pictures from various points on the petri dish were stitched together using Adobe Photoshop CS2. Note smooth muscle cells (SMCs) can be seen around the microthread, however there are not many cells past the halfway point.

7 Discussion

This section serves to interpret the results of the polyaniline and microthread mesh testing.

7.1 Polyaniline

The team had great difficulty producing uniform films of polyaniline. The films obtained using the initial protocol from Dr. Gouma's laboratory were very cracked. Initially, it was suspected that the air currents in the fume hood caused the films to crack as they dried. Therefore, the procedure described in Appendix H (revised 01/21/08) was used to prevent air currents from disrupting the drying film. This procedure involved placing the slides or tissue culture discs in covered Petri dishes that had holes cut in the top to allow diffusion of NMP. However, the revised procedure resulted in films that were just as cracked as previous films and took longer to dry.

After reviewing the literature, 4% wt/vol PANI solutions were used, rather than 12% wt/vol solutions [91, 92]. The PANI films produced were much less cracked and more uniform than the previously made films. However, the films made using 4% wt/vol solutions of PANI were stuck to the disc and could not be removed without destroying them.

It is believed that the cracking of PANI films that was observed during drying was due to the poor vaporization characteristics of NMP. NMP has a higher boiling point than other solvents such as N-dimethylformamide (DMF) and tetrahydrofuran (THF) and thus has inferior vaporization characteristics. The non-uniform diffusion of NMP from the PANI solution likely created regions of localized stresses. As the NMP diffused at a faster rate in certain areas of the film, this caused the film to be pulled in certain directions, resulting in localized fractures during drying. The films made using a higher concentration of PANI were more cracked because the higher concentration of polymer further impeded the diffusion of NMP.

In order to prevent the cracking of PANI films, an oven and/or vacuum may be used to improve the uniformity of the diffusion of NMP [91]. Using the vacuum chamber,

as described in Section 6.1, relatively uniform, un-cracked films of PANI were produced on both slides and tissue culture discs.

Upon producing uniform PANI films, the films were doped in 1M HCl for 15 minutes to make them electroactive [91]. However, the doping process caused the films to crack and split almost immediately. When slivers of PANI were doped (obtained from batches of cracked PANI films when solvent casting 12% wt/vol solutions), the slivers were able to withstand the doping process. The explanation for the difference observed between the PANI films on the slides and discs and the PANI slivers likely stems from the residual stresses present in the films. The films that were not able to withstand the doping process were attached to the glass surfaces, forcing them to remain in a flat state, which is not the natural state of most polymers. The films likely cracked because the chloride ions in the HCl attacked the bonds within the PANI, which caused the film to unfold into various conformations dictated by the residual stresses that were present. These films were also unable to withstand washing in PBS.

A higher molecular weight polymer (65kD) was used to potentially prevent cracking of the film during doping. When mixing a 4% wt/vol solution of PANI, the polymer powder did not completely dissolve into solution. Despite minimizing the amount of un-dissolved powder that was cast onto the glass slides, the films produced were rough and contained surface irregularities. In order to ensure that the solution is homogenous and completely dissolved, it can be passed through a filter to remove any un-dissolved polymer powder [91].

The doped slivers of PANI that were produced using solutions of 12% wt/vol were tested to determine their contractile properties. The PANI slivers were tested under field and point stimulation with voltages ranging from 0V to 10V. However, no visible contraction was observed in any of the samples. There are numerous factors that may explain the inability to produce a visible contraction of the PANI slivers. The electric stimulation may have been inadequate. For the field stimulation setup, the electric field between the wires may not have been directly in line with the PANI strip, thus decreasing the amount of current being applied. Furthermore, the resistance of the PBS solution was measured with a digital multimeter and found to be on the order of 10^6 Ohms. Since the resistance of the solution was so high, the amount of current that actually reached the

PANI may not have been large enough to cause a contraction. With point stimulation, it was difficult to maintain contact between the tips of the wires and the ends of the PANI strip because the PANI was so brittle and prone to cracking. Additionally, contraction of PANI films may have been microscopic and may require more sophisticated equipment to visualize.

In conclusion, processing of polyaniline was very difficult. Uniform films could be produced, but could not be easily handled due to their brittle nature. Also, processing these films proved problematic because the films could not be easily doped or washed in PBS. When contractile testing was performed on slivers of PANI, no visual contraction could be observed. Although research has shown that PANI can be electrically stimulated, the team's field research has only confirmed PANI's ability to be chemically stimulated (see Appendix A). The possible microscopic contraction from an electrical stimulus or chemical stimulation is not practical for this left ventricular restraint device.

7.2 Biocompatibility of Microthreads

The biocompatibility of the microthreads in the device is an important design constraint. The acid-soluble type I collagen threads used were made from rat tail tendon. Although it was not feasible to test the biocompatibility of the microthreads with cardiac cells or human mesenchymal stem cells due to time constraints, the biocompatibility with other cell types has been confirmed by previous research.

Cornwell tested the migration of transduced human dermal fibroblasts along the length of collagen microthreads [19]. Cornwell reported that acid-soluble type I collagen threads had fibroblast migration rates similar to native threads from rat tail tendons. Cornwell cited the work of Koob et al. to further confirm the biocompatibility of collagen microthreads. Koob et al. tested the attachment of bovine tendon fibroblasts to type I collagen microthreads produced with a crosslinking agent [93, 94]. Koob et al. found that about 86% of fibroblasts attached to culture dishes coated in collagen microthreads compared to 100% of fibroblasts attached to the polystyrene tissue culture dish control. The tendon fibroblast proliferation rates on the collagen microthreads were reported to be about half of the fibroblast proliferation rate on the polystyrene tissue culture dish. Additionally, no difference in fibroblast morphology between cells on the microthreads

or on the dish was reported [93, 94]. Since this research indicates that microthreads are viable for use in cell delivery, regenerative agents, such as stem cells, could also be incorporated into the device to aid in the regeneration of the myocardium [19].

7.3 Mechanical Properties of Microthread Mesh

The calculations in Section 5.5 and their results in Section 6.3 are simple approximations for a LV restraint device made of microthreads. Several assumptions about the heart and the microthreads were made to simplify the analysis. It was assumed that the dilated heart is a hollow sphere and that the fibers in the left ventricle are oriented vertically. The fibers are actually oriented at slight angles from the vertical axis [95]. The assumption that the fibers are oriented vertically was made to allow the values for the heart wall stiffnesses to correspond to the vertical and horizontal axes in the fiber and cross fiber directions, respectively. It was assumed that the values reported for the stiffness of hearts with right ventricular hypertrophy could be used in the case of left ventricular hypertrophy [95]. It was also assumed that the heart wall tissue is linearly elastic, which is not entirely valid because most biological tissues are viscoelastic.

In addition to the assumptions made about the properties of the heart wall, assumptions were also made about the microthreads. It was assumed that all microthreads have the same diameter, length, and elastic modulus. As reported by Cornwell, the error reported for the elastic modulus of uncrosslinked microthreads is $\pm 1.2\text{MPa}$ [19]. Although this error is relatively large (30%), this assumption is valid as a first approximation. The error reported for the cross-sectional area of microthreads is very small ($\pm 5790 \mu\text{m}^2$) and therefore this assumption is valid. The assumption that all of the microthreads had the same length is not entirely valid because the threads are wrapped around a sphere. Microthreads that are wrapped around the center of the sphere will be longer than those that are wrapped around the ends of the sphere. However, this assumption accounts for a very small change in the volume of the microthreads because the threads are so small.

It was expected that more threads would be required in the fiber direction than in the cross fiber direction because the stiffness of the heart wall is greater in the fiber direction than in the cross fiber direction [95]. The stiffness of the composite material

was taken to be 10% greater than the native heart wall because it is expected that a small increase in stiffness would provide structural support to prevent dilatation, but at the same time would not interfere greatly with the contraction of the healthy myocardium. In order to account for a factor of safety or to increase the stiffness of the heart wall-microthread composite even further, the number of microthreads required could be recalculated. Also, the stiffness was increased equally both in the fiber and cross fiber directions in order to keep the model relatively simple. However, the stiffness may not need to be equally increased in both directions.

7.4 Cell Migration Assay

A cell migration assay was performed to observe the migration and proliferation of smooth muscle cells (SMCs) with PDGF coated microthreads in comparison to control samples. As described in Section 6.4, SMCs were observed surrounding the PDGF coated microthreads. Although it appeared that the cells migrated to the thread and subsequently proliferated around the thread, cell migration was not apparent when the entire petri dish was observed. As seen in Figure 24, which shows a characteristic picture of the entire dish seven days after plating, many cells were observed on the side of the dish where the cells were initially plated, with very few cells between this side of the dish and the microthread. This observation may be attributed to the fact that the PDGF concentration was likely very low on the side of the dish where the cells were initially cultured. Since the PDGF concentration may have been too low to promote cell migration, the cells likely proliferated where they were initially plated, with little migration towards the microthread. In order to observe a more significant amount of cell migration, a smaller petri dish could be used because the PDGF concentration would not be as low on the side of the dish where the cells are plated.

When performing the cell migration assay, a major problem that was consistently observed with each trial was overgrowth of the cells. In all trials, within a week the cells had begun to overgrow the petri dish and were observed to lift off the plate in sheets and fold over. As previously described, the cells appeared to proliferate on the side of the dish where they were initially plated with very little migration to the other side of the dish where the microthread was located. The proliferation of cells on one side of the dish

caused them to overgrow and lift off the plate in this area. To prevent the overgrowth of cells, a smaller petri dish could be used so that the cells are more likely to migrate to the microthread before they overgrow the dish.

In the last trial, very few cells were observed surrounding the PDGF coated microthread B. This observation appeared to be an anomaly because all other PDGF coated microthreads in all trials were surrounded by cells. When being glued on to the tissue culture treated petri dish, this particular thread appeared to be pulled toward the dish as if it were attracted by some type of static electricity phenomenon. It was very difficult to lay the microthread down in the marked position and once it was laid down, it was no longer hydrated. This phenomenon may have affected the ability of this particular microthread to promote cell migration and proliferation.

8 Conclusions

By utilizing an engineering design process, a contractile LV restraint device was designed, using PANI as the major component of the device. However, preliminary testing demonstrated that PANI films could not be manufactured with the appropriate material properties. The films could not be doped and could not be removed from the glass slides because they were brittle and hard to handle. Additionally, during preliminary testing of the contractile properties of PANI slivers, no visible contraction was observed. Because PANI could not be doped and did not visually contract with the application of an electrical stimulus, it was not a viable material for a contractile LV restraint.

Since a contractile LV restraint could not be developed within the time constraints, preliminary designs were re-evaluated to select a passive restraint design to pursue. A mesh restraint was developed with microthreads chosen as the material for the device and PDGF selected as the therapeutic agent. Calculations based on the rule of mixtures demonstrated that 34 microthreads would be required for the device and therefore it would be feasible to build a prototype for future testing. Based on first order approximations, a pressure vessel-based equation indicated that the mesh restraint will reduce dilatation of the heart. A cell migration assay was performed and demonstrated that the migration and proliferation of smooth muscle cells was higher with PDGF coated microthreads.

In this design project, a LV restraint that reduces dilatation and delivers a therapeutic agent was developed. Initial calculations demonstrated that the mesh will reduce dilatation of the heart. Results from the cell migration assay indicate that the device has the potential to promote angiogenesis *in vivo*. By promoting angiogenesis, the device will provide an environment conducive to myocardial regeneration. Additionally, since it has previously been shown that microthreads are viable for use in cell delivery, regenerative agents, such as stem cells, could also be incorporated into the device to aid in the regeneration of the myocardium [19]. Although more research and testing is needed to optimize the microthread mesh restraint and ensure its functionality, this design project provided an important first step in the development of a microthread mesh restraint that promotes angiogenesis.

9 Future Recommendations

This project describes the beginning of the many steps needed to develop a fully functional left ventricular restraint device that promotes angiogenesis. Promising results have been shown but further investigation is needed to ultimately develop this device for use in patients with heart failure.

9.1 Polyaniline Device

Initially, PANI was selected as the major component of the device because it would provide contractile support to the failing heart. Though the idea seemed promising, major problems prevented further development of designs that involved PANI.

The protocol used produced PANI films that were brittle and hard to handle. Uniform PANI films could be produced, however they could not be removed from the slide and cracked during the doping process. Additionally, when small pieces of cracked PANI films were doped and then electrically stimulated, no visual contraction was observed. In order to determine if the PANI films contract when stimulated electrically, the set up that was used could be verified or more sophisticated stimulation and detection equipment could be used. Since the protocol did not produce uniform films that could be doped or contract when stimulated, PANI was not a viable material for this project due to time constraints. In order to use PANI in a contractile LV restraint device, further investigation is needed to develop a protocol for manufacturing PANI with more appropriate material properties.

9.2 Microthread Device

In order to develop a LV restraint that promotes angiogenesis, microthreads coated in PDGF were tested for their ability to promote migration and proliferation of smooth muscle cells. Results from the cell migration assay demonstrated an increased rate of migration and proliferation of smooth muscle cells was higher with the PDGF treated microthreads than the control samples. Further testing should be performed to quantitatively compare the rate of migration and proliferation on all samples. In order to do this, smooth muscle cells could be stained and counted at specific regions of the petri

dish at certain time points. Statistical analysis should be performed to verify that the increase in migration and proliferation of the cells with the PDGF treated microthreads is statistically significant compared to the other samples. Also, further investigation should be performed to optimize the concentration of PDGF on the microthreads. Since PDGF is expensive, the concentration should be as minimal as possible but should still promote cell migration and proliferation. The ability of regenerative agents, such as human mesenchymal stem cells, to be incorporated into the device should also be investigated.

Due to time constraints, a microthread device could not be manufactured and mechanical testing could not be performed. The rule of mixtures demonstrated that the device will reduce dilatation of the heart wall. However, the rule of mixtures was used as a first order approximation and therefore, more complex calculations involving finite element analysis could be used to obtain more accurate results. Additionally, experimentation should be performed to confirm these findings and to ensure that the device can withstand the forces it would be subjected to after implantation. Uniaxial tensile testing could be used as a first approximation to characterize the ultimate tensile stresses, strains, and fatigue life of the mesh. Following uniaxial testing, multi-axial tensile testing should be performed to ensure that the mesh can withstand the orientation of the forces within the heart wall. Fatigue testing is vital because the mesh will be subjected to repeated loading once implanted.

In order to determine the safety and efficacy of the device *in vivo*, testing should be performed in small animal models and then large animal models. The ability of the device to promote angiogenesis *in vivo* should be confirmed by examining the blood vessel density before and after implantation [10]. Finally, the ability of the mesh to provide structural support and prevent dilatation should be confirmed *in vivo*.

The results presented demonstrate that PDGF coated microthreads could be used to develop a LV restraint that promotes angiogenesis. Although all the desired testing could not be accomplished due to time constraints, this research provides a strong foundation for future development in creating a left ventricular restraint device that promotes angiogenesis for patients that suffer from heart failure.

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APPENDIX A – Notes From Stony Brook Trip

On December 5, 2007, our MQP team visited The State University of New York at Stony Brook to consult with Dr. Pelagia-Irene (Perena) Gouma and her graduate student Koushik Ramachandran, who work in the Department of Materials Science and Engineering. The main goals of our visit included learning about the procedures and equipment used to make electroactive polyaniline (PANI).

The team had a meeting with Koushik in which he presented their research concerning PANI. He also answered important questions relating to the methods of PANI synthesis and other concerns we had based on our literature review on PANI. Koushik also demonstrated the procedure used to make PANI films. Specifically, the items listed below were explained in the question and answer session with Koushik:

1. There are two types of PANI powders: an emeraldine base or an emeraldine salt. The difference between the two is that the salt is conductive whereas the base form is insulating.
2. PANI can be made to contract by two general means: exposure to chemical vapors (acetone, hexane, ethanol, etc.) or by the application of an electrical stimulus while submerged in an electrolytic solution.
3. PANI typically must be mixed with other materials, such as cellulose acetate (CA) or gelatin, to improve its poor mechanical properties.
4. The form of PANI they were making consisted of PANI salt with CA in various proportions. CA is incorporated into the PANI because previous literature reported that it supports cell growth and enhances the mechanical properties of PANI. The PANI:CA was dissolved in acetone (solvent) and cast upon a glass slide to form a thin film.
5. The PANI:CA was shown to curl upon chemical stimulation from acetone and chemical vapors, but it did not respond to electrical stimulation. Koushik noted that a high resistance of 10 kOhm was measured for many of the PANI:CA samples.
6. In order to make PANI electroactive, the emeraldine base must be dissolved in the solvent N-methyl-pyrrolidinone (NMP). The emeraldine salt cannot be used because it does not dissolve in the NMP, forming an irregular and discontinuous film when cast.
7. To make the PANI base electroactive it must be doped with chemicals such as hydrochloric acid (HCl), camphorsulfonic acid, or m-cresol. The addition of protons and changes in state allow the PANI to contract.
8. As the film becomes thicker, it becomes harder to activate. For PANI:CA that has been solvent cast the more solvent used in the procedure, the more porous the resulting material becomes and thus it is easier to activate via chemical stimulation.

Procedure for making PANI:CA films:

1. A 50:50 PANI:CA solution is prepared by dissolving 0.625 g of PANI emeraldine salt and 0.625 g of CA in 1 mL of acetone. The resulting solution is 12.5% PANI by volume.

2. The PANI:CA solution is sonicated for about 10-15 minutes to obtain a uniform mixture.
3. The solution is carefully poured over a glass slide (or other substrate) and spread out using a small wooden stick.
4. The acetone is allowed to evaporate for about 5 minutes.
5. The thin PANI:CA film is then peeled off using tweezers.

Procedure for making electroactive PANI:

The procedure for solvent casting the PANI emeraldine base is similar to the procedure described above. A solution is mixed using 12% PANI by volume in NMP, which is then poured onto a glass slide. The NMP is allowed to evaporate from the PANI solution overnight because NMP takes longer to evaporate than acetone. Furthermore, the glass slide with the PANI and NMP mixture on it could be exposed to higher temperatures to speed up the process of evaporation. After the PANI film is made, it is doped using HCl (or another dopant) by submerging it in the dopant for a few minutes.

APPENDIX B – Group Rated Pairwise Comparison Chart

	Minimal Cost	Available Materials	Appropriate Mechanical Properties	Simple Implantation Procedure	Easily Produced	Appropriate Contractile Properties	SCORE	SCORE +1	Weighted %
Minimal Cost	X	0	0	0	0	0	0	1	4.8%
Available Materials	1	X	0	1	0.5	0	2.5	3.5	16.7%
Appropriate Mechanical Properties	1	1	X	1	1	1	5	6	28.6%
Simple Implantation Procedure	1	0	0	X	0	0	1	2	9.5%
Easily Produced	1	0.5	0	1	X	0	2.5	3.5	16.7%
Appropriate Contractile Properties	1	1	0	1	1	X	4	5	23.8%
						TOTAL	15	21	100%

APPENDIX C – Stress Calculations

In patients with high ventricular pressure, meridional wall stress, $\sigma = 161 \times 10^3 \text{ dyn/cm}^2 = 16.1 \text{ kPa}$. Meridional wall stress is defined as the force per unit area acting from the apex to base of the heart [87].

Meridional wall stress can be expressed by the following function:

$$\sigma = \frac{F}{A} = \frac{F}{l \times t}, \text{ where } t = \text{wall thickness, } F = \text{wall force, and } l = \text{length of the wall}$$

The average wall thickness for patients with high ventricular pressure, $t = 17 \text{ mm} = 0.017\text{m}$ [87]

Wall tension, F/l (N/m) can be expressed as:

$$\frac{F}{l} = \sigma \times t$$

Incorporating a safety factor (SF) of 5, the wall tension can be calculated:

$$\frac{F}{l} = \sigma \times t \times SF = 16.1\text{kPa} \times 17\text{mm} \times 5 = 1.37\text{N/mm}$$

APPENDIX D – Fatigue Calculations

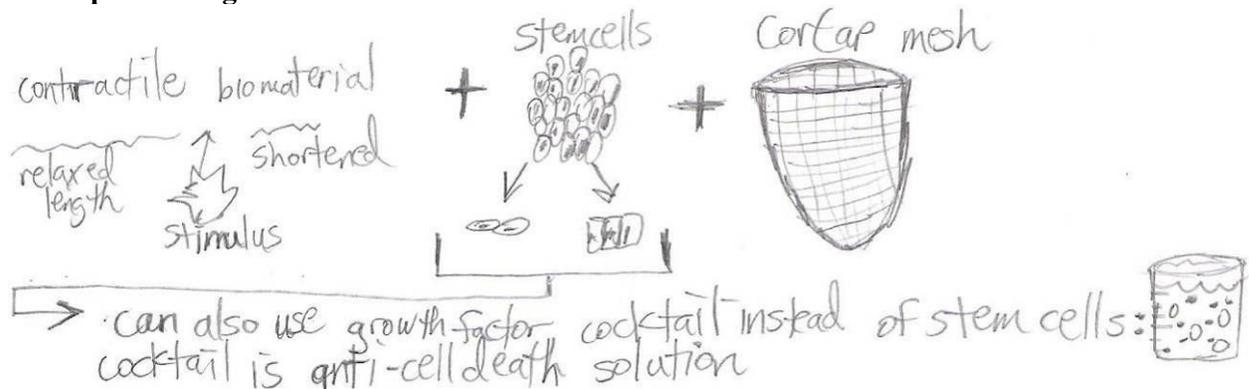
A number of studies have reported the mortality rate for patients with heart failure to be approximately 59-68% after the first two years. These studies often report the results of data obtained from hospitals and therefore typically reflect more severe cases of heart failure [89]. Since our device is intended for patients with class III or IV heart failure, we believe that this data will accurately reflect the mortality of patients intended to be treated with our device. Thus, our device must be able to withstand cyclic loading for a minimum of two years.

The heart beats an average of 75 beats/min [21]. The total number of cycles our device must withstand is the number of heart beats that occur in two years:

$$\frac{75 \text{ beats}}{1 \text{ min}} \times \frac{60 \text{ min}}{1 \text{ hr}} \times \frac{24 \text{ hr}}{1 \text{ day}} \times \frac{365 \text{ days}}{1 \text{ yr}} \times 2 \text{ years} = 78,840,000 \text{ cycles}$$

APPENDIX E – Conceptual Designs

Conceptual Design 1:



A contractile biomaterial, such as PANI, stem cells, and a CorCap™ mesh are combined into one device. The PANI aids in systolic contraction and the stem cells serve to regenerate the myocardium. The CorCap™ mesh will provide passive restraint to the heart. A cell-sustaining “anti-death” solution could be used in place of the stem cells.

Pros:

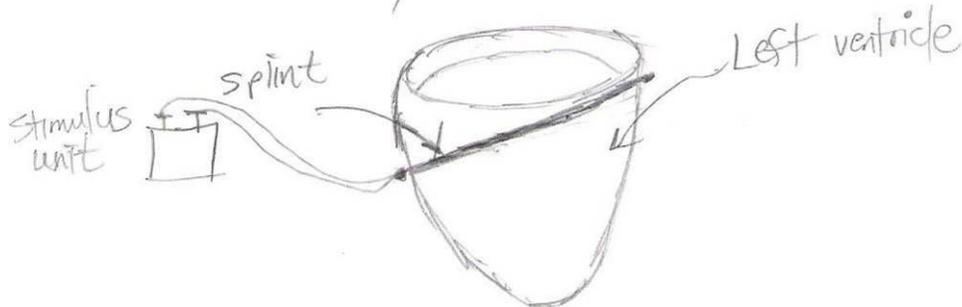
- Improves heart function through a variety of treatments
- Localized delivery of the therapeutic agent directly to the infarcted area

Cons:

- May be difficult to maintain the effectiveness of each individual treatment in a combined device
- May be difficult to simulate contraction that propagates through the mesh like the native heart
- Complex design
- Regulatory approval of this device may be difficult to obtain

Conceptual Design 2:

Polymer (PANI) thread splint similar to 'nut and bolt' idea, but with only the splint.



A PANI thread splint crosses through the left ventricle from front to back without passing through the septum and is sutured to the myocardium on each end. An electrical stimulus from a pacemaker causes the PANI thread to contract during systole.

Pros:

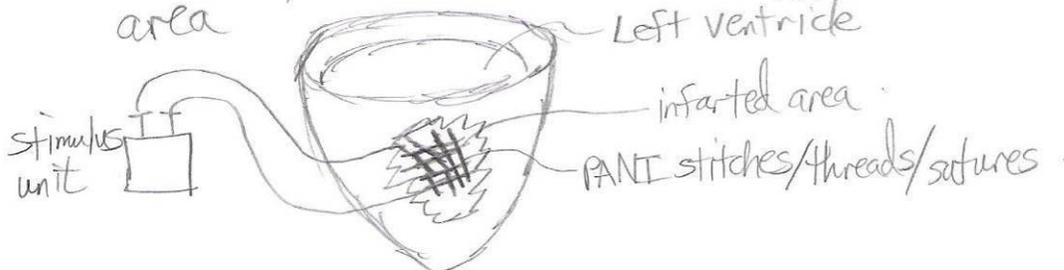
- Simple design

Cons:

- Passive restraint provided by the thread splint may be limited
- The amount of contractile assistance may be limited in this configuration
- The thread may disrupt myocardium function
- Splint may induce blood clot formation

Conceptual Design 3:

Sutures/Threads/Cross-stitch of PANI around infarcted area



PANI threads are cross-stitched and sutured into place in the infarcted area of the heart. An electrical stimulus causes the PANI to contract during systole.

Pros:

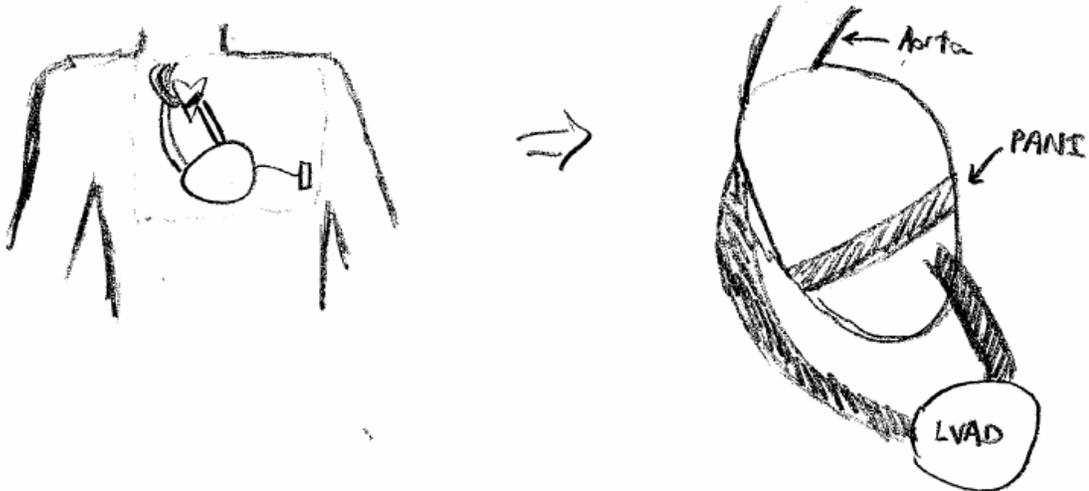
- Localized delivery and contractile assistance
- Similar to heart patches, which have been thoroughly tested

Cons:

- Surgical cross-stitching of the PANI may be difficult
- Sutures in the infarcted area may cause further damage
- The amount of contractile assistance may be limited by this configuration

Conceptual Design 4:

LVAD with PANI stimulated by alcohol.



LVAD that pumps blood/ "alcohol"
to stimulate the PANI.

A modified left ventricular assist device is used in conjunction with PANI. A PANI hoop is wrapped around the heart and is chemically stimulated to contract by alcohol. A modified LVAD contains a special fluid circuit that delivers the alcohol to the PANI.

Pros:

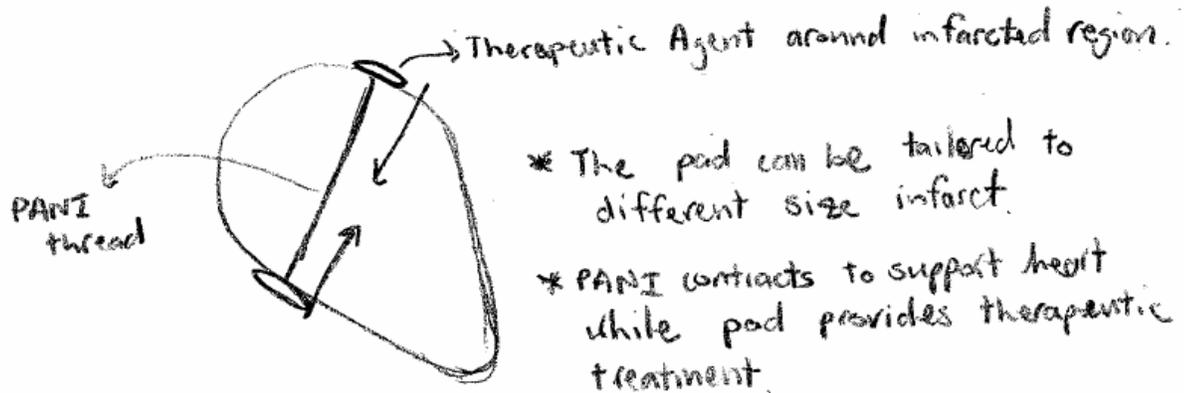
- High probability of beneficial LV remodeling due to generous unloading of the LV

Cons:

- Complex design
- Alcohol could leak from the fluid circuit and cause toxicity
- It may be expensive to modify a LVAD to circulate alcohol

Conceptual Design 5:

Nut and Bolt with PANI Thread. The nut is made out of therapeutic treatment. Pad could be biodegradable.



A PANI thread splint crosses through the left ventricle from front to back without passing through the septum near the site of the infarction. It is attached to two end pads, which serve to anchor the splint on the heart and hold a therapeutic treatment. The therapeutic treatment, such as stem cells, will regenerate the heart tissue while the PANI splint provides contractile assistance and passive restraint.

Pros:

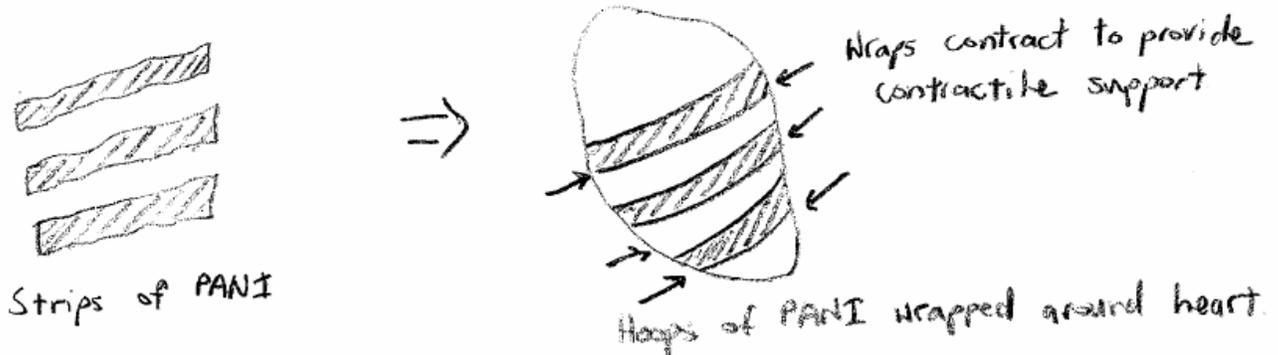
- The epicardial pads can be tailored to various infarct sizes
- Localized delivery of the therapeutic agent directly to the infarcted area

Cons:

- The thread may disrupt myocardium function
- May be difficult to attach PANI to the end pads
- The splint may induce blood clot formation

Conceptual Design 6:

PANI Hoops LV Restraint



PANI strips are arranged in hoops around the circumference of the heart from its apex to the atrioventricular groove. A programmed electrical stimulus causes the PANI hoops to contract starting with the one closest to the apex. The PANI hoops also provide passive restraint for the heart during diastole.

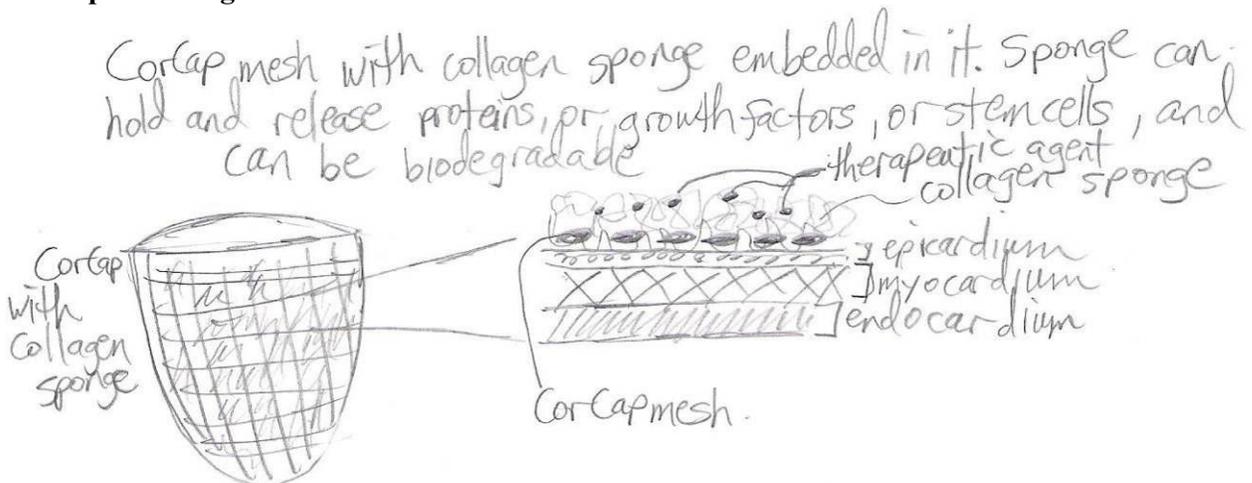
Pros:

- A high degree of contractile assistance is provided by this configuration

Cons:

- It may be difficult to contract each hoop at the appropriate time, resulting in arrhythmias
- Contraction of the device may produce right ventricular dysfunction due to unloading
- It may be difficult to incorporate a therapeutic agent into this design

Conceptual Design 7:



A CorCap™ mesh is combined with a collagen sponge and therapeutic treatments such as growth factors or stem cells. The CorCap™ mesh provides passive restraint and

serves as a platform for the collagen sponge. The collagen sponge with the therapeutic agent is embedded into the mesh around the site of the infarction.

Pros:

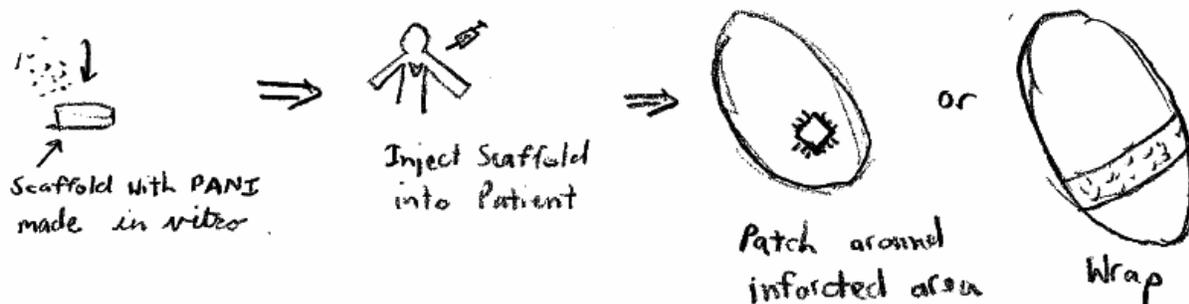
- Localized delivery of the therapeutic agent directly to the infarcted area
- Can be biodegradable

Cons:

- Does not provide contractile assistance
- Embedding the sponge and treatment agent into the mesh at the site of the infarct may be difficult

Conceptual Design 8:

Injectable Fibrin Glue Scaffold Seeded with PANI as a Patch/Wrap.



A fibrin glue scaffold containing PANI is made *in vitro*. The scaffold is then injected into the patient and then either formed into a patch over the infarcted area or a wrap. The PANI is electrically stimulated to contract during systole.

Pros:

- Simple implantation and delivery procedure
- A number of shapes and forms can be made from injectable fibrin glue

Cons:

- Preparation of PANI-impregnated fibrin glue may be difficult
- It may be difficult to obtain an optimal PANI fiber arrangement upon injection of the fibrin gel

Conceptual Design 9:



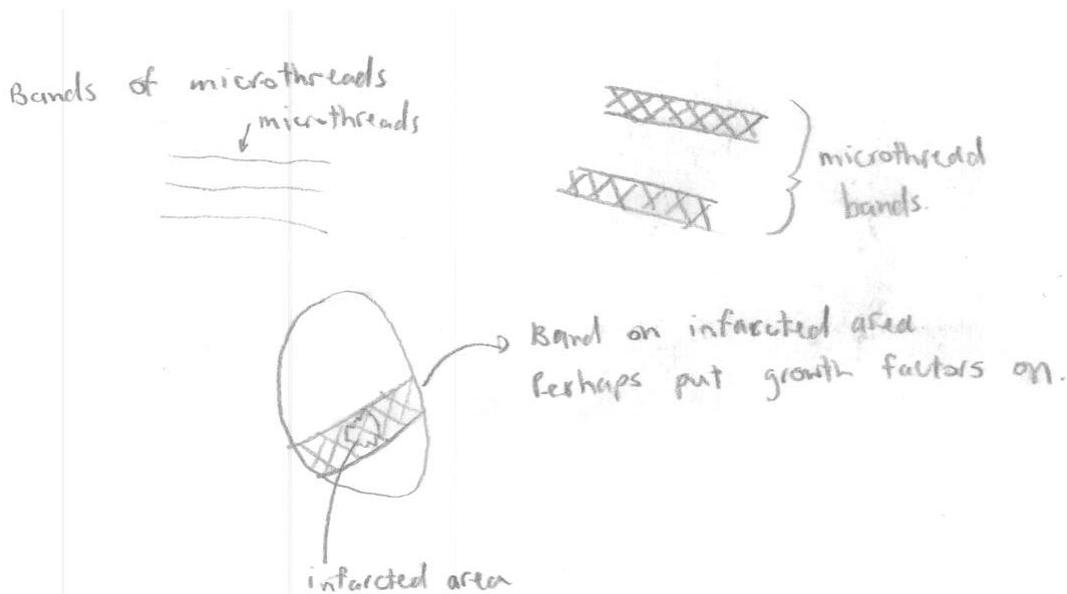
A mesh similar to the CorCap design is made from a passive material, such as microthreads. Growth factors or cells are incorporated into the microthreads that will be placed over the infarcted area.

Pros:

- Simple implantation procedure
- Will provide a large degree of structural support
- Local delivery of therapeutic agent to the infarcted area

Cons:

- May be difficult to construct the intricacies of the mesh design
- No contractile support



A mesh is made from a passive material, such as microthreads. Unlike the wrap design, the mesh will act as a band and will not wrap around the entire heart. Growth

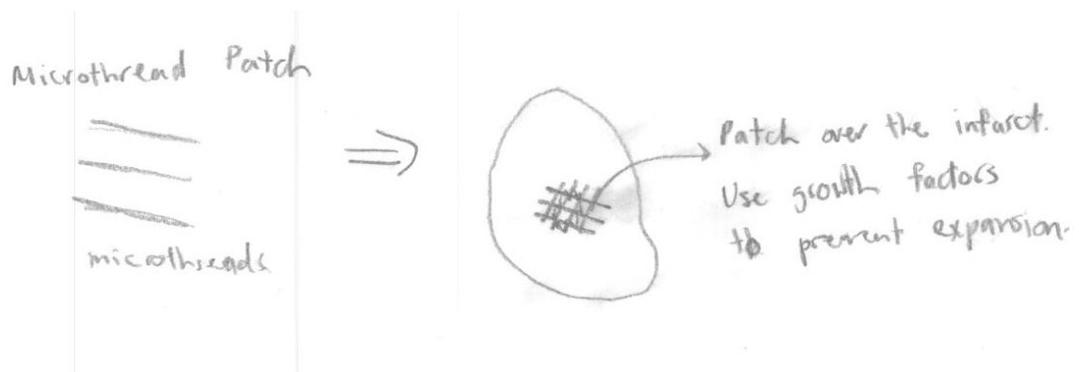
factors or cells are incorporated into the microthreads that will be located directly over the infarcted area.

Pros:

- Simple implantation procedure
- Simple construction
- Local delivery of therapeutic agent to the infarcted area

Cons:

- Will not provide as much mechanical support as the mesh design
- No contractile support



A patch is made from a passive material, such as microthreads. Growth factors or cells are incorporated into the microthreads.

Pros:

- Local delivery of the therapeutic agent to the infarcted area
- The patch can be constructed more easily than the mesh or band design

Cons:

- May be difficult to attach to the heart
- Will provide little structural support
- No contractile support

APPENDIX F– Evaluation Matrix

Using the constraints and objectives identified in Section 4.2, an evaluation matrix was used to evaluate the most feasible design alternatives. To evaluate the constraints, a yes or no question was used to determine if the design met the applied constraints. If the design would not meet the constraint, it was not considered a viable design alternative. For each objective, the designs were given a score of 0 to 5, where a rating of 0 indicated that the design would not meet the objective at all and 5 indicated that the design would completely meet the objective. For each objective and constraint, the basis for evaluation is described below.

Constraint: Biocompatible

Basis for evaluation: The ability of the device to not induce an inflammatory response and to be non-toxic and hemocompatible.

Constraint: Cost (\$624)

Basis for evaluation: The materials for the device and manufacturing cost must be under \$624.

Constraint: Time (April, 2008):

Basis for evaluation: It must be feasible to manufacture and test the design within the given time constraint.

Objective: Minimal cost

Basis for evaluation: The ability of the design to be produced at the lowest cost possible, which is dependent on the cost of the individual materials, their availability, and the expenses incurred in processing/testing them.

Objective: Available materials

Basis for evaluation: The ability of the design to incorporate easily obtainable materials.

Objective: Appropriate mechanical properties

Basis for evaluation: The ability of the device to provide the necessary mechanical support and have the necessary tensile strength, elastic properties, fatigue, and wear resistance.

Objective: Simple implantation procedure

Basis for evaluation: The ability of the device to be easily implantable, compatible with current surgical techniques, and require a short patient recovery time.

Objective: Easily produced

Basis for evaluation: The ability of the device to be manufactured easily and quickly with as few steps as possible.

Objective: Appropriate contractile properties

Basis for evaluation: The ability of the device to contract with the native heart, without producing arrhythmias.

Constraints and Objectives	Weight	Contractile biomaterial, wrap restraint, and cells or growth factors	Contractile biomaterial, nut & bolt restraint, and cells or growth factors on end caps	Contractile biomaterial, hoops or strips, cells or growth factors	Contractile biomaterial, patch, cells or growth factors	Passive wrap restraint with cells or growth factors
C: Biocompatible	---	Y	Y	Y	Y	Y
C: Cost (\$624)	---	Y	Y	Y	Y	Y
C: Time (April, 2008)	---	Y	Y	Y	Y	Y
O: Minimal Cost	4.8%	3(0.048)=0.144	3(0.048)=0.144	3(0.048)=0.144	4(0.048)=0.192	3(0.048)=0.144
O: Available Materials	16.7%	2(0.167)=0.334	2(0.167)=0.334	2(0.167)=0.334	2(0.167)=0.334	4(0.167)=0.668
O: Appropriate Mechanical Properties	28.6%	4(0.286)=1.144	3(0.286)=0.858	2(0.286)=0.572	1(0.286)=0.286	4(0.286)=1.14
O: Simple Implantation Procedure	9.5%	4(0.095)=0.38	2(0.095)=0.19	3(0.095)=0.285	3(0.095)=0.285	3(0.095)=0.285
O: Easily Produced	16.7%	2(0.167)=0.334	3(0.167)=0.501	4(0.167)=0.668	4(0.167)=0.668	2(0.167)=0.334
O: Appropriate Contractile Properties	23.8%	2(0.238)=0.467	4(0.238)=0.952	2(0.238)=0.476	3(0.238)=0.952	0(0.238)=0
TOTAL	100%	2.803	2.979	2.479	2.717	2.575

Constraints and Objectives	Weight	Passive band restraint with cells or growth factors	Passive patch with cells or growth factors	Contractile biomaterial, LVAD	Passive wrap restraint, collagen sponge to hold growth factors or cells	Contractile biomaterial and fibrin glue, patch, wrap, or band
C: Biocompatible	---	Y	Y	Y	Y	Y
C: Cost (\$624)	---	Y	Y	N	Y	Y
C: Time (April, 2008)	---	Y	Y	N	N	N
O: Minimal Cost	4.8%	3(0.048)=0.144	4(0.048)=0.192	N/A	N/A	N/A
O: Available Materials	16.7%	4(0.167)=0.668	4(0.167)=0.668	N/A	N/A	N/A
O: Appropriate Mechanical Properties	28.6%	2(0.286)=0.572	1(0.286)=0.286	N/A	N/A	N/A
O: Simple Implantation Procedure	9.5%	4(0.095)=0.380	3(0.095)=0.285	N/A	N/A	N/A
O: Easily Produced	16.7%	4(0.167)=0.668	4(0.167)=0.668	N/A	N/A	N/A
O: Appropriate Contractile Properties	23.8%	0(0.238)=0	0(0.238)=0	N/A	N/A	N/A
TOTAL	100%	2.432	2.099	N/A	N/A	N/A

Explanation of Evaluation

Design #1: Contractile biomaterial, wrap restraint, and cells or growth factors

- Biocompatible – (Y) The device is expected to be non-toxic, hemocompatible, and to not induce an inflammatory response.
- Cost – (Y) The materials and manufacturing processes required are expected to be within the project budget of \$624.
- Time – (Y) It is feasible to manufacture and test the design within the given time frame.
- Minimal cost – (3) The materials should be able to be purchased and processed at a relatively low cost. However, since the mesh encloses the entire heart, a large amount of material may be utilized, which would increase the cost of producing the device.
- Available materials – (2) The materials for the device are available for purchase, but may require a significant amount of processing and manufacturing.
- Appropriate mechanical properties – (4) The wrap restraint will provide a large amount of mechanical support because it prevents expansion of the entire heart.
- Simple implantation procedure – (4) The restraint will be implanted by wrapping it around the heart and then securing it to the heart. This can be done using a minimally invasive surgical technique and will therefore require a short patient recovery time.
- Easily produced – (2) It will be difficult to produce the intricacies of the complex mesh design. The contractile biomaterials may be difficult to be made into a mesh.
- Appropriate contractile properties – (2) It will be difficult to simulate a contraction that propagates through the mesh similar to the propagation of contraction through the native heart.

Design #2: Contractile biomaterial, nut & bolt restraint, and cells or growth factors on end caps

- Biocompatible – (Y) The device is expected to be non-toxic, hemocompatible, and to not induce an inflammatory response
- Cost – (Y) The materials and manufacturing processes required are expected to be within the project budget of \$624.
- Time – (Y) It is feasible to manufacture and test the design within the given time frame.
- Minimal cost – (3) The materials should be able to be purchased and processed at a relatively low cost. However, since this design incorporates two different parts (the splint and end cap) and potentially two different types of materials, additional costs in combining these parts or materials may be incurred.
- Available materials – (2) The materials for the device are available for purchase, but may require a significant amount of processing and manufacturing.
- Appropriate mechanical properties – (3) The nut & bolt restraint will provide limited mechanical support. Also, stress concentrations may result at the interface between the end caps and the splint.

- Simple implantation procedure – (2) The device will be very difficult to implant because the splint will penetrate the ventricular wall. Also, patient recovery time may be prolonged due to the invasiveness of this design.
- Easily produced – (3) The parts of the nut and bolt design can be more easily produced than the mesh design. However, incorporating the end caps onto the ends of the splint will be difficult.
- Appropriate contractile properties – (4) The contraction of this device will be easiest to control because the propagation of the contraction does not need to be as precisely controlled as with the mesh.

Design #3: Contractile biomaterial, hoops or strips, cells or growth factors

- Biocompatible – (Y) The device is expected to be non-toxic, hemocompatible, and to not induce an inflammatory response.
- Cost – (Y) The materials and manufacturing processes required are expected to be within the project budget of \$624.
- Time – (Y) It is feasible to manufacture and test the design within the given time frame.
- Minimal cost – (3) The materials should be able to be purchased and processed at a relatively low cost.
- Available materials – (2) The materials for the device are available for purchase, but may require a significant amount of processing and manufacturing.
- Appropriate mechanical properties – (2) The hoops or strips will provide limited mechanical support.
- Simple implantation procedure – (3) The device can be implanted relatively simply by wrapping the hoops or strips around the heart.
- Easily produced – (4) The hoops or strips can be produced much more easily than the other design alternatives.
- Appropriate contractile properties – (2) The propagation of contraction will be difficult to control if there are multiple hoops or strips.

Design #4: Contractile biomaterial, patch, cells or growth factors

- Biocompatible – (Y) The device is expected to be non-toxic, hemocompatible, and to not induce an inflammatory response.
- Cost – (Y) The materials and manufacturing processes required are expected to be within the project budget of \$624.
- Time – (Y) It is feasible to manufacture and test the design within the given time frame.
- Minimal cost – (4) The materials should be able to be purchased and processed at a relatively low cost. This design has the highest rating because the patch will require a very small amount of material.
- Available materials – (2) The materials for the device are available for purchase, but may require a significant amount of processing and manufacturing.
- Appropriate mechanical properties – (1) The patch will provide little mechanical support to the entire heart because it will be placed only over the infarct.
- Simple implantation procedure – (3) The device may be difficult to attach to the heart because it will only be held in place by suturing.

- Easily produced – (4) The simple design of the patch can be produced relatively easily.
- Appropriate contractile properties – (3) The propagation of contraction will not need to be precisely controlled due to the localized nature of the patch.

Design #5: Passive wrap restraint with cells or growth factors

- Biocompatible – (Y) The device is expected to be non-toxic, hemocompatible, and to not induce an inflammatory response.
- Cost – (Y) The materials and manufacturing processes required are expected to be within the project budget of \$624.
- Time – (Y) It is feasible to manufacture and test the design within the given time frame.
- Minimal cost – (3) The materials should be able to be purchased and processed at a relatively low cost.
- Available materials – (4) The materials for the device are available for purchase and may require some processing and manufacturing.
- Appropriate mechanical properties – (4) The wrap restraint will provide the necessary mechanical support to the entire heart.
- Simple implantation procedure – (3) The restraint will be implanted by wrapping it around the heart and then securing it to the heart. This can be done using a minimally invasive surgical technique and will therefore require a short patient recovery time.
- Easily produced – (2) It may be difficult to produce the intricacies of the complex mesh design.

Design #6: Passive band restraint with cells or growth factors

- Biocompatible – (Y) The device is expected to be non-toxic, hemocompatible, and to not induce an inflammatory response.
- Cost – (Y) The materials and manufacturing processes required are expected to be within the project budget of \$624.
- Time – (Y) It is feasible to manufacture and test the design within the given time frame.
- Minimal cost – (3) The materials should be able to be purchased and processed at a relatively low cost.
- Available materials – (4) The materials for the device are available for purchase and may require some processing and manufacturing.
- Appropriate mechanical properties – (2) The band restraint will provide some mechanical support, but may cause bulging at the top and bottom of the band.
- Simple implantation procedure – (4) The restraint will be implanted by wrapping it around the heart and then securing it to the heart. This can be done using a minimally invasive surgical technique and will therefore require a short patient recovery time.
- Easily produced – (4) The device will be easier to manufacture than the wrap restraint because it is a bit more simplistic in design.

Design #7: Passive patch with cells or growth factors

- Biocompatible – (Y) The device is expected to be non-toxic, hemocompatible, and to not induce an inflammatory response.

- Cost – (Y) The materials and manufacturing processes required are expected to be within the project budget of \$624.
- Time – (Y) It is feasible to manufacture and test the design within the given time frame.
- Minimal cost – (4) The materials should be able to be purchased and processed at a relatively low cost. The patch will require a smaller amount of material and thus a lower cost than other design alternatives.
- Available materials – (4) The materials for the device are available for purchase and may require some processing and manufacturing.
- Appropriate mechanical properties – (1) The patch will provide limited mechanical support because it will be placed only over the infarct.
- Simple implantation procedure – (3) The device may be difficult to attach to the heart because it will only be held in place by suturing.
- Easily produced – (4) The simple design of the patch can be produced relatively easily.

Design #8: Contractile biomaterial, LVAD

- Biocompatible – (Y) The device is expected to be non-toxic, hemocompatible, and to not induce an inflammatory response.
- Cost – (N) The materials and manufacturing processes required are not expected to be within the project budget of \$624.
- Time – (N) It is not feasible to manufacture and test the design within the given time frame.

Design #9: Passive wrap restraint, collagen sponge to hold growth factors or cells

- Biocompatible – (Y) The device is expected to be non-toxic, hemocompatible, and to not induce an inflammatory response.
- Cost – (Y) The materials and manufacturing processes required are expected to be within the project budget of \$624.
- Time – (N) It is not feasible to manufacture and test the design within the given time frame.

Design #10: Contractile biomaterial and fibrin glue, patch, wrap, or band

- Biocompatible – (Y) The device is expected to be non-toxic, hemocompatible, and to not induce an inflammatory response.
- Cost – (Y) The materials and manufacturing processes required are expected to be within the project budget of \$624.
- Time – (N) It is not feasible to manufacture and test the design within the given time frame.

APPENDIX G- Protocols for Cell Culture

Culturing Human Mesenchymal Stem Cells (HMSCs)	
Purpose	To culture HMSCs for biocompatibility testing.
Specifications	N/A
Materials and Equipment	<p>Materials</p> <ul style="list-style-type: none"> • Dulbecco’s Modified Eagle Medium (DMEM) • 10% fetal bovine serum (FBS) • T-75 culture flasks • 0.25% trypsin • Hank’s Balance solution • Sterile pipettes: 10mL, 1mL • Aspirating pipettes • 70% ethanol <p>Equipment</p> <ul style="list-style-type: none"> • Sterile hood • Light microscope
Procedure	<p>Culturing Cells</p> <ol style="list-style-type: none"> 1. Spray ethanol in hood and wipe down to disinfect area. 2. Aspirate all media from cell culture flask. Do not pass hands over any opened containers because this may contaminate the solutions. 3. Fill culture flask with 10mL of fresh media. 4. Observe cells under a microscope to see if they are attached. 5. Feed cells approximately every 3-4 days. <p>Splitting Cells</p> <ol style="list-style-type: none"> 1. Spray ethanol in hood and wipe down to disinfect area 2. Aspirate all media from cell culture flask. Do not pass hands over any opened containers because this may contaminate the solutions. 3. Rinse flask with either 10mL of Hank’s Balance solution or DMEM. 4. Add 1mL of trypsin. 5. Place flask under microscope and watch for cells to detach (approximately 2-3 minutes). 6. Once most of the cells are visibly detached, add cell culture

	<p>media with 10% FBS.</p> <ol style="list-style-type: none"> 7. Pipette cells and media into a centrifuge tube and agitate them. 8. Place in centrifuge at 600RPMs for 3 minutes to spin the cells down. 9. Aspirate the media and add in 10mL of fresh media. 10. Re-suspend/aliquot cells into desired number of flasks and add cell media for a total volume of 10mL. 11. Feed cells 24 hours after splitting and check under microscope to ensure that the cells have plated and no contamination has occurred.
Expected Results	N/A
Methods of Analysis	N/A
References	Protocol obtained from Professor Gaudette's laboratory

Culturing Rat Aortic Smooth Muscle Cells (SMCs)	
Purpose	To culture SMCs for cell migration assays with microthreads.
Specifications	N/A
Materials and Equipment	<p>Materials</p> <ul style="list-style-type: none"> • Dulbecco's Modified Eagle Medium (DMEM) • 10% fetal bovine serum (FBS) • T-75 culture flasks • 0.25% trypsin • Hank's Balance solution • Sterile pipettes: 10mL, 1mL • Aspirating pipettes • 70% ethanol <p>Equipment</p> <ul style="list-style-type: none"> • Sterile hood • Light microscope

Procedure	<p>Culturing Cells</p> <ol style="list-style-type: none"> 1. Spray ethanol in hood and wipe down to disinfect area. 2. Aspirate all media from cell culture flask. Do not pass hands over any opened containers because this may contaminate the solutions. 3. Fill culture flask with 10mL of fresh media. 4. Observe cells under a microscope to see if they are attached. 5. Feed cells approximately every 2 days. <p>Splitting Cells</p> <ol style="list-style-type: none"> 1. Spray ethanol in hood and wipe down to disinfect area 2. Aspirate all media from cell culture flask. Do not pass hands over any opened containers because this may contaminate the solutions. 3. Rinse flask with either 10mL of Hank's Balance solution or DMEM. 4. Add 1mL of trypsin. 5. Place flask under microscope and watch for cells to detach (approximately 7 minutes). 6. Once most of the cells are visibly detached, add cell culture media with 10% FBS. 7. Pipette cells and media into a centrifuge tube and agitate them. 8. Place in centrifuge at 600RPMs for 3 minutes to spin the cells down. 9. Aspirate the media and add in 10mL of fresh media. 10. Re-suspend/aliquot cells into desired number of flasks and add cell media for a total volume of 10mL. 11. Feed cells 24 hours after splitting and check under microscope to ensure that the cells have plated and no contamination has occurred.
Expected Results	N/A
Methods of Analysis	N/A
References	Protocol obtained from Professor Rolle's laboratory

APPENDIX H – Protocol for Producing PANI films

Solvent Casting Polyaniline Films	
Purpose	To produce thin films of polyaniline.
Specifications	N/A
Materials and Equipment	<p>Materials</p> <ul style="list-style-type: none"> • Emeraldine base polyaniline, average Mw of 10kD • N-methyl-2-pyrrolidone, biotech grade • 1M HCl • PBS • Drierite • Parafilm • Petri dishes • Glass slides • Micropipette and tips <p>Equipment</p> <ul style="list-style-type: none"> • Fume hood • Shaker • Vacuum chamber • Tweezers • Small bottles with caps • Nitrogen tank
Procedure	<p>Making PANI:NMP solution</p> <ol style="list-style-type: none"> 1. Mix 12% wt/vol of PANI in NMP in a small bottle with a cap 2. Place the bottle on the shaker to dissolve overnight. 3. Store remaining PANI in an inert gas environment by adding nitrogen gas to the bottle, sealing it with parafilm, and placing the bottle in a vacuum chamber containing drierite. <p>Pouring PANI:NMP solutions onto casting surface</p> <ol style="list-style-type: none"> 1. Place 1-2mL of PANI solution onto each glass slide using a micropipette. 2. Leave the slides in a fume hood overnight to allow for evaporation of the NMP solvent. <p>Doping of PANI films</p>

	<ol style="list-style-type: none"> 1. Remove dry PANI films from each slide using tweezers. 2. Wash the films 3 times with PBS for 5 min. 3. Place each film in a petri dish. 4. Pour 1M HCl in each petri dish until the film is submerged. 5. Remove the films using tweezers after 15 min of doping. 6. Place films in another petri dish to dry.
Expected Results	Thin films of electroactive PANI
Methods of Analysis	N/A
References	Protocol obtained from Dr. Gouma's laboratory at Stony Brook University (Appendix A)

Solvent Casting Polyaniline Films, Revised 1/21/08	
Purpose	To produce thin films of polyaniline.
Specifications	N/A
Materials and Equipment	<p>Materials</p> <ul style="list-style-type: none"> • Emeraldine base polyaniline, average Mw of 10kD • N-methyl-2-pyrrolidone, biotech grade • 1M HCl • PBS • Small bottles with caps • Four-well chamber slides • 12mm tissue culture discs coated with poly-D-lysine • Tape • Micropipette and tips • Parafilm • Drierite • Petri dish • Small bottles with caps <p>Equipment</p> <ul style="list-style-type: none"> • Fume hood • Shaker

	<ul style="list-style-type: none"> • Vacuum chamber • Box cutter • Tweezers • Nitrogen tank
Procedure	<p>Making PANI:NMP solution</p> <ol style="list-style-type: none"> 1. Mix solutions of 12% wt/vol and 4% wt/vol of PANI in NMP in a small bottle with a cap. 2. Place the bottle on the shaker to dissolve overnight. 3. Store remaining PANI in an inert gas environment by adding nitrogen gas to the bottle, sealing it with parafilm, and placing the bottle in a vacuum chamber containing drierite. <p>Pouring PANI:NMP solutions onto casting surface</p> <ol style="list-style-type: none"> 1. Use a box cutter to make holes in the cover of a petri dish. 2. Put each chamber slide and tissue culture disc into a petri dish cover or bottom. 3. With chamber slides, pour 1-2mL of 4% wt/vol PANI solution into each well using a micropipette. 4. Repeat step 3 with 12% wt/vol PANI solution. 5. With tissue culture discs, pour 35μL of 4% wt/vol PANI solution onto the disc using a micropipette. 6. Repeat step 5 with 12% wt/vol PANI solution. 7. Place a cover on the petri dish and place tape around the crease to seal the top and bottom halves. 8. Leave the dish in a fume hood to allow for evaporation of the NMP solvent. <p>Doping of PANI films</p> <ol style="list-style-type: none"> 1. Remove dry PANI films from each chamber slide using tweezers. 2. Wash the films 3 times with PBS for 5 min. 3. Place each film in a petri dish. 4. Pour 1M HCl in each petri dish until the film is submerged. 5. Remove the films using tweezers after 15 min of doping. 6. Place films in another petri dish to dry.
Expected Results	Thin films of electroactive PANI
Methods of Analysis	N/A

References	[91, 92]
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Solvent Casting Polyaniline Films, Revised 1/29/08	
Purpose	To produce thin films of polyaniline.
Specifications	N/A
Materials and Equipment	<p>Materials</p> <ul style="list-style-type: none"> • Emeraldine base polyaniline, average Mw of 10kD • N-methyl-2-pyrrolidone, biotech grade • 1M HCl • PBS • Glass slides • 12mm tissue culture discs coated with poly-D-lysine • Micropipette and tips • Parafilm • Drierite • Petri dish <p>Equipment</p> <ul style="list-style-type: none"> • Small bottles with caps • Shaker • Fume hood • Plastic container • Vacuum chamber • Tweezers • Nitrogen tank
Procedure	<p>Making PANI:NMP solution</p> <ol style="list-style-type: none"> 1. Mix 4% wt/vol of PANI in NMP in a small bottle with a cap 2. Place the bottle on the shaker to dissolve overnight. 3. Store remaining PANI in an inert gas environment by adding nitrogen gas to the bottle, sealing it with parafilm, and placing the bottle in a plastic container filled with nitrogen gas and drierite. <p>Pouring PANI:NMP solutions onto casting surface</p> <ol style="list-style-type: none"> 1. Place the tissue culture discs on glass slides. 2. Add 35µL of the 4% wt/vol of PANI solution onto the discs using a micropipette.

	<ol style="list-style-type: none"> 3. To ensure uniform diffusion of NMP out of solution, place some of the slides in a vacuum chamber, applying a light vacuum and allowing the air to circulate throughout the chamber (either using a splitter pipe or cracking the chamber open slightly). 4. Place the other slides in the fume hood. 5. Leave the slides in the vacuum chamber or fume hood overnight to allow diffusion of the NMP solvent. <p>Doping of PANI films</p> <ol style="list-style-type: none"> 1. Place the slides containing dry PANI films into a petri dish. 2. Wash 3 times with PBS for 5 min. 3. Place the slides into another petri dish and pour 1M HCl in the dish until the slides are submerged. 4. Remove the slides using tweezers after 15 min of doping. 5. Place the slides in another petri dish to dry.
Expected Results	Thin films of electroactive PANI
Methods of Analysis	N/A
References	[91, 92]

Solvent Casting Polyaniline Films, Revised 1/31/08	
Purpose	To produce thin films of polyaniline.
Specifications	N/A
Materials and Equipment	<p>Materials</p> <ul style="list-style-type: none"> • Emeraldine base polyaniline, average Mw of 10kD • N-methyl-2-pyrrolidone, biotech grade • 1M HCl • PBS • Glass slides • Parafilm • Drierite • Petri dish

	<ul style="list-style-type: none"> • Micropipette with tips <p>Equipment</p> <ul style="list-style-type: none"> • Small bottles with caps • Shaker • Fume hood • Plastic container • Vacuum chamber • Tweezers
<p>Procedure</p>	<p>Making PANI:NMP solution</p> <ol style="list-style-type: none"> 1. Mix 4% wt/vol of PANI in NMP in a small bottle with a cap. 2. Place the bottle on the shaker to dissolve overnight. 3. Store remaining PANI in an inert gas environment by adding nitrogen gas to the bottle, sealing it with parafilm, and placing the bottle in a plastic container filled with nitrogen gas and drierite. <p>Pouring PANI:NMP solutions onto casting surface</p> <ol style="list-style-type: none"> 1. Add 300μL of the 4% wt/vol of PANI solution onto the slides using a micropipette. 2. To ensure uniform diffusion of NMP out of solution, place the slides in a vacuum chamber, applying a light vacuum and allowing the air to circulate throughout the chamber (either using a splitter pipe or cracking the chamber open slightly). 3. Leave the slides in the vacuum chamber overnight to allow diffusion of the NMP solvent. <p>Doping of PANI films</p> <ol style="list-style-type: none"> 1. Place the slides containing dry PANI films into a petri dish. 2. Wash 3 times with PBS for 5 min. 3. Place the slides into another petri dish and pour 1M HCl in the dish until the slides are submerged. 4. Remove the slides using tweezers after 15 min of doping. 5. Place the slides in another petri dish to dry.
<p>Expected Results</p>	<p>Thin films of electroactive PANI</p>
<p>Methods of Analysis</p>	<p>N/A</p>
<p>References</p>	<p>[91, 92]</p>

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Solvent Casting Polyaniline Films – Optimal Protocol	
Purpose	To produce thin films of polyaniline.
Specifications	N/A
Materials and Equipment	<p>Materials</p> <ul style="list-style-type: none"> • Emeraldine base polyaniline, average Mw of 10kD • N-methyl-2-pyrrolidone, biotech grade • 1M HCl • PBS • Glass slides and/or 12mm glass tissue culture discs • Micropipette with tips • Parafilm • Drierite • Petri dish <p>Equipment</p> <ul style="list-style-type: none"> • Small bottles with caps • Shaker • Vacuum chamber • Tweezers
Procedure	<p>Making PANI:NMP solution</p> <ol style="list-style-type: none"> 1. Mix 4 wt%/vol of PANI in NMP in a small bottle with a cap. 2. Place the bottle on the shaker to dissolve overnight. 3. Store remaining PANI in an inert gas environment by adding nitrogen gas to the bottle, sealing it with parafilm, and placing the bottle in container filled with nitrogen gas and drierite. <p>Pouring PANI:NMP solutions onto casting surface</p> <ol style="list-style-type: none"> 1. If using 12mm tissue culture discs, place 35μL of the solution onto the discs using a micropipette. 2. If using glass slides place 300μL of the solution onto the slides using a micropipette. 3. To ensure uniform diffusion of NMP out of solution, place the slides or discs in a vacuum chamber, applying a light vacuum and allowing the air to circulate throughout the chamber (either using a splitter pipe or cracking the chamber open slightly). 4. Leave the discs and/or slides in the vacuum chamber overnight

	<p>to allow diffusion of the NMP solvent.</p> <p>Doping of PANI films</p> <ol style="list-style-type: none"> 1. Place the discs and/or slides containing dry PANI films into a petri dish. 2. Wash 3 times with PBS for 5 min. 3. Pour 1M HCl in the petri dish until the discs or slides are submerged. 4. Remove the discs/slides using tweezers after 15 min of doping.
Expected Results	Thin films of electroactive PANI
Methods of Analysis	N/A
References	[91, 92]

APPENDIX I- Protocol to Test the Contractile Properties of PANI

Contractile Testing of PANI	
Purpose	To determine the contractile properties of PANI
Specifications	<ul style="list-style-type: none"> • Contraction rate of 75 beats/min [21] • Contraction duration < 0.3s [21]
Materials and Equipment	<p>Materials</p> <ul style="list-style-type: none"> • PANI films • PBS • Petri dish • Tape <p>Equipment</p> <ul style="list-style-type: none"> • Grass stimulator • High speed camera • Wires (stripped at ends)
Procedure	<p>Setup</p> <ol style="list-style-type: none"> 1. Tape one end of the wires onto the bottom of the petri dish such that they are opposite each other with about 2in in between. 2. Place the other end of the wires into the output terminals of the Grass stimulator. 3. Pour PBS in the petri dish and place the film of PANI in line with the wires, making sure that the PANI is submerged. <p>Test for visual contraction</p> <ol style="list-style-type: none"> 1. Using a single pulse trigger with a pulse duration of 150ms start. at 1V and slowly increase the voltage in increments of 0.5V up to 10V. 2. Repeat step 1 until a visual contraction is observed. <p>Measuring the contraction length and time</p> <ol style="list-style-type: none"> 1. Place markers on the PANI strip and apply an electrical stimulus as described previously. 2. Record the contraction with a high speed camera and calculate the change in length.

	<p>3. From the video recording, determine the contraction duration.</p> <p>Determining the contraction force</p> <ol style="list-style-type: none"> 1. Setup the experiment as described above and attach a force transducer to the PANI. 2. Apply an electrical stimulus to the PANI and observe the maximum force during contraction.
Expected Results	The force, duration, and length of contraction of PANI films
Methods of Analysis	N/A
References	None

APPENDIX J-Protocol for Manufacturing Microthreads

Making Soluble Collagen Type I Microthreads	
Purpose	To make acid-soluble type I collagen threads
Specifications	N/A
Materials and Equipment	<p>Materials</p> <ul style="list-style-type: none"> • 3mL of collagen solution obtained from rat tail tendon • 500mL of FFB (fiber formation buffer) • 500mL of FIB (fiber incubation buffer) • 5mM HCl <p>Equipment</p> <ul style="list-style-type: none"> • Extrusion pump • Forceps • Glass trays (approximately 1in by 2inc) • Water bath • 0.86mm inner diameter polyethylene tubing • Rotating vessel • Cardboard box
Procedure	<p>Preparation</p> <ol style="list-style-type: none"> 1. Obtain collagen solution by processing rat tail tendon. 2. Dissolve collagen solution in 5mM HCl (final concentration of 10mg/mL) in a rotating vessel at 4°C. <p>Extruding threads</p> <ol style="list-style-type: none"> 1. Turn on water bath and set to 37°C. 2. Place glass tray filled with 500mL of FFB in water bath. 3. Using the syringe, take up ~3mL of collagen solution. Attach the polyethylene tubing to the syringe and connect the syringe to the extrusion pump. <i>Note: Avoid taking up non-dissolved solute into the syringe as it will clog the tubing.</i> 4. Set the extrusion pump to a volume of 3mL with 0.4mL/minute and turn on. 5. Once collagen solution reaches the end of the tubing, slowly drag the tip over the bottom of the tray from one end to the other. <i>Note: It may be difficult to see the microthread coming out and the speed at which the tip should be dragged is relative to the speed of the collagen traveling through the tube.</i> 6. After the desired number of microthreads is extruded, cover

	<p>the glass tray with saran wrap. Cover the water bath and wait for 24 hours.</p> <ol style="list-style-type: none"> 7. Move the microthreads to FIB for 24 hours. 8. Take out microthreads using forceps and hang them on the edges of a cardboard box overnight.
Expected Results	Acid-soluble collagen threads (about 20cm in length and 50 μ m in diameter).
Methods of Analysis	N/A
References	[19]

APPENDIX K-Microthread Mesh Calculations

The equation for the rule of mixtures with a constant strain model is:

$$E_C = E_1 \bar{V}_1 + E_2 \bar{V}_2 + E_3 \bar{V}_3 + \dots \text{ [Eq. 1]}$$

In Eq. 1, E_C is the stiffness of the composite material, $\bar{V}_1, \bar{V}_2, \bar{V}_3, \dots$ are the volume fractions of each constituent material, and E_1, E_2, E_3, \dots are the stiffnesses of each constituent material. The volume fraction of each material is the fraction of the total volume that the material occupies [90].

In this case, the composite material consists of the heart wall and a microthread mesh. Thus, Eq. 1 can be written as follows:

$$E_C = E_H \left(\frac{V_H}{V_H + V_T} \right) + E_T \left(\frac{V_T}{V_H + V_T} \right) \text{ [Eq. 2]}$$

In Eq. 2 E_C = composite stiffness, V_H = volume of the heart wall, V_T = volume of the microthreads, E_H = stiffness of the heart wall, E_T = stiffness of the microthreads. Since the microthreads will be wrapped around the entire length of the heart, the length of the threads is equal to the length of the heart and thus the volume fraction can be simplified to an area fraction:

$$E_C = E_H \left(\frac{A_H}{A_H + A_T} \right) + E_T \left(\frac{A_T}{A_H + A_T} \right) \text{ [Eq. 3]}$$

The following assumptions were made to simply the calculations:

- The heart is a hollow sphere
- All microthreads have the same properties (length, cross-sectional area, and stiffness)
- The stiffness of the right and left ventricles is the same
- The heart wall is linear elastic
- The stiffness of the heart wall is constant in the direction of the fibers and is constant in the direction perpendicular to the fibers.
- Fibers in the heart wall run vertically from apex to base

In order to better visualize the cross-sectional area of each material within the composite, a cross-sectional view of the heart wall was drawn and can be seen in Figure 26.

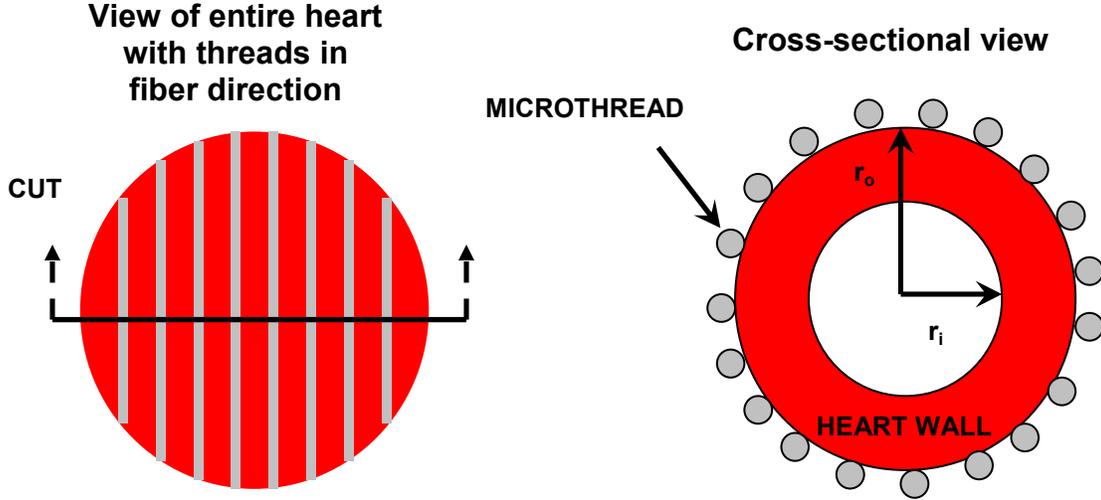


Figure 26: Cross-Sectional View of the Composite Material

Since the heart was assumed to be spherical, the cross-sectional area of the wall was calculated by subtracting the area of inner circle from the area of the outer circle:

$$A_H = \pi(r_o^2 - r_i^2) \text{ [Eq. 4]}$$

The area of the microthreads was calculated by multiplying the cross-sectional area of one thread (A_C) by the total number of threads (N):

$$A_T = A_C N \text{ [Eq. 5]}$$

From Hooke's law for springs in parallel, the total stiffness of the system is the sum of the stiffness of each spring. Since the microthreads are placed in parallel, the total stiffness of the microthreads is the stiffness of one thread multiplied by the number of threads. Therefore, Eq. 3 can be re-written as follows by substituting Eq. 4 and 5:

$$E_C = E_H \left[\frac{\pi(r_o^2 - r_i^2)}{\pi(r_o^2 - r_i^2) + A_C N} \right] + N \times E_T \left[\frac{A_C N}{\pi(r_o^2 - r_i^2) + A_C N} \right] \text{ [Eq. 6]}$$

The following values were assumed from literature and were substituted into Eq. 6:

$$E_T = 4.0 \text{MPa [19]}$$

$$A_C = 25,900 \mu\text{m}^2 \text{ (for hydrated microthreads) [19]}$$

$$r_o = 60 \text{mm [38]}$$

$$r_i = 43\text{mm} \text{ (} t = 17\text{mm) [87]}$$

Since the stiffness of the heart is different in the fiber and cross fiber directions, the calculations must be performed in both directions using the appropriate value for stiffness:

$E_H = 90,000\text{Pa}$ in the fiber direction for a heart with right ventricular hypertrophy [95]

$E_H = 25,000\text{Pa}$ in the cross fiber direction for a heart with right ventricular hypertrophy [95]

The composite stiffness (E_C) was taken to be 10% greater than the stiffness of the heart wall, which is equal to 99,000Pa in the fiber direction and 27,500Pa in the cross fiber direction. It was found that **N = 21.8 microthreads** in the fiber direction and **N = 11.5 microthreads** in the cross fiber direction.

After calculating the number of microthreads required for the stiffness of the composite to be 10% greater than that of the heart wall, the structural properties of the mesh were confirmed to ensure that the mesh would reduce the dilatation of the heart wall. From basic mechanics:

$$\sigma = \frac{F}{A} = \frac{T \times t}{A_{\text{total}}} = E \times \varepsilon \quad [\text{Eq. 7}]$$

In Eq. 7 σ = stress, F = force, A = area, T = wall tension, t = wall thickness, E = wall stiffness, and ε = strain. Simplifying Eq. 7 gives the following:

$$\varepsilon = \frac{T \times t}{E \times A} \quad [\text{Eq. 8}]$$

In order to decrease the amount of dilatation, the mesh should result in a decrease in the strain. In Eq. 8 the wall tension and wall thickness remain constant and therefore in order for the strain to decrease, the product of the stiffness and area must increase.

With the heart wall alone:

$$E \times A = E_H \pi (r_o^2 - r_i^2) \quad [\text{Eq. 9}]$$

With the addition of the microthread mesh restraint:

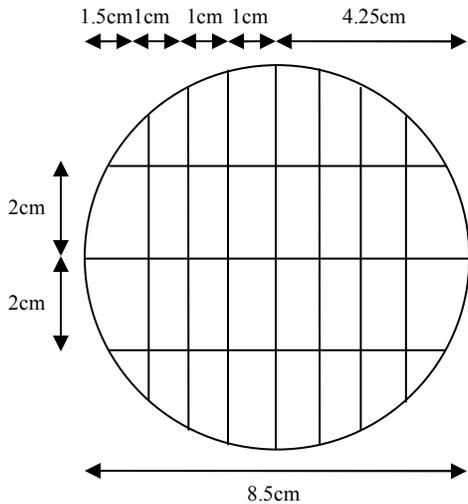
$$E \times A = E_C [\pi (r_o^2 - r_i^2) + A_C N] \quad [\text{Eq. 10}]$$

The product of stiffness and area was calculated with the heart wall alone and with the microthread mesh using Eq. 9 and Eq. 10 and the values were obtained from

literature, as described above. In the fiber direction, for the heart wall alone **EA is 495.1N** and with the microthread mesh **EA is 544.6N**. In the direction perpendicular to the fibers (cross fiber direction), for the heart wall alone **EA is 137.5N** and with the microthread mesh **EA is 151.3N**. In both directions EA has increased and therefore the mesh will be able to reduce the dilatation of the heart wall.

APPENDIX L-Protocol for Cell Migration Assay

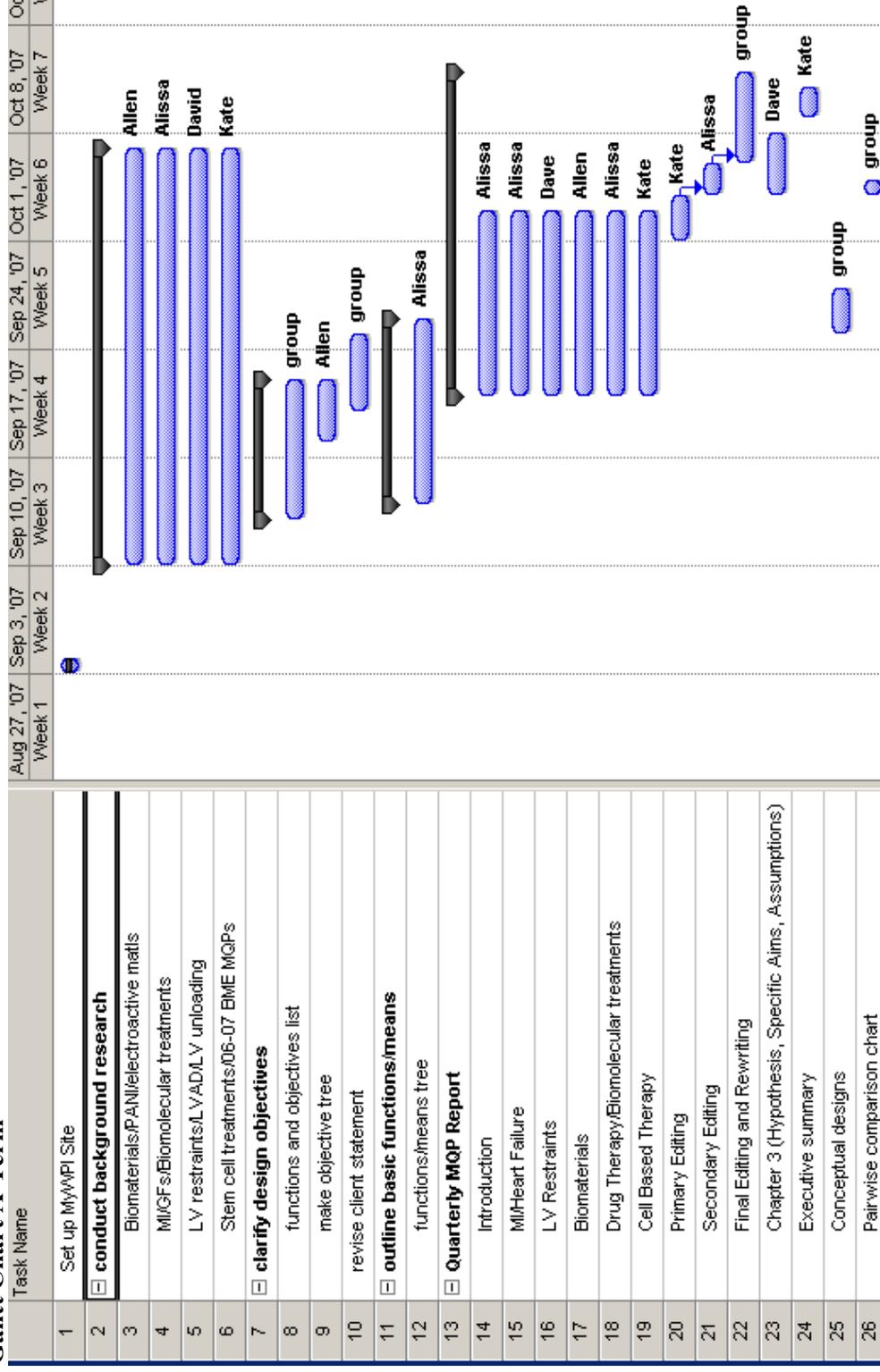
Cell Migration Assay	
Purpose	To compare the rate of migration of SMCs to microthreads coated in PDGF to uncoated microthreads.
Specifications	N/A
Materials and Equipment	<p>Materials</p> <ul style="list-style-type: none"> • Silicone elastomer • PDMS curing agent • Petri dishes • 70% ethanol • PBS • PDGF solution (10ng/mL PDGF-ββ in DMEM + 20% FBS + 1% pen/strep) • Microthreads • Sterile silicone glue • Cell culture media (DMEM + 10% FBS) • 0.25% trypsin <p>Equipment</p> <ul style="list-style-type: none"> • Vacuum chamber • Oven set to 50°C • Scalpel • Autoclave • Sterile hood • Inverted light microscope with camera • Adobe Photoshop CS2
Procedure	<p>Making PDMS blocks</p> <ol style="list-style-type: none"> 1. Mix silicone elastomer and PDMS curing agent in a ratio of 10:1. 2. Pour PDMS mixture into petri dishes. 3. Place in a vacuum chamber to remove air bubbles. 4. Place in an oven set to 50°C for approximately 2 hours. 5. Remove the PDMS from the petri dish and cut in half with a scalpel. (The diameter is 8.5cm so the PDMS should be cut at 4.25cm.) Remove the ends of the half circles with a scalpel by cutting 2cm from the ends of the half circles. 6. Sterilize the PDMS blocks in an autoclave. <p>Microthread preparation</p>

	<ol style="list-style-type: none"> 1. Sterilize microthreads in 70% ethanol for 24 hours. 2. Wash microthreads in sterile PBS three times for 5 minutes per wash. 3. For PDGF coated microthreads, place the threads in the PDGF solution for 24 hours and subsequently wash the threads in PBS 3 times for 5 minutes per wash. <p>Performing the cell migration assay</p> <ol style="list-style-type: none"> 1. Attach the microthreads to one side of the petri dish using sterile silicone glue. 2. Draw gridlines on the cover of the petri dish, as outlined below: <div style="text-align: center;">  </div> <ol style="list-style-type: none"> 3. Place the PDMS blocks in the petri dish under the sterile hood. 4. Trypsinize cells and count them using a hemocytometer. 5. Culture SMCs on the side of the plate opposite the microthreads placing ~50,000 cells on each plate. 6. Allow the cells to plate down for 24 hours. 7. Aspirate the media to remove any floating cells. 8. Remove the PDMS block and add fresh media. 9. Visualize the plate using an inverted light microscope with a camera adapter to observe the migration and proliferation of the cells. 10. Take pictures of each plate at the intersection of each grid line. 11. Stitch the pictures together using Adobe Photoshop CS2.
<p>Expected Results</p>	<p>The cells cultured in the plates with PDGF coated microthreads will migrate more quickly than cells cultured with control samples.</p>
<p>Methods of Analysis</p>	<p>N/A</p>

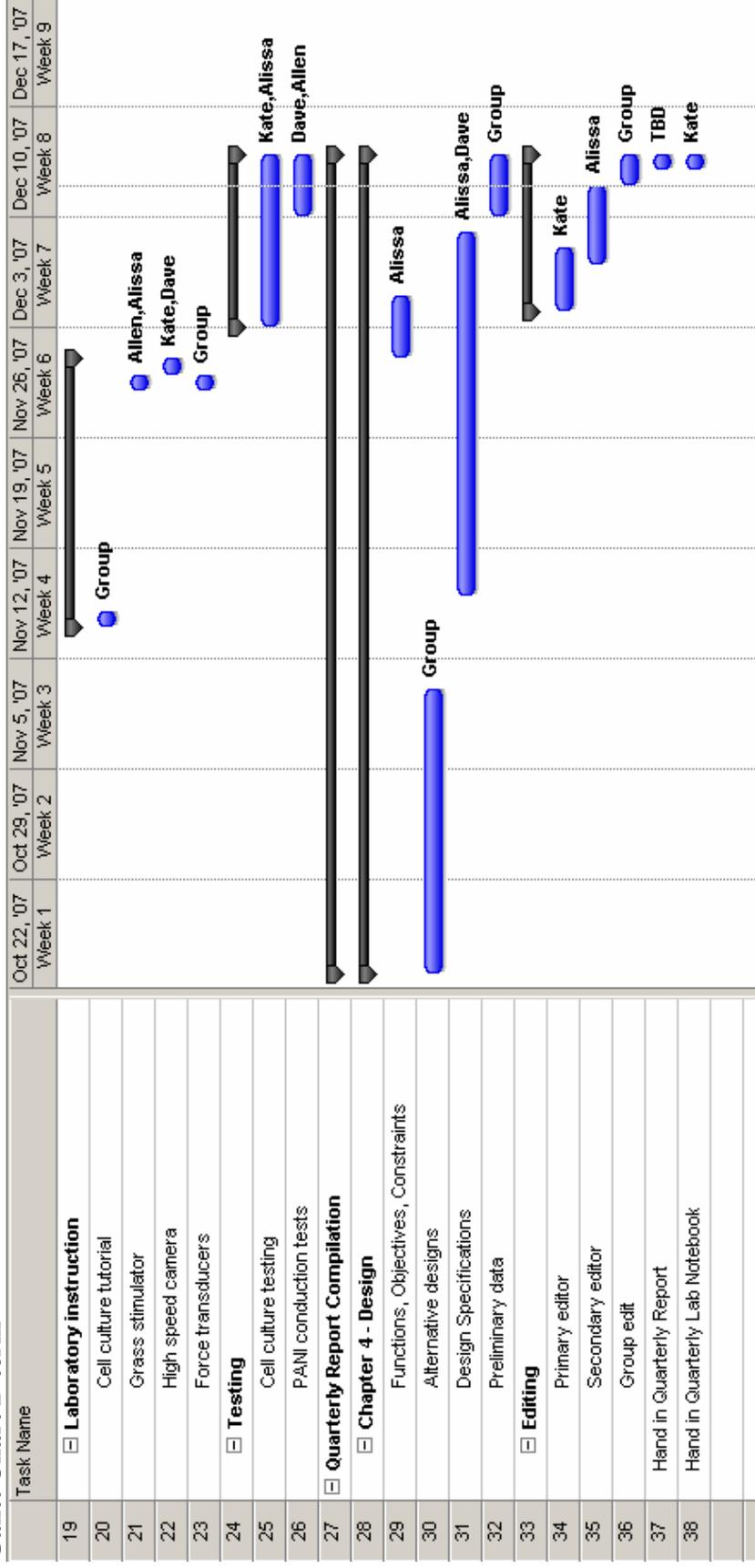
References	Protocol obtained from Professor Gaudette's laboratory.

APPENDIX M – Gantt Charts

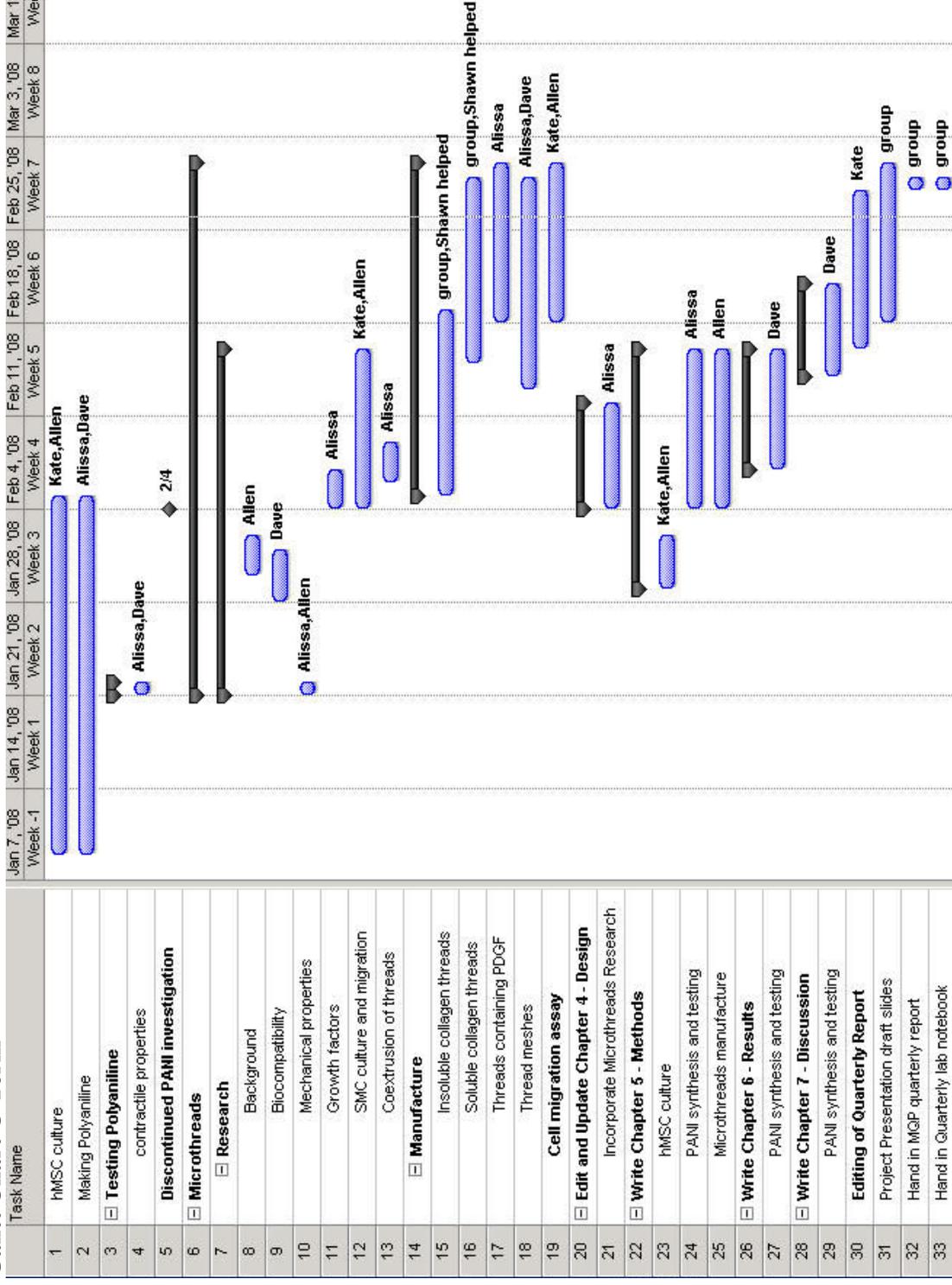
Gantt Chart A-Term



Gantt Chart B-term



Gantt Chart C-Term



Gantt Chart D-Term

