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Development of a Protective Sheath for Delivering Cell Seeded Microthreads to the Heart

Kyley Jean Chornoby
Worcester Polytechnic Institute

Mark Christopher Kowaleski
Worcester Polytechnic Institute

Rebecca Rey Holmberg
Worcester Polytechnic Institute

Development of a Protective Sheath for Delivering Cell Seeded Microthreads to the Heart

A Major Qualifying Project
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the Degree of Bachelor of Science

Kyley Chornoby

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Rebecca Holmberg

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Mark Kowaleski

_____

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Report Submitted To:
Professor Glenn Gaudette, Ph.D.
Graduate Student Mike Fakharzadeh
Worcester Polytechnic Institute

Abstract

Stem cell delivery has shown promise in cardiac regeneration. One method of delivery is the use of human mesenchymal stem cells (hMSCs) seeded on biological microthreads. However, during delivery, the cells are often damaged due to exposure to shear stresses created by the heart wall. The purpose of this project was to design a protective sheath encompassing the threads to increase the number of cells successfully delivered. The team developed a sheath that can be easily manufactured as well as quickly assembled with the microthreads and suture needle. Additionally, the sheath possesses enough mechanical stability to withstand the forces placed on it during surgery. To accomplish this, the team selected several biomaterials based on literary research and then conducted various tests to determine the properties required of the biomaterial in order for it to protect the cell-loaded microthreads. This included establishing mechanical strength and delivery rates of the sheath. The team discovered that a protective sheath made from an electrospun variable form of polyethylene terephthalate (PET) provided sufficient mechanical stability to protect the hMSC-loaded microthreads during delivery to the heart.

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*Note: all group members contributed to writing each section of this report.

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Introduction

The heart must be able to remain functional and pump blood to the lungs and the rest of the body in order to maintain life. However, as the population continues to age, complications from heart disease can cause serious health problems or death. Myocardial infarction (MI), or more commonly known as a heart attack, affects approximately 1.2 million Americans each year. Five million Americans experience heart failure every year, which is estimated to contribute to 300,000 deaths (Berger, 2009). Myocardial infarction causes cell death within the heart wall, and if severe enough, MI can eventually lead to heart failure. This in turn, can sometimes require a heart transplant, but the demand for transplantable hearts is much higher than the available supply. Thus, there is great need for better treatment of heart disease and heart failure.

Regenerative medicine and the use of stem cells are currently being explored in researchers' applications for treating heart disease. Various types of stem cells exist, but it is embryonic, induced pluripotent, and human mesenchymal stem cells that hold the most potential in myocardial regeneration applications. Each of these three types of stem cells possesses advantages and disadvantages, but human mesenchymal stem cells are currently the most used because they have been shown to improve cardiac function (Fox, 2009, Amado et al, 2005, and Pittenger, 2004).

Scientists have been developing and testing stem cell delivery methods. However, many of these methods have limitations including a lack in site specificity and efficiency in delivering viable stem cells to the target region (Murphy, 2008). The most common delivery techniques being used today include intravenous injection, endocoronary infusion, intramyocardial injection, and implantable scaffolds.

A new technique is also being developed involving the use of biodegradable microthreads. The stem cells are seeded on the microthreads, which are then sewn through the infarcted area of the heart. This allows for greater control of implantation and engraftment, providing the surgeon with the ability to precisely guide the microthreads to the exact location within the heart wall. Despite recent successes with delivering viable stem cells to the infarct, the fibrin microthread technique exposes the stem cells to shear stresses during implantation. This often results in removal or damaging of the stem cells.

It is the purpose of this team to design, develop, and test a suitable form of protection for the cells seeded on the biodegradable microthreads. This design will reduce the shear stresses placed on the cells during implantation and in turn, increase the quantity and quality of viable cells to the infarcted area.

Background and Literature Review

In this chapter we begin by introducing the general anatomy and pathology of the heart and the need for addressing heart complications such as myocardial infarction and heart failure. Second, we provide a review of regenerative medicine and stem cells, which currently are receiving great attention from the medical and scientific communities as solutions to these cardiac complications. We explore five methods presently used to deliver stem cells to damaged myocardium, and we investigate several biomaterials that hold potential for developing a method that better protects the cells during fibrin microthread delivery. We present this information in the following sections.

The Heart

The heart is a muscular organ responsible for pumping blood throughout the body. As a vital organ, the heart operates in conjunction with the circulatory system to provide oxygenated blood to other organs and supplying energy to power cellular processes. It is divided into four chambers: the left and right atria and the left and right ventricles. The atria receive the blood, whereas the ventricles pump the blood from the heart to different organs of the body completing the circulatory cycle (American Heart Association, 2003).

The heart plays a vital role in maintaining a healthy life. However, certain complications can arise such as ischemia, blocked arteries, or arrhythmias, all of which can lead to serious health problems.

Heart Failure and Myocardial Infarction

Myocardial infarction occurs when blood flow from the blood vessels to the heart is blocked or impeded, thus interrupting the oxygen supply to the myocardium (Mayo Clinic, 2010). Consequently, the heart muscle dies. Heart failure is a condition in which the heart is unable to pump enough blood throughout the body (Berger, 2009). The leading cause of heart failure due to MI occurs when roughly 25% of the cardiomyocytes in the ventricle die (Murry, et al., 2006). When the blood is incapable of reaching other parts of the body, additional problems may arise such as congestion in the lungs, liver, gastrointestinal tract, and limbs. Insufficient blood flow leads to a lack of oxygen and nutrition, damaging the organs and reducing their ability to function properly. Each year, approximately 1.2 million Americans suffer from MI and about 5 million have heart failure contributing to an estimated 300,000 deaths (Berger, 2009). The estimated total cost of heart failure in the United States in 2008 was approximated to be more than $35 billion (Cowie, 2000). On any given day, approximately 4,000 people are in need of a heart transplant (Mayo), but only 2,210 and 2,192 were performed in the United States in 2007 and 2006, respectively (American Heart Association, 2009). Due to the increasing need for heart transplants as well as the limited supply of donors, only half of the patients in need actually receive transplants. Because of the large number of deaths each year due to heart failure and the limited amount of heart transplant donors, medical advancements are imperative. More specifically, researchers are continuously in search of new methods of cardiac regeneration.

Regenerative Medicine

Regenerative medicine is a relatively new field of science involving the creation of functional and living tissues in order to address a wide range of medical issues. It helps natural processes work faster, as well as aids in the repair and re-growth of natural tissues and organs. It typically involves experimentation with stem cells, biomaterials, or the manipulation of cell-signaling. Regenerative medicine holds the potential for scientists to develop replacement organs for those in need of transplants as well as discovering new ways to address certain diseases (National Institutes of Health, 2009).

Stem Cells

Stem cells can be found all over the body, and they possess the ability to differentiate into new cell types. They are different from other cells in the body because they are unspecialized and can continually divide via cell division. Additionally, through the use of certain additives and precisely controlling their living environment, some stem cells can be manipulated into differentiating into a certain cell type (Fox, 2009).

The ability of stem cells to regenerate into certain cells and tissues is very controversial within the scientific community, especially concerning adult stem cells. Some believe stem cells can undergo a process called transdifferentiation. This means a stem cell can turn into a cell that is entirely different from its previous state (Orlic, et al, 2002). For example, a stem cell found in the brain might be believed to transdifferentiate into a skin cell. Others, however, claim that this process of transdifferentiation is impossible; they believe stem cells remain within their original cell lines (Murry, et al, 2004). Yet another group of scientists believe stem cells do not differentiate at all; they believe stem cells secrete growth factors which direct other nearby cells to grow, replicate, and repair (Lee and Makkar, 2004). Additionally, stem cells can be categorized by their potency levels. Some stem cells are pluripotent, meaning they can become any type of cell within the body; these are typically called embryonic stem cells (ESCs). Multipotent stem cells are unable to participate in transdifferentiation, which most scientists term "adult stem cells." Unipotent stem cells simply self-renew themselves and have already differentiated into their specific cell-type. Both of these cell types are considered to have "limited differentiation" capabilities because they can only specialize into certain cell types (National Institutes of Health, 2009).

Embryonic, induced pluripotent, and mesenchymal stem cells are the primary three types of stem cells with prior success in cardiac regeneration.

Embryonic Stem Cells

Embryonic stem cells are pluripotent, giving them high potential in regenerative medicine and various medical applications. Not only have they been shown to differentiate successfully into cardiomyocytes, but once differentiated, embryonic stem cells have been shown to express heart proteins, possess myofibrillar organization, and the ability to contract (Schuldt, et al., 2008). However, using ESCs requires scientists to destroy blastocysts, which is where this type of stem cell is located; consequently, many people object to using ESCs because of the ethical, religious, and political controversies associated with them. Additionally, while ESCs' high potency provides them with the ability to become any type of cell, it unfortunately also makes them more likely to

proliferate uncontrollably and lead to the formation of teratomas (National Institutes of Health, 2009).

Induced Pluripotent Stem Cells

Another type of stem cell with potential for myocardial regeneration is induced pluripotent stem cells (IPSCs). They were developed in 2007 when, Shinya Yamanaka, a doctor in Japan turned regular adult cells back into embryonic stem cells. Embryos were not used in this process, thus avoiding the embryonic stem cell controversy. To create these IPSCs, Yamanaka located the gene switches responsible for programming human skin stem cells, and he turned those same switches on in the adult skin cells. This caused the skin stem cells to turn back to their pre-differentiated status as embryonic stem cells. With years of experimentation, Yamanaka discovered the four genes responsible for reverting differentiated cells back to an embryonic, pluripotent state. Problems, however, began to surface as further research was conducted on these new stem cells. One of the four genes Yamanaka identified was an oncogene. Therefore, when the IPSCs were placed within mice, cancerous growths began to form (Fox, 2009). Additional research is being done to eliminate the need for the oncogene in producing IPSCs; however, achieving successful implantation of these cells without triggering the formation of tumors currently requires further research and experimentation.

Human Mesenchymal Stem Cells

Human mesenchymal stem cells (hMSCs) are derived from bone marrow and are able to differentiate into different types of cells including fat, cartilage, and bone. Many studies have been conducted to determine whether or not hMSCs can also differentiate into cardiomyocytes, or heart muscle cells. Despite this research, the actual mechanism by which hMSCs operate and help to restore heart function still remains controversial. Some believe the cells simply generate new myocardium through either transdifferentiation, fusion with existing living cardiomyocytes, or secretion of growth factors (Lee and Makkar, 2004). Others believe the hMSCs help release myocardial wall tension at the area of infarct, thereby allowing the heart to more easily contract (Luciano, 2006). Either way, the studies show that implantation of hMSCs to damaged heart tissue helps increase heart functionality. Additionally, using hMSCs in cardiac regeneration applications does not require the patient to take immunosuppressant drugs because the stem cells come directly from the patient. hMSCs have also been shown to increase angiogenesis, or the growth of capillaries, which helps increase blood flow around the heart and supports myocardial regeneration efforts (Steinhoff, 2009). hMSCs, therefore, hold much promise in the field of cardiac regeneration.

Delivery Methods

Currently there are several accepted delivery techniques for stem cells to infarcted areas of the heart. They are intravenous injection, intracoronary infusion, intramyocardial injection, and scaffold implantation. A new approach to scaffold delivery is biological microthreads.

Intravenous Injection

Intravenous injection (IV) is a technique using a suspension of stem cells that is directly injected into a vein of the subject. This allows the cells to circulate throughout the heart and the rest of the body. This method has been of particular interest because IV

is minimally invasive when compared with other methods currently used and/or investigated (Wolf, et al, 2009). Studies have demonstrated that hMSCs delivered through intravenous delivery migrate to the injured target site of the heart (Pittenger, et al, 2004). However, other studies have observed a large majority of the cells never reaching the heart infarct. Immediately following injection, cells are often trapped in the lungs, which can lead to severe lung damage (Fischer, et al, 2008). While some of the stem cells do in fact reach their intended target site, others are often found migrating to other filtering organs including the liver, spleen, and kidneys (Fischer, et al, 2008). Within the first hour of injection 50-60 % are caught in the lungs, four hours later 42% in the spleen, 21% in the liver and only 6% in the lungs (Fischer, et al, 2008). This lack of site localization is the major drawback of intravenous injection. Acknowledging this distinct disadvantage, researchers have hypothesized that although the majority of the delivered cells are trapped in the lungs, the hMSCs secrete TSG-6, an anti-inflammatory protein, which results in a decreased infarct size (Lee, et al, 2009). In spite of this, there is a major concern with this method in which hMSCs have the potential to continue to differentiate and proliferate. A low percentage of the stem cells reach the infarcted region of the heart, while a high percentage of them are distributed throughout the body, causing potential for abnormal growths to occur in any area of the body where the cells are trapped (Freyman, et al, 2006). Since it is difficult to track the migration and location of the delivered cells, tumors may develop in areas where they are least expected. Therefore, despite the fact that this method is minimally invasive, IV carries many risks and disadvantages.

Intracoronary Infusion

The second method of stem cell delivery to the heart, intracoronary infusion utilizes a stoppage of blood flow in order to engraft stem cells (Perin, et al, 2008). This requires the use of an angioplasty balloon in order to successfully engraft the stem cells. This angioplasty balloon is inserted in a coronary artery upstream of the infarct and inflated, which stops arterial blood flow. A solution containing the stem cells is then infused behind the inflated balloon. After two minutes, blood flow is restored to the coronary artery by deflating the balloon. This process is repeated multiple times until the entire solution of stem cells is delivered (Freyman, et al, 2006). Although intracoronary infusion is more site-specific than the intravenous injection technique, a majority of the cells get distributed throughout the body away from the intended target site. This is shown with an engraftment rate of approximately 3%. Furthermore, after one to two hours of injection, stem cell retention rate ranged between 1.3% and 5.3% (Laflamme and Murry, 2005). This is due to the huge influx of blood when the balloon is deflated, causing the stem cells to be washed away. Intracoronary infusion has also been shown to decrease arterial blood flow, which can lead to further myocardial damage (Freyman, et al, 2006). Cells that were found to attach to the myocardium were localized around the edge of the infarct or area of healing. Cells were absent from the center of the injury site, showing low cell survival in the ischemic area of the heart (Perin, et al, 2008)

Intramyocardial Injection

Another method of stem cell delivery is intramyocardial injection. This technique utilizes multiple direct injections of a stem cell suspension into and around the infarct. Studies have shown that this is a practicable method of delivery, and complications have yet to be observed other than some stem cells leaking through the injection track when

the heart contracts. Furthermore, these studies have hint to researchers that improved myocardial contractions occur post injection (Laflamme and Murry, 2005). It is the most localized injected delivery method because the stem cells do not migrate as far away from the injection site as the intravenous injection and intracoronary infusion techniques (Freyman, et al, 2006). With intramyocardial injection however, the stem cells are found to migrate within the tissue and do not remain at the specific intended target site because some travel back out of the injection tract as the heart contracts.

Scaffold

The fourth method of stem cell delivery used in myocardial regeneration efforts involves using a scaffold or a cardiac patch. The implantation of a scaffold replaces or covers infarcted tissue with a tissue engineered graft. This graft acts like a template for the newly transplanted cells (Alperin, et al., 2005). Stem cells are seeded on the scaffold, which can be made of a variety of biodegradable biomaterials. That material, from which the scaffold is made, however, must be able to support cardiac function, survive the ischemic period after the infarct, and withstand the demanding mechanical properties of the heart. For example, some patches are constructed of natural type I collagen, synthetic polymers such as PLGA or polyurethane, or composites of both natural and synthetic materials (Alperin, et al., 2005). This method allows for the stem cells to be implanted directly and uniformly on the site of the infarct, leading to higher engraftment rates. Previous studies have shown engraftment rates of approximately 23% (Simpson, et al., 2007). In another study, few to no stem cells were found to have migrated to other organs (Park, et al., 2005). However, the seeding of a scaffold requires significant incubation time and access to a bioreactor. Another complication with the scaffold is the need for an invasive procedure to suture the patch onto the infarct area (Park, et al., 2005).

Biodegradable Microthreads

Thread-like scaffolds are used to imitate the fundamental fibrous structural elements found in native tissues such as tendon, ligament, and dermis. Fibrin is a natural biomaterial used for creating biopolymer microthreads. It contains cell-signaling properties that arbitrate the initial phase of tissue regeneration by promoting cell migration, attachment, and proliferation from the wound margin. It comes from a combination of fibrinogen and thrombin. The biodegradable microthreads can be produced from a mixture of bovine fibrinogen and bovine thrombin with the presence of calcium under a constant flow, allowing the thrombin to cleave a peptide on the fibrinogen molecule to facilitate fibrin polymerization and thread formation. This method is a novel technique for tissue regeneration. However, it is unknown whether or not this material provides both sufficient mechanical integrity and biodegradation for new tissue in-growth (Pins and Cornwell, 2007).

Fibrin can be used to prevent blood loss, promote granulation tissue formation as a guide for the migration and proliferation of fibroblasts, and work as a sponge for cytokines and growth factors. Fibrin has the ability to promote repopulation and regeneration, as well as a high binding affinity for cytokines, growth factors, proteases and protease inhibitors, which promote key cell functions for wound healing. All together, fibrin, growth factors, and other bioactive molecules act to promote cellular infiltration and remodeling to ultimately regenerate tissue (Cornwell, 2007).

Fibrin is a novel biopolymer and a natural structural protein of healing tissues. It can be made from autologous materials, and fibrinogen can be isolated from a patient's own blood, ensuring no immunological rejection.

However, fibrin has a limited initial mechanical strength for high load bearing situations. When used *in vitro*, the mechanical integrity and strength properties are dependent upon the initial concentration of fibrinogen (Cornwell, 2007).

Biodegradable microthreads aligned in bundles significantly aid in tissue regeneration. hMSCs can be seeded on these microthreads, which have been tested in various experiments to confirm that the hMSCs remained viable, were still able to proliferate, and maintained their ability to differentiate (Murphy, 2008).

Currently, biodegradable microthreads attached to a surgical needle are being used because this assembly mimics the common surgical procedure of placing a suture into a patient. This allows the surgeon to use a familiar technique without needing to be retrained. Biodegradable microthreads, with less than a 100μm diameter each, provide contact guidance, alignment, and orientation of the seeded cells (Cornwell, 2007). The needle-thread combination and small size of the threads also allows the surgeon to precisely place the hMSCs in the exact location of the infarct. Additionally, the biodegradable microthreads support all hMSC mechanisms, whether it is paracrine signaling or differentiation or transdifferentiation (Murphy, 2008).

In Table 1, the engraftment rates from studies conducted using various animal models are summarized.

Table 1: Engraftment Rates from Studies Conducted Using Various Animal Models (DiTroia, et al., 2008)

Delivery Method	Engraftment Percentage
Intravenous injection	3%
Intracoronary infusion	6%
Intramyocardial injection	12%
Scaffold	23%
Biodegradable microthreads	65-70%

Potential Biomaterials

This section explores potential biomaterials from which the protective sheath can be made.

Polyurethane

Polyurethane is a segmented polymer with alternating hard segments and soft segments. Each polyurethane molecule consists of a macrodiol (or polyol) or backbone, a diisocyanate, and a chain extender (Tatai, et al, 2007). The backbone of polyurethane is the short segment, and it provides the polymer with flexibility. Both the diisocyanate and the chain extender are part of the hard segment. They contribute to the polymer's strength, and they act as crosslinking elements (Wright, 2006).

The ability to tailor polyurethane to exact specifics is a great advantage. By changing the chemicals and the ratio between soft segments and hard segments, it is possible to manipulate the physical and chemical properties of this polymer. Additionally, polyurethane is biodegradable, however, it can take weeks or years depending on how it was manipulated (Tatai, et al, 2007).

There are two main types of polyurethanes based on their structures, and the basic molecule of polyurethane can be seen in Figure 1. The first type is an aromatic polyurethane, which contains benzene rings. This gives the polymer high tensile strength and strong chemical resistance. The second type, aliphatic polyurethane, has a backbone consisting of a hydrocarbon. Because of this, aliphatic polymers are more flexible than other polyurethanes (Wright, 2006).

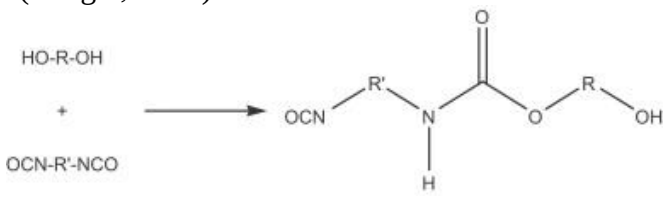

Figure 1: Polyurethane Molecule (Tatai, et al., 2007)

Medical grade polyurethane was first purposed as a biomaterial in 1967 (Boretos, Pierce, 1967). It is chosen in many applications because of its mechanical properties, blood compatibility and tailorability. It has been used in blood bags, heart valves, and vascular grafts (Kanyanta and Ivankovic, 2009).

Polytetrafluoroethylene (PTFE)

Polytetrafluoroethylene (PTFE), also known as Teflon, is a polymer with repeating CF_2-CF_2 chains as shown in Figure 2.

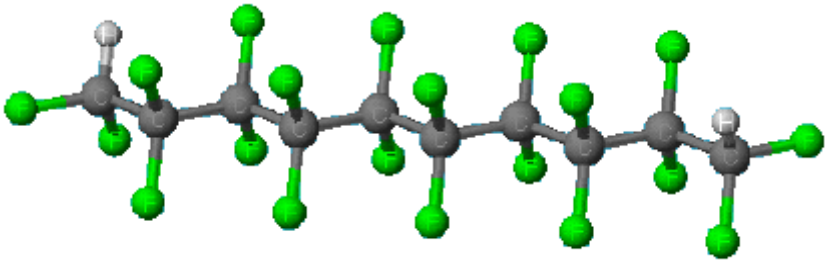

Figure 2: Chemical Structure of PTFE (Lenntech, 2009)

PTFE holds extraordinary characteristics that make it the ideal choice for a variety of products and applications. PTFE has one of the lowest coefficients of friction compared to any other material (Plastomer Technologies, 2009). It is abrasion resistant, meaning it is adaptable to harsh environments, flame resistant with a high melting point, and chemically inert and pure (Plastomer Technologies, 2009). It is also resistant to many chemicals, corrosion, weather, UV, and adherence (Lenntech, 2009). PTFE possesses great dielectric properties as well. This means that PTFE, as an insulating material, can withstand high voltages before it breaks down (Lenntech, 2009). PTFE is serviceable over a wide range of temperatures without a significant change in its physical characteristics or mechanical properties (Faughnan, et al., 1998). Table 2 lists a variety of PTFE's properties.

Table 2: List of Properties for PTFE (Plastomer Technologies, 2009)

Property	Units	Virgin PTFE	Reprocessed PTFE	25% Glass PTFE
Specific Gravity	N/A	2.14-2.20	2.15-2.20	220-230
Tensile Strength	PSI	1500-3500	1500-2400	2000-3000
Elongation	%	250-350	75-200	100-260

Hardness	Durometer "D"	50-60	N/A	55-65
Water Absorption	%	0.001	N/A	0.013
Coeff. of Friction (Static)	N/A	0.04	N/A	0.085
Dielectric Constant	N/A	2.00	2.26	2.4
Dielectric Strength	Volts	1000	450	235
Coeff. of Thermal Expansion	In./In./Ft.	5.5 x 10.3	N/A	2.75 x 10.3
Coeff. of Thermal Conductivity	Btu/hr/ftz	1.7	N/A	3.12

PTFE has a long service life, retaining its properties over a long period of time, even when exposed to extreme temperatures, UV light and oils, oxidizing agents and solvents, or in water (Plastomer Technologies, 2009). In previous studies, PTFE has been used as a graft material for ophthalmic plastic surgery (Karesh, 1987). In this study, PTFE was found to be a non-antigenic, autoclavable, inert polymer that can be incorporated into surrounding tissue through fibrous ingrowth (Karesh, 1987).

PTFE is widely used in many different applications. These include resins, additives, coatings, and films (Lenntech, 2009). The most common market application is a non-stick coating for cookware (Dupont Teflon). PTFE is also applied to a variety of different industries such as semiconductor, medical, chemical, automotive, electrical, aerospace, filtration, wire and cable, and petrochemical (Plastomer Technologies, 2009). More specifically, PTFE is used in labs for piping, tubing, and different containers. For example, PTFE tubing has been used for preconcentration techniques to determine specific levels of different chemicals in water samples (Som-Aum, 2002). Likewise, PTFE has been used in another study as a filter tube for a similar preconcentration procedure to analyze trace elements in an aqueous sample (Murakami, 2006).

Polyethylene terephthalate (PET)

Polyethylene terephthalate (PET) is a non-degradable thermoplastic polymer most commonly found in packaging materials. It is used in the packaging of foods, beverages, drugs, and cosmetics (Limam, et al., 2005). Its wide variety of use in these applications is due to its favorable physical, mechanical, and chemical properties. It is highly resistant to chemicals, impermeable to gas and water vapor, transparent, very resistant to fatigue, and can be made into films or fibers. Additionally, it is very light in weight, has a high melting point, and is resistant to creasing (Ceretti, et al., 2009 and Yang, et al., 2009).

Made from terephthalic acid and ethylene glycol, PET has been found to contaminate the contents of its containers due to the migration of plasticizers. These plasticizers are esters of phthalic acid, which are added to PET to increase its flexibility. Because they are not covalently bonded to the PET, they are easily released into the container's contents, and they have been linked to testicular and liver damage, liver cancer, and have formed teratomas in rodent models.

Despite these complications, PET has been explored as a biomaterial in biocompatible, hemocompatible, antimicrobial surfaces, textiles, heart valve sewing rings, and vascular tissue engineering applications (Goddard, et al., 2007). For example, it has also been used in passive diastolic restraining (Chen, et al., 2008). Furthermore, experimentation with surface modifications to PET has helped to increase its poor wettability and adhesive properties (Yang, et al., 2009).

One study observed the effects of gamma sterilization on meshes made from PET and compared them to those made from polypropylene that underwent the same sterilization procedure. Through the use of various microscopy techniques, it was observed that the PET remained undamaged while the polypropylene meshes experienced significant damage. This study demonstrates that devices made from PET are easily sterilized and do not undergo any physical changes when being sterilized (Bracco, et al., 2005).

Potential Sheath Production Techniques

This section explores the various methods by which fibers can be made from polymers. The first method explained is electrospinning, followed by injection molding and extrusion.

Electrospinning

The process of electrospinning involves using high voltages and jet stream of the desired polymer to make fibers. The fibers' diameter can range anywhere between 1nm and 1μm depending on the polymer being used and the voltage. The process is generally cost effective; however, inexperienced technicians may experience difficulty in maintaining a stable and constant jet stream of polymer (Ramakrishna, et al., 2005). Figure 3 depicts a schematic of the components involved in electrospinning and how they work together.

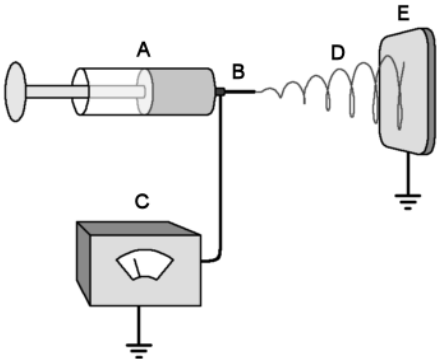

Figure 3: Process of Electrospinning (Ramakrishna, et al., 2005)

The polymer is placed in solution form within a syringe (A) with a metal needle. This needle (B) is connected to a voltage supply (C). As the polymer is ejected from the syringe chamber and out of the needle, the combination of force and voltage creates fibers (D) that can be collected on a target (E) (Ramakrishna, et al., 2005).

Injection Molding

The process of injection molding is the most common polymer molding technique which accounts for 33% or all plastics production. The process is mainly made for large scale production. However they main fault of injection molding is plastics will deform during the cooling process especially with thin wall plastics (Chen, Turng, 2005). This process can be seen in Figure 4.

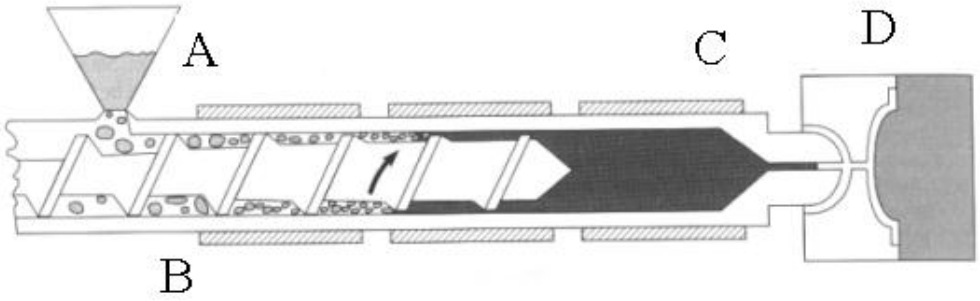

Figure 4: Process of Injection Molding (Strong, 2000)

The process starts by loading the thermoplastic pellets into the hopper (A). The pellets are fed and melted together (B) by the screw. The liquid plastic then comes to the nozzle (C). Finally it enters the mold (D) (Strong, 2000).

Extrusion

Extrusion is a process in which materials are subjected to a specific cross-sectional profile. The material is drawn through a die of a fixed cross-section as seen in Figure 5. The advantages of this method are its ability to create complex cross-sections for different applications, and also its ability to work with brittle materials, since the material only undergoes compressive and shear stresses. Common extruded materials are metals, polymers, ceramics and concrete.

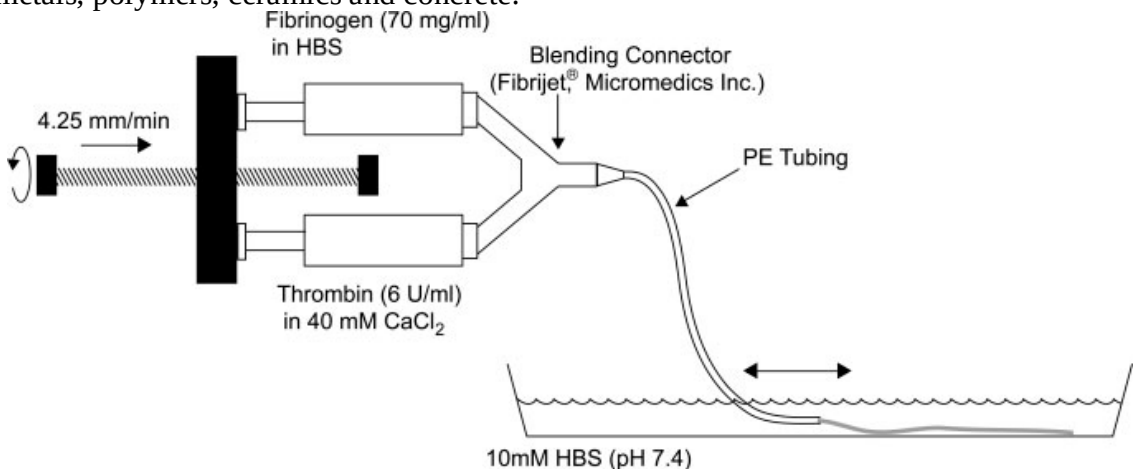

Figure 5: Extrusion Process (Pins and Cornwell, 2007)

In previous studies, extrusion has been applied to produce structures of specific diameter and length. In relation to this project, solutions of fibrinogen and thrombin have been combined to form fibrin, and then coextruded at a specific rate through polyethylene tubing to create the thread-like structure as shown in Figure 5 (Cornwell, K, 2007). Within five minutes, the threads form at the bottom of the bath. This is a continuous procedure, capable of producing an indefinitely long material, depending on the length of the thread needed. Therefore, this process is proven to be efficient and capable of producing intricate structures for different applications.

Project Approach

The following sections present the project hypotheses, assumptions, and goals.

Project Hypotheses

Hypothesis 1: The addition of a protective sheath will protect seeded hMSCs thereby increasing the number of viable cells available for cardiac regeneration.

Rationale: When pulled through the heart, cells are subjected to shear stresses that can damage them or sever them from the fibrin microthread delivery vehicle.

Specific Aim: Design a protective sheath that prevents shear stresses from damaging or severing seeded hMSCs from the biodegradable microthreads.

Hypothesis 2: The addition of a protective sheath will increase the mechanical integrity of the biodegradable microthreads.

Rationale: The current microthread delivery technique includes threads of collagen as well as fibrin. The collagen provides mechanical integrity, however, collagen increases thread diameter, but does not enhance the number of seeded cells. Therefore, no collagen threads and the addition of more biodegradable microthreads will increase the number of hMSCs that are actually seeded on the threads. A protective sheath will provide the lost mechanical support due to the lack of collagen in the microthread bundle.

Specific Aim: Design a protective sheath that increases the mechanical integrity of the biodegradable microthreads

Project Assumptions

To develop a working prototype of our design, specific project assumptions were established.

Assumption 1: It is assumed that hMSCs will successfully encourage cardiac regeneration when delivered to an infarcted region of the heart.

Assumption 2: It is assumed that fibrin is the best material for the adhesion of hMSCs and microthread delivery.

Assumption 3: It is assumed that the microthread delivery technique is the preferred method of stem cell delivery.

Assumption 4: It is assumed that the preferred cell-seeding technique is dynamic rotational seeding on bundle microthreads.

Project Goals

The goal of this project is to design a protective sheath for microthread stem cell delivery to the heart. To accomplish this goal, the following specific aims were developed.

- Design a protective sheath that prevents shear stresses from damaging or severing seeded hMSCs from the biodegradable microthreads, and
- Design a protective sheath that increases the mechanical integrity of the fibrin microthreads by exploring different mechanical properties of different materials.

Product Design

This chapter discusses the primary attributes that must be met in order for the device to be considered successful. The objectives, functions, constraints, and specifications helped create a more detailed and thorough client statement. Once the client statement was extensively revised, design alternatives were generated.

Objectives, Functions, Specifications, and Constraints

From the initial client statement and extensive research, design functions, objectives, specifications, and constraints were developed for creating a technology to address the problems with the current treatment options for breast cancer.

Objectives

To establish the following design objectives, the question "*What must the device be?*" was answered.

Objective #1: The device must be user-friendly.

The final device would not be commercially viable without the first objective. The sheath must be easy to use by the client, and therefore easily inserted into the heart. It must be easily applied to the needle as well as easily sterilized prior to implantation. It must also be reliable, in which no complications arise during the attachment of the sheath to the needle or during surgery. Finally, the sheath must possess the ability to be easily altered for use with a smaller needle both in diameter and length.

Objective #2: The device must be inexpensive.

For manufacturing purposes, the sheath must be inexpensive.

Objective #3: The device must be storable.

The sheath must be storable in order for it to be easily transported and stocked in different laboratory settings.

Objective #4: The device must be compatible.

The protective sheath design must be safe for the technician to assemble, to handle, and to use. The device must also be compatible with the attached materials, such as the biodegradable microthreads and the needle. Additionally, the sheath must be easily integrated into the current procedures without requiring disruption of the flow of the surgery. Most importantly, the sheath must be safe for the patient, and in that respect, the sheath must cause minimal inflammatory response, minimal necrosis to the surrounding cells and tissues, and minimal damage to the heart wall during delivery.

Objective #5: The device must be degradable or easily removable.

Once the protective sheath has successfully been implanted in the heart wall the sheath must either degrade or be removed. If the sheath is degradable it must break down within a specific time and not have any harmful byproducts. If the sheath is to be removed the sheath must not damage the cells as it slides past the cells.

Objective #6: The device should be multi-functional.

The sheath must not only protect the hMSCs from the shear forces during implantation, but it must also be able to incubate the stem cells.

Objective #7: The device must be mechanically sound and durable.

The sheath must be mechanically superior compared to the existing properties of the fibrin collagen bundles which have a UTS of 0.13N-0.14N. An acceptable factor of safety would be greater than two. The sheath must be durable so repeat use will not cause failure.

Functions

To establish the following design functions, the question "*What must the device do?*" was answered.

Function #1: The device must deliver the hMSCs to the target site.
Function #2: The device must protect the hMSCs during delivery.
Function #3: The device must contain the hMSCs during pre-surgery procedures and during delivery.
Function #4: The device must provide mechanical stability.
Function #5: The device must either degrade or be removable.
Function #6: The device must incubate the cells during pre-surgery procedures.

Constraints

The following design constraints were established. If the device did not meet these constraints, the device would have failed.

Sheath Size

The sheath must be able to contain 0.5-0.6mm bundle (without collagen).
The sheath's outer diameter must be less than 1mm.
The sheath must be able to hold a minimum of 8 fibrin threads within it.

Percentage of Engrafted Cells

40% or more cells must be successfully engrafted (40% of 8,000 cells).

Budget

The total budget for the creation of this device is $468.

Time

This device must be completed before April 22, 2009.

Specifications

The following design specifications were developed in order to make this device function.

- 8,000 viable cells must be loaded on the thread
- Greater than 40% engraftment of delivered cells
- User interface time (time needed to assemble sheath with rest of apparatus) must add no more than 30min/bundle
- Total time of making sheath must take less than 24hours
- Greater than 50% of the cells must remain viable after delivery
- Greater than 90% of the cells must be contained/remain on the thread after sheath placement
- Cells must remain viable when contained within the sheath for (x) amount of time
- Must have 10-20N failure load (Fakharzadeh, 2009)
- If to be degradable, it must fully degrade within one day and minimize negative response
- If to be removable, greater than 50% of the cells must remain viable once sheath is removed

Client Statements

Initial Client Statement: Design a method to protect cells seeded on biodegradable microthreads delivered to the heart.

Revised Client Statement: Design a protective sheath to guard human mesenchymal stem cells seeded on biodegradable microthreads delivered to the heart. The sheath must be removable or degradable without causing any negative response. It must be user-friendly, biocompatible, easily sterilized, transportable, and scalable that will be no larger than 1mm in diameter to fully encompass a 0.5-0.6mm bundle of microthreads. The sheath must also successfully deliver 40% or more of the stem cells seeded on the microthreads through the heart wall to the tissue without damaging or shearing off the hMSCs.

Conceptual Designs

The following section includes the six design alternatives developed during the course of several brainstorming sessions and in combination with what was learned from the literature

Design Concept 1: Cone

The cone conceptual design would use a hard metal or a polymer hollow cone. The needle would go from the base of the cone out through the tip of the cone as shown in Figure 6.

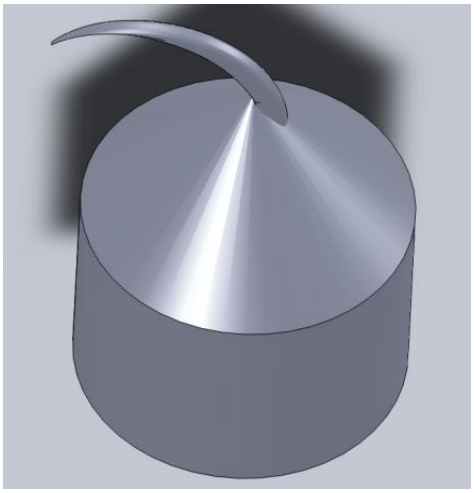

Figure 6: Cone Design Concept

The needle would then be held in place with the threads dangling out the base of the hollow cone with approximately 1cm of the cone overshadowing the microthreads. When the needle and cone are sewn through the heart, the cone would act like a wedge. By spreading the heart tissue away, a track would be created through which the microthreads would follow. Once the needle and the cone have passed through the heart wall, the cone can be removed, which would allow for the threads to be cut from the needle.

Design Concept 2: Stent

The stent conceptual design was developed from the concept of a cardiovascular stent. However, this design would be made of a solid material instead of the typical wire braided mesh construction of a typical stent. At the top of the stent where the needle is attached, a hole in the sheath would allow for the surgeon to cut the threads from the needle after the device had been drawn through the heart tissue. A pull tab, attached at the opposite end, would allow the surgeon to easily grip and remove the entire sheath. These features of the stent conceptual design can be seen in Figure 7.

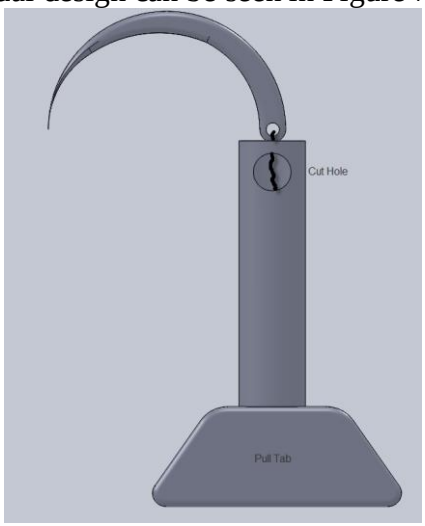

Figure 7: Stent Design Concept

Design Concept 3: Bandage

This conceptual design would utilize a flat thin polymer sheet. A thin line of glue at the top edge of the top right corner would run down the right side of the sheet. To protect the threads, the needle would be placed on the glue at the top left corner. The needle would be oriented so that the tip and half of the needle would lie outside the sheet while the needle eye and microthreads would lie on the sheet. The sheet, the needle, and the threads would then be rolled up, creating a tube around the threads. The glue on the right side would then seal the tube. A cutting line would show the surgeon how far to pull the needle through the heart wall and where to cut the needle from the threads. This design alternative can be seen in Figure 8.

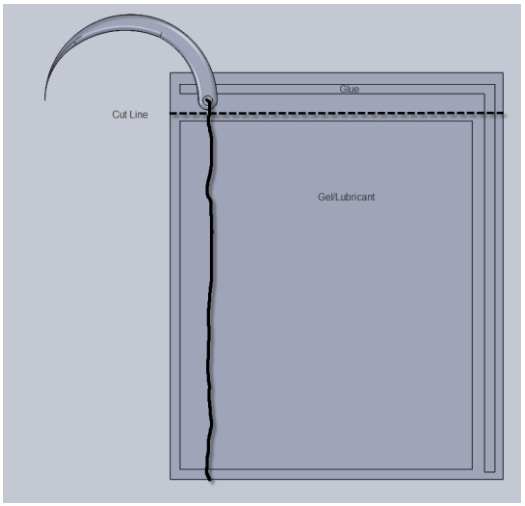

Figure 8: Bandage Design Concept

The tube could be either biodegradable or could be removed. To remove the tube, it can be pulled out one end while the surgeon holds the thread at the other end.

Design Concept 3: Blanket

The cape design would use a square thin flat sheet as can be seen in Figure 9. The needle would go through the center of the square and the threads dangling below. When sewn through the heart the sheet would collapse around the threads protecting them. The cape, needle and threads will be cut once the needle is through the heart wall. The cape could be biodegradable or removable. The cape would continue through the heart while the threads would be held in place.

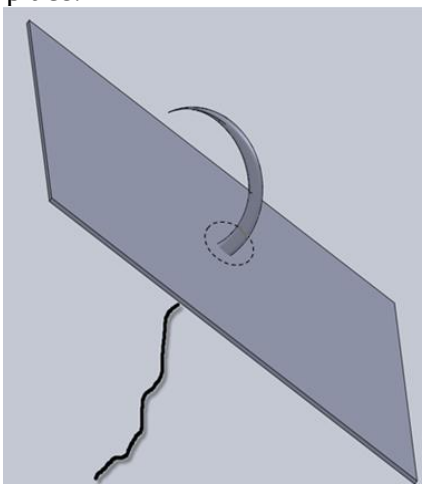
Figure 9: Blanket Design Concept

Design Additions

Small pull tabs can be attached to the sheath or to the threads. This would aid the surgeon in gripping the thread or the sheath allowing for more control. It would also act as a stopper for the threads. When a sheath is being removed the pull tab would give the surgeon a better grip making it easy to pull out the sheath. Any combination of the pervious designs could be used in the final design. For example the cones, tube with a pull tab.

Evaluation of Design Alternatives

In order to determine which design concept would work best for protecting hMSCs during microthread delivery to the heart, a matrix was developed to compare all four design concepts. However, before this comparative matrix was used, a pairwise comparison chart, completed by the design team, the client, and the user were filled out as can be seen in Appendices F, G, and H. A blank pairwise comparison chart is show in Table 3 on the next page.

Table 3: Blank Pairwise Comparison Chart

	User-friendly	Compatible	Inexpensive	Storable	Degradable or easily removable	Multi-functional	Mechanical integrity or durability	Total
User-friendly	X							
Compatible		X						
Inexpensive			X					
Storability				X				
Degradable or easily removable					X			
Multi-functional						X		
Mechanical integrity and durability							X	

Once the pairwise comparison charts were completed and compiled, the design concept comparative matrix was then completed as shown in Table 4. All constraints had to be met by each design, otherwise it was not considered. The objectives, in weighted order, were listed, and each design was ranked on how well it would accomplish each individual objective.

Table 4: Design Concept Comparative Matrix

			Conceptual Designs			
			Cone	**Stent**	**Bandage**	**Blanket**
Constraints	Contain 0.5-0.6mm bundle		√	√	√	√
	Sheath outer diameter <1mm		√	√	√	√
	Minimum 8 fibrin threads fit inside		√	√	√	√
	40% of 8,000 cells successfully engrafted		√	√	√	√
	Budget: $468		√	√	√	√
	Completed by April 22nd		√	√	√	√
Objectives	Value per Weighted Objective					
	7 √'s	Compatible	√√√√√√√	√√√√√√√	√√√√√√√	√√√√√√√
	6 √'s	Mechanical integrity	√√√√√√	√√√√√√	--	--
	5 √'s	Degradable/Removable	--	√√√√√	√√√√√	--
	4 √'s	User-Friendly	--	√√√√	--	√√√√
	3 √'s	Multi-Functional	--	--	--	--
	2 √'s	Inexpensive	--	--	√√	√√
	1 √'s	Storability	√	√	√	√
Totals			**14**	**23**	**15**	**14**

As can be seen from the results of Table 4 in the comparative design concept matrix, the stent design concept had the highest total result. Thus, this is the design that will receive further exploration.

Design Considerations

While the team and the client identified the conceptual design that would accommodate all of the constraints and objectives as seen in Table 4, additional design considerations were made during the design process. Through addressing these other design considerations, two new designs began to emerge. In the following sections, the four design considerations are discussed, all of which led to the creation of the team's two prototypes.

Sheath Type: Removable versus Degradable

The team considered two types of sheaths—removable and degradable. The team decided to use a removable sheath because it would allow the hMSCs to be in immediate contact with the heart tissue. From literary research, using a biodegradable sheath might require

too much time for it to degrade, thereby preventing immediate exposure of the hMSCs to the heart wall. Additionally, the presence of a biodegradable sheath over that period of time may restrict transportation of nutrients and removal of waste, compromising the viability of the hMSCs.

Assembly Methods

One of the biggest challenges the team faced was determining how to get the seeded microthreads into the sheath was the sheath was created. Two approaches were considered including slipping the sheath over the suture needle and cutting the sheath. The first approach, called the Sock Method, requires the technician to first cut the sheath tubing to 3cm in length. The sheath is then slipped over the tip of the needle and then progressively moved the sheath down the needle and over the seeded microthreads. The second approach, also known as the Cut-Down Method, requires two technicians, a surgical scalpel blade, and two pairs of tweezers. After a piece of the sheath tubing was cut to a length of 3cm, one end was inserted onto one of the tweezer prongs. Carefully using the scalpel, the tubing was cut all the way along one side of the sheath keeping the cut as straight as possible. Using the both technicians and both sets of tweezers, the cut tubing was held open, while the seeded microthreads were placed inside. By releasing the tubing, the sheath closed around the microthreads.

Needle Attachment Methods

Another challenge the team had to overcome was how to attach the size 20 curved suture needles to the designed protective sheath. Several options were explored including the incorporation of a pressure-fitted sheath, utilizing a draw-string created from surgical sutures, Krazy Glue, and heat.

The team explored the use of two different pressure fittings to secure the needle to the sheath. As can be seen in Figures 10 and 11, a barbed and a wedge fit were considered.

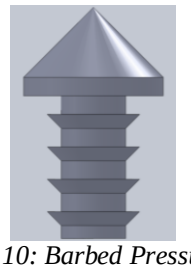

Figure 10: Barbed Pressure Fit

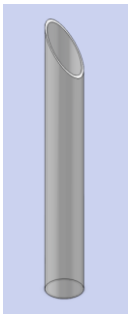

Figure 11: Wedge Pressure Fit

The drawstring needle attachment method incorporated the use of a surgical suture needle to simply tie the curved suture needle to the protective sheath. The curved

suture needle is inserted into the sheath, and a suture is tied around the sheath and the needle.

The use of Krazy Glue was also considered, which when sterilized, is more often known as Dermabond within the medical community. The active ingredient, 2-octyl cyanoacrylate, is responsible for the glue's immense adhesive abilities. After inserting the eye of the curved suture needle into the sheath and holding it with a pair of tweezers, 5µL of the glue was pipetted onto the needle-sheath interface. The assembly was held within the tweezers for approximately one minute and then left to dry.

Lastly, the team explored the possibility of using heat to melt the sheath to the needle. When experimenting with the heat attachment method, the team was careful to move the sheath far up along the suture needle so as not to accidentally burn the cells or the microthreads contained within the sheath.

These design considerations resulted in the creation of two sheath prototypes—one made from polyethylene teraphthalate (PET) and one from polytetrafluoroethylene (PTFE). The details of these two designs are described in the next chapter.

Two Prototype Designs

The team developed two final designs that encompassed the most favorable features of the conceptual designs. The first sheath, Design I, was made of PET, while the second sheath, Design II, was made of PTFE. Each design had its advantages and disadvantages, and in this section, both designs are further explained in detail.

Design I: PET Sheath

The first design was a removable sheath made of electrospun PET from BioSurfaces Incorporated (Ashland, MA) with an inner diameter of 2mm and a length of 3cm (Figure 12). It was assembled by the Sock Assembly Method as previously described, and 1cm of the PET sheath overlapped the base and eye of the needle. Heat was applied to attach the sheath to the needle.

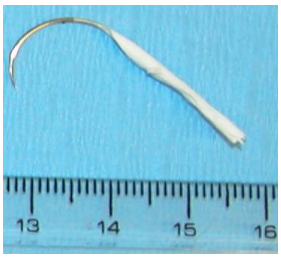

Figure 12: Design I (PET Sheath)

Design II: PTFE Sheath

The second design was also a removable sheath made from extruded PTFE ordered from MocroSoly Technology Corporation (Eatontown, NJ) (Figure 13). The inner diameter, however, was 1mm, the outer diameter was 1.58mm, and the length was again 3cm. It was assembled through the Cut-Down Assembly Method, and the needle was attached to the sheath by applying 5µL of Krazy Glue. The interface was then held within the tweezers for approximately one minute and then left to dry.

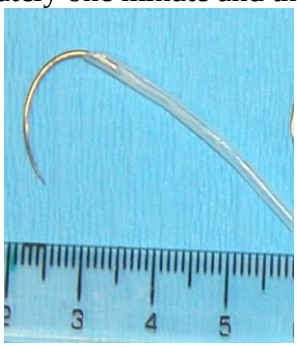

Figure 13: Design II (PTFE Sheath)

Methodology

This chapter contains the methods by which the materials were tested and chosen, in order to create a protective sheath that prevented seeded hMSCs from damage and removal during microthread cell delivery to the heart. Furthermore, this chapter shares how the device was evaluated and how the results were verified.

To perform the necessary tests on the two selected sheath designs, one made from polyethylene teraphthalate (PET) and one from polytetrafluoroethylene (PTFE), the team had to maintain healthy, viable hMSCs throughout the project, as well as produce the biological microthreads. Additionally, the cells were seeded on the microthreads using a rotational seeding device and quantified to determine seeding efficiency. The team performed a uniaxial tensile test on the two sheath designs to determine the failure loads for the needle-sheath interfaces. Finally, the sheaths were pulled through the ventricular wall of a rat heart to determine whether or not they would successfully protect and deliver the cell-seeded microthreads to the area of interest.

Cell Culture

A majority of the experiments conducted in this project required the use of cultured stem cells. Experiments were performed using passages 5-13 hMSCs cultured in Dulbecco's Modified Eagle's Medium (DMEM) with 10% Fetal Bovine Serum (FBS) and 1% Penicillin/Streptomycin (P/S). To maintain sterility, all cell culture experiments were performed aseptically in a biological safety hood. An exact cell culture protocol can be viewed in Appendix A. All cells were incubated at 5% CO_2 and 37ºC in tissue culture treated flasks.

Fibrin Microthread Production

Fibrin microthreads were self-assembled from solutions of fibrinogen and thrombin. The two solutions were extruded through polyethylene tubing at a pump speed of 0.23mL/min into a bath of 10mM HEPES, pH 7.4. Individual threads of less than 100μm diameter each were produced and bundled into groups of eight with 0.6-0.8mm thickness by twisting the microthreads together and allowing them to dry. The exact microthread bundling protocol can be viewed in Appendix C and the process can be seen in Figure 14.

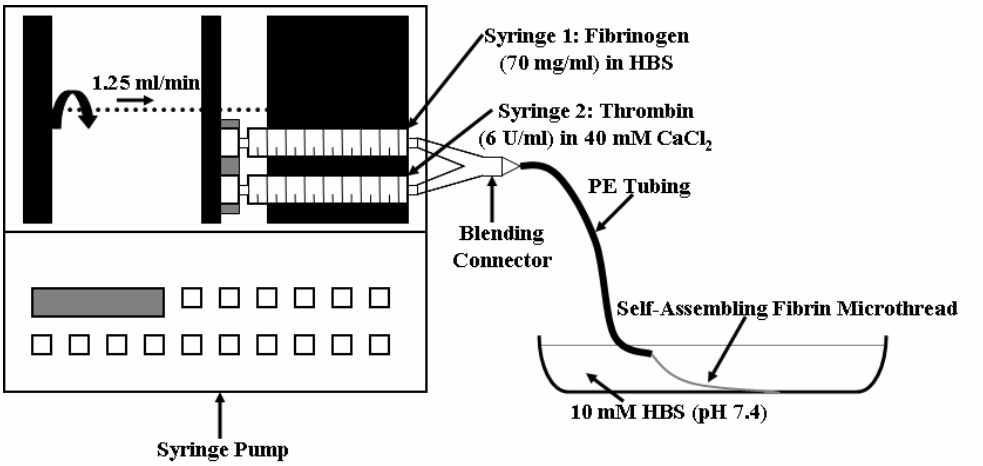

Figure 14: Schematic Drawing of Coextrusion System for Producing Self-Assembled Fibrin Microthreads

Microthread Sterilization

The microthread bundles were assembled by threading the microthreads through the eye of the curved suture needle. Once placed within the 1.98mm ID silastic bioreactor, slide clamps were placed at each end of the assembly. By using 3mL syringes, the microthreads were rehydrated with 100µL of phosphate buffered saline (PBS) for ten minutes, sterilized with 300µL of 70% ethanol for thirty-five minutes, and rinsed three times with 300µL of sterile deionized water. Using a 3mL syringe, all the sterile deionized water was removed, and the microthreads were dynamically seeded within thirty minutes. An exact microthread sterilization protocol can be seen in Appendix D.

Dynamic Seeding: Rotational Method

Following sterilization, each bioreactor was injected with 100µL of cell suspension at a concentration of 100,000cells/100µL using a 1cc syringe and a 27 gauge needle. The tubes were placed in 50mL conical tubes (with holes to allow gas exchange) and attached to a MACSmix tube rotator at 4rpm. The tubes were placed in an incubator at 5% CO_2 and 37ºC and rotated for 24 hours. An exact seeding protocol can be seen in Appendix E.

Cell Quantification Methods

Many of the tests that were conducted required the use of cultured hMSCs. To detect how many of these hMSCs were successfully delivered and engrafted after delivery, several assays were considered to quantify the hMSCs.

MTS Assay

The MTS assay is used to measure cell viability and quantity in a living culture. The byproducts of living cells react with a tetrazolium compound which results in a purple formazen dye. The amount of dye that is present is directly proportional to cell metabolic activity and cell quantity.

After seeding the microthreads, the threads were placed in individual wells in a 96-well plate. 100µL of media and 20µL of MTS solution were also added to the wells. The absorbance of the dye, read at 490nm can be compared to the standard curve of cell quantities as demonstrated in Table 5.

Table 5: MTS Assay Absorbancies (DiTroia, et al., 2000)

Plate Well	**Concentration (cells/mL)**	**Cells/well (0.1mL)**	**To obtain (total 0.325mL; 0.1 mL in triplicate + 0.025mL left over)**
A	35,000	3,500	0 ml media + 0.325ml stock
B	30,000	3,000	0.046ml media + 0.279ml stock
C	25,000	2,500	0.093ml media + 0.232ml stock
D	20,000	2,000	0.139ml media + 0.186ml stock
E	15,000	1,500	0.186ml media + 0.139ml stock
F	10,000	1,000	0.232ml media + 0.093ml stock
G	5,000	500	0.279ml media + 0.046ml stock
H	0	0	0.325ml media + 0ml stock

Hoechst Nuclear Stain

To test cell quantity, the use of a fluorescent dye such as Hoechst nuclear stain can be used. By staining the microthreads with the Hoechst dye, the seeded cells can be quantified under an optical microscope. The dye binds to the DNA within the cells, which when excited at 390nm, will emit a blue light at approximately 490nm.

To conduct a Hoechst nuclear stain, after seeding the microthreads, they are washed in DPBS for 15 minutes. The threads are then fixed using 4% paraformaldehyde for 15 minutes. A dilution of 1:6000 Hoechst and DPBS is added, and the solution is let to sit for another 15 minutes. Finally the threads are washed in DPBS for 15 minutes. The threads are then mounted on glass slides in order to view them under the optical microscope.

Hemocytometer (Fakharzadeh, 2009)

The last method of cell quantification is the use of a hemocytometer. The media is removed from the flask, then 5mL of DPBS is added and the flask is incubated for 5 minutes. After the 5 minutes, the DPBS is removed and another 5mL of trypsin is added to the flask of cells and left to incubate for 5 minutes. After incubation, cell detachment is ensured through the use of a microscope. Five mL of media is added, and the mixture is transferred to a 15mL conical tube. This tube is centrifuged for 5 minutes at 1,000rpm. The media is then aspirated and 0.5mL of media is added. Ten 10µL of the cell mix is mixed with 10µL of trypan blue dye in a microcentrifuge tube. A coverslip is placed on the hemocytometer and 10µL of the cell/trypan blue solution is added to one square of the hemocytometer. This is placed under microscope and the cells are counted in the middle square. If 100 cells are observed before counting all five squares, counting may cease. However, if less than 100 cells are observed in that first square, counting is continued into the other squares. In order to determine the number of cells in the flask Equation 1 and Equation 2 were used.

Equation 1: Determining Number of Cells in Flask I

$$\frac{Number\ of\ cells\ counted}{Number\ of\ squares\ counted} * 10^4 * 2 = \frac{Cells}{1\ mL\ of\ media}$$

Equation 2: Determining Number of Cells in Flask II

$$Total\ number\ of\ cells = \frac{Cells}{1\ mL\ of\ media} * Cells\ suspension\ volume\ (0.5\ mL)$$

Mechanical Testing

The microthreads, un-seeded and attached to a size 20 suture needle, were placed in the Instron machine in order to determine the failure load at the needle-thread interface. The assembly underwent a uniaxial tensile test at a rate of 1mm/min to failure. The same testing was performed on assemblies with the two sheath designs. The sheaths were 3cm long, and the tests provided the load at failure for the sheath-needle interface for five trials for each sheath. An exact protocol for the Instron test can be viewed in Appendix J.

Proof-of-Concept: Surgery with Both Prototype Designs

In order to test the feasibility of our project, the microthreads were sutured through the ventricular wall of a paraformaldehyde-fixed rat heart. The same procedure was performed with the two sheath designs encompassing the microthreads to determine whether or not the sheaths would successfully pass through the heart wall as well as protect the threads during suturing.

Results

Each design was subjected to mechanical testing and a proof-of-concept test involving suturing it through the heart. The mechanical testing of the PTFE sheath resulted in a failure load of 4.25 ± 3.16N (Table 7) while the PET sheath had a failure load of 2.58 ± 0.50N (Table 6).

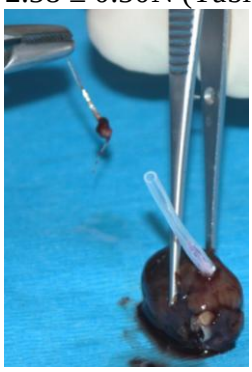

Figure 15: PTFE Sheath

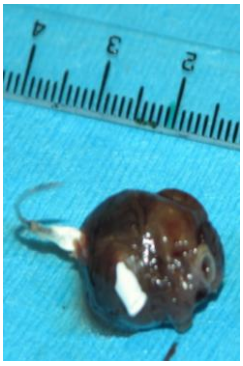

Figure 16: PET Sheath

Table 6: PET Sheath Failure Loads

Test #	Load at Yield
1	2.29 N
2	3.31 N
3	2.72 N
4	1.97 N
5	2.59 N
Average	2.58 ± 0.502 N

Table 7: PTFE Sheath Failure Loads

Test #	Load at Yield
1	8.21 N
2	1.11 N
4	1.04 N
5	4.73 N
6	6.17 N
Average	4.26 ± 3.16 N

Based on previous research, the failure load of the actual microthread bundle was between 0.13-0.14N. Thus, the team was able to develop a factor of safety of 30 and 18 for the PTFE and PET sheaths, respectfully. The PTFE was unable to pass through the heart (Figure 15), despite it having a higher load at failure yield strength of 4.25N. The electrospun PET sheath however, did successfully pass through the heart (Figure 16).

Discussion: Design Verification

Lastly, the PET sheath could be attached to the needle through the use of heat. This process was extremely simple to perform, inexpensive, and took less than five seconds. By melting the sheath to the needle, a smooth interface was created as well as a very strong attachment (as is demonstrated by the Instron results). The PTFE sheath, however, could not be melted to the needle and required the use of Krazy Glue. This was a complicated, messy, and time-consuming process.

After the team assembled the PET and PTFE sheaths, obtained their load at failure strengths, and conducted a proof-of-concept test in a paraformaldehyde fixed rat heart, it was clear which design would best address the needs of the revised client statement. The team decided to pursue working with Design I: The PET Sheath for several different reasons.

Electospun PET has many desirable properties for use in cardiac applications in comparison to PTFE. PET is an appropriate and biocompatible material for suturing through the heart. The PET sheath can be custom-made through the process of electrospinning. This allows the sheath to be customized to the sizes necessary depending on the patient. Furthermore, electrospinning the PET allows for the sheath to be extremely flexible and porous. This is advantageous because it allows for oxygen diffusion during incubation in a bioreactor. The PTFE sheath, however, requires extrusion equipment to create it, and it must be ordered online where it is available only in predetermined sizes and thicknesses.

Additionally, the ease of assembly at which the PET sheath can be constructed was yet another reason the team decided to use the PET sheath design. The assembly of the PET sheath proved much easier than assembling the PTFE sheath. The PET sheath required only one technician, whereas the PTFE sheath required two technicians to hold the sheath open when inserting the seeded microthreads. Furthermore, the PET sheath was flexible and easy to handle, while the PTFE sheath was extremely inflexible and slippery due to its high coefficient of friction.

Another reason the PET sheath design was further pursued was because of the mode of needle attachment. This was done through the use of heat, a process which was extremely simple to perform, inexpensive, and required less than five seconds. By melting the sheath to the needle, a smooth interface was created as well as a very strong attachment (as is demonstrated by the Instron results). The PTFE sheath, however, could not be melted to the needle and required the use of Krazy Glue. This was a complicated, messy, and time-consuming process. Controlling the placement of the glue was difficult due to the capillary action in the PTFE sheath and often, the glue travelled so far down the sheath, it made contact with the seeded microthreads. Because the drying time of the glue was unknown, incomplete bonding between the needle and the sheath occurred several times making experimentation complicated.

After determining which material, assembly method, and needle attachment strategy would work best for delivering hMSCs to infarcted regions of the heart, the team was finally able to conduct two more tests to further validate the PET sheath design. The first test involved the use of the Instron uniaxial testing machine to determine the failure loads of the PET sheath and the PTFE sheath at their needle-sheath interfaces. Despite the fact that the PTFE sheath had a higher mean failure load than the PET sheath at 4.26N

and 2.58N, respectively, this was not indicative that the PTFE sheath was necessarily better for surgery through a heart wall. Additionally, the PTFE sheath also had a higher standard deviation (with n=5) than the PET sheath at ±3.16N and ±0.502N, respectively. This suggests that the bonding of the glue to the PTFE sheath was inconsistent, resulting in some sheaths with very high failure loads while others had very low failure loads. The PET sheath, with a much lower standard deviation, indicates that the strength of the PET sheath was consistent from sheath to sheath resulting in it being more reliable.

The proof-of-concept test further validated why the electospun PET sheath met the team's goals and objectives. Only the PET sheath was able to pass through the stiff paraformaldehyde fixed rat heart. This was due to the PET sheath's soft and flexible nature in combination with its smooth needle-sheath interface, resulting in low amounts of friction and shear stresses during surgery. Conversely, the PTFE sheath did not successfully pass through the heart. This was attributed to the large, rigid, and uneven interface between the PTFE sheath and the suture needle, consequently requiring much more force to suture through the heart.

Conclusions

Based on the results of these tests, the team determined that the PET sheath was the best design to protect the cell-loaded microthread bundles during delivery through the heart wall (Figure 17). The sheath was removable so that the hMSCs would be directly exposed to the myocytes. It was made out of electrospun PET to allow oxygen diffusion as well as flexibility when being sutured through the heart wall. The sock method provided ease of assembly. Heat was applied to attach the sheath to the needle to form a close, firm, smooth interface. Finally, it was 3cm in length and had a 2mm inner diameter to fully encompass the microthreads.

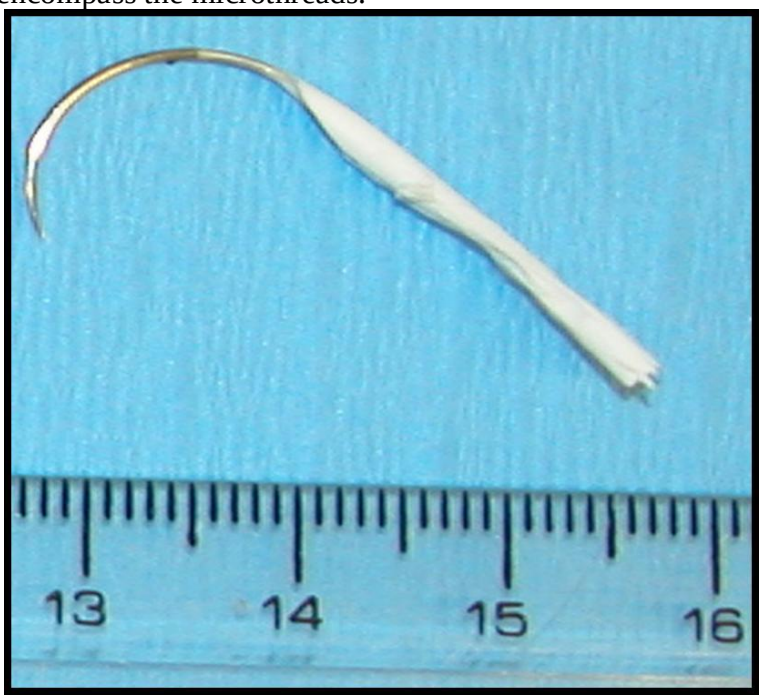

Figure 17: PET Sheath Final Design

With these attributes, we believe that the presence of our protective sheath will in turn increase the engraftment rate as well as evenly distribute the hMSCs to the area of interest, therefore improving cardiac function.

Regulations and Ethical Issues

When designing a device to be used in an animal, it is necessary to acknowledge that the device must be carefully studied and examined before it can be used on that animal. The primary concern is the safety of the animal—for this particular design, a rat. In all of the conceptual designs, the safety of the rat was considered; thus all of the conceptual designs and the two prototype sheaths were created with an inner diameter of 2mm. This consideration minimized the damage to the rat's heart wall during surgery. Furthermore the sheaths were made to accommodate a size 20 curved suture needle, which is the standard size for suturing microthreads through the ventricle of a rat. Additionally, the Institutional Animal Care and Use Committee's (IACUC) guidelines for ethical conduct in the care and use of animals in research were referenced (Gaudette, 2008).

Before the mass production of the final PET sheath design, it would first need to be tested on multiple rats to determine and to explore potentially unforeseen problems. It is necessary to determine at what point the device is safe enough to use on a rat and when the risk of damage is warranted by the benefits of the test. Through contact with IACUC, the device could be reviewed as well as the proposed animal testing procedures to determine if testing is educationally necessary and receive approval.

Whenever any medical device is created, it must meet certain regulatory standards in order for it to go to market for use in public practice. The Food and Drug Administration (FDA) regulates all medical devices. While the FDA does not extensively concern itself with animal regulations, the sheath itself could eventually be used in human applications. Therefore, it would need approval from the FDA. It would most likely be classified as a Class III device since the safety of the device cannot be assured simply by general controls and invasive surgery is required for use.

Future Recommendations

While the proof-of-concept experiments proved helpful in determining which prototype design worked best in an actual surgical setting, additional experiments and testing should be completed to further improve the PET sheath design.

One future recommendation would be to use a PET sheath with an inner diameter of 1mm. In the team's experiments, the sheath had a diameter of 2mm, which is not ideal for suturing through a rat heart. However, the ability of PET to be electrospun makes it easy to create sheaths of varying diameters to accommodate different patients.

A second future recommendation would be to perform cell quantifications numerically determine if the sheath is successful at delivery the hMSCs to the infarcted region of the heart. Following the sterilization protocol (Appendix D) the team would sterilize several bioreactor assemblies, each containing one bundle of eight fibrin and four collagen microthreads and a size 20 curved suture needle. Once completely sterilized, each of the assemblies would be seeded with hMSCs (Appendix E) using the previously stated equations to determine the amount of cells applied. Once seeded in the dynamic rotational seeding device for twenty-four hours, the team would remove the assemblies from the rotational seeding device. Two bundles of microthreads would be removed from their bioreactors and threaded through the bottom left ventricle of a live Sprague-Dawney female rat heart and pulled through the epicardium and immediately removed through the top of the left ventricle. After removing the microthreads from the heart, the team would count the number of cells still remaining on the threads using the hemocytometer method (Appendix A). This would provide the team with a baseline cell count to which the other two threading experiments would be compared.

The second two threading experiments would include threading several more fibrin-collagen microthread bundles through the rat's left ventricle; bundles would be contained within the PTFE center cut design, while the other bundles would be contained within the PTFE wedge with electrospun PET. After the assemblies are threaded completely through and removed from the ventricle, the team would again use the hemocytometer method (Appendix A) to count the number of cells. The baseline cell count, obtained without any protective sheath, could be compared to the cell counts of the experiments with the protective sheaths to determine whether the sheaths were effective in protecting and delivering the hMSCs.

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Appendices

The following section contains supplemental information to what is contained in the report.

Appendix A: Cell Culture Protocol (Fakharzadeh, 2009)

Materials

- STERILE media: DMEM (Dulbecco's Modified Eagle's Medium) with 10% FBS Fetal Bovine Serum) and 1% P/S (Penicillin and Streptomycin)
- STERILE DPBS (Dulbecco's Phosphate Buffered Saline), 1X
- Trypsin EDTA, 1X
- 10 μL, 100 μL, and 1000 μL pipettes
- Pipette boy
- STERILE 10 μL, 100 μL, and 1000 μL pipette tips
- STERILE T75 or T25 cell culture flask
- STERILE Pasteur pipettes
- STERILE serological pipettes
- STERILE 15 mL conical tube
- Trypan blue solution in PBS (50% trypan blue solution, 50% PBS)
- 0.7 mL microcentrifuge tube
- Hemocytometer

Procedure

1. Look at cells under microscope to ensure at least 80% confluency
2. Remove media from flask
3. Add 5mL DPBS to flask
4. Incubate for 5 minutes in hood
5. Remove DPBS from flask
6. Add 5mL trypsin to flask
7. Incubate for 5 minutes in incubator
8. Look at flask under inverted microscope to ensure cell detachment
9. Add 5mL media to flask and transfer cell suspension to 15mL conical tube
10. Centrifuge cell suspension at 1000 RPM for 5 minutes (don't forget counter weight)
11. Prepare hemocytometer and allow it to dry
12. Aspirate supernatant (everything except cell pellet at bottom of conical tube)
13. Add 0.5mL of media (make sure to mix solution)
14. Add 10μL of cell suspension to 10μL of trypan blue solution in 0.7mL microcentrifuge tube
15. Put coverslip on hemocytometer and add 10μL of combined trypan blue solution/cell suspension to one square
16. Count cells with microscope starting on the middle square segment.
17. Count cells in the 5 squares. If you reach 100 cells counted before counting all 5 squares you may stop counting. Make sure you count all of the cells in each square you choose to count (don't stop half way!)

18. If you do not count 100 cells in the 5 squares you must add the remainder of the trypan blue solution/cell suspension to the other square on the hemocytometer
19. Repeat step 16 if you need to count more squares
20. Use the following equation to determine the number of cells in your flask:

$$\frac{Number\ of\ cells\ counted}{Number\ of\ squares\ counted} * 10^4 * 2 = \frac{Cells}{1\ mL\ of\ media}$$

$$Total\ number\ of\ cells = \frac{Cells}{1\ mL\ of\ media} * Cells\ suspension\ volume\ (0.5\ mL)$$

21. If seeding is desired, use the following equation to determine the required total volume of cell suspension:

$$\frac{Cells\ required}{Seeding\ volume} = \frac{Total\ number\ of\ cells}{Y}, Solve\ for\ Y$$

So, for example when I seed my microthreads I need a volume of 100µL per seeding tube and I want 100,000 cells in that 100µL of cell suspension. So I would plug the following values into this equation, assuming that I have 500,000 cells:

$$\frac{100{,}000\ cells}{100\ \mu L} = \frac{500{,}000\ total\ cells}{Y}$$

For this example Y would equal 500µL. Since I already added 500µL to the cells after centrifugation I don't need to add any more media in this case. So whenever you start with a cell suspension of 500µL, you need to subtract that from the Y value you solved for
(Y – 500).

22. If only subculture is desired, use the equation above to make a cell concentration of $\frac{500{,}000\ cells}{1\ mL}$
23. After seeding or when subculturing the remaining cell suspension must be transferred into either T75 or T25 flasks. 500,000 cells should be put into a T75 flask and 160,000 cells should be put into a T25 flask to ensure proper growth
24. After cells suspension has been added to the appropriate size flask(s) calculate the amount of media that you must add to create a total volume of 10mL for T75 flasks or 3mL for T25 flasks.
25. Put cells back in incubator and feed them the NEXT DAY

Appendix B: Cell Feeding Protocol (Fakharzadeh, 2009)

Materials

- Gloves
- DMEM media (10% FBS and 1% PIS)
- Container of Pasteur Pipettes
- 10mL surgical pipette

Procedure

1. Place the DMEM into the water bath until it reaches 37*C (can aliquot this out in order to decrease the heating time)
2. Spray hood, tools, and vacuum tube with EtOH
3. Touching only one Pasteur pipette at a time, remove one from the container and attach it to the vacuum tube
4. Retrieve the cells from the incubator and check them in the microscope for contamination
5. Retrieve the DMEM from the water bath and wipe it down with EtOH
6. Wipe gloves with EtOH
7. Using the vacuum with the attached Pasteur pipette, suck the old DMEM media up from the cells
8. Using the surgical pipettes, place 3mL of the heated DMEM into the container holding the cells
9. Surgical pipette goes in the biohazard waste
10. Pasteur pipette goes in the sharps container
11. Wipe down everything with EtOH

* TO AVOID CONTAMINATION, DO NOT PASS HAND OVER ANYTHING!

Appendix C: Microthread Bundling Protocol (Fakharzadeh, 2009)

Materials

- Gloves
- Overhead projector screen
- Black mat
- 2 pairs of forceps
- 2 pieces of tape
- DI H_20
- Fibrin threads
- Needle
- Scissors

Procedure

1. Lay 8 fibrin threads of the same length on the overhead projector screen as close as possible
2. Push the 8 ends as close as possible and tape them down on the overhead projector
3. Note: we have no need for the collagen threads because we will have the sheath to protect them therefore, we don't need the added stability/strength associated with the collagen threads
4. With your dominant hand at the tape end and your non-dominant hand holding the threads taught, smooth the DI H_2O along the threads using a plastic pipette
5. Place a kimwipe underneath the threads so they don't stick to the projector screen
6. Cut the threads at the un-taped end so they are all the same exact length
7. Let the threads dry
8. Measure 4cm of the threads starting from the un-taped end and cut the threads
9. Pass the threads through the needle using the forceps
10. Wrap the two halves of the thread bundle around itself
11. Wet the threads using lots of DI H_2O
12. Twist both strands together and rewet
13. Let the bundle dry

Appendix D: Microthread Sterilization Protocol (Fakharzadeh, 2009)

Materials

- PBS (phosphate buffered saline)
- 70% ethanol
- STERILE DI (deionized) water
- STERILE 3 mL syringes
- Tissue culture dish
- Prepared microthreads

Procedure

1. Remove side clamp and add 100 μL PBS with 3 mL syringe to each microthread bundle
2. Replace side clamp
3. Allow to hydrate for 10 minutes
4. Remove side clamp and add 300 μL of 70% ethanol with new 3 mL syringe to each microthread bundle to ensure that all PBS has been removed
5. Replace side clamp
6. Allow to sterilize for 35 minutes
7. Remove side clamp and add 300 μL of STERILE DI water with new 3 mL syringe to each microthread bundle to ensure that all ethanol has been removed
8. Replace side clamp
9. Allow to hydrate for 10 minutes
10. Repeat steps 7-9 two more times
11. Use new 3 mL syringe to remove all DI water
12. Seed microthreads within 30 minutes

Appendix E: Microthread Seeding Protocol (Fakharzadeh, 2009)

Materials

- 1.98 mm I.D. Silastic tubing
- (2) side clamps
- Size 18 half circle tapered suture needle
- Biological microthread bundle (collagen/biodegradable microthreads)
- PBS
- Sterile PBS
- 70% Ethanol
- (6) sterile syringes
- 27G needle (without sharp tip)
- Sterile 1 cc syringe
- Cell suspension (100,000 cells/100 μL)
- 50 mL conical tube

Procedure

1. Use a new syringe to expel all sterile PBS from the bioreactor immediately before seeding
2. Use a new syringe (1 cc maximum) to inject 100 μL of cell suspension (my cell concentration is 100,000 cells/100 μL) into the bioreactor
3. After seeding, remove 27G needle from bioreactor
4. Place bioreactor in 50 mL conical tube
5. Place bioreactor in MACSmix tube rotator and rotate at 4 RPM (lowest setting) for 24 hours

Appendix F: Design Team's Pairwise Comparison Chart

Pairwise Comparison Chart for Main Objectives								
	User-friendly	Compatible	Inexpensive	Storability	Degradable/easily removable	Multi-functional	Mechanical integrity/durability	**Total**
User-friendly	x	0	1	1	0.5	1	0.5	**4**
Compatible	1	x	1	1	1	1	1	**6**
Inexpensive	0	0	x	1	0	1	0	**2**
Storability	0	0	0	x	0	1	0	**1**
Degradable/easily removable	0.5	0	1	1	x	1	0.5	**4**
Multi-functional	0	0	0	0	0	x	0	**0**
Mechanical integrity/durability	0.5	0	1	1	0.5	1	x	**4**

Appendix G: Client's Pairwise Comparison Chart

Pairwise Comparison Chart for Main Objectives								
	User-friendly	**Compatible**	**Inexpensive**	**Storability**	**Degradable/easily removable**	**Multi-functional**	**Mechanical integrity/durability**	**Total**
User-friendly	X	0	1	1	1	0	0	**3**
Compatible	1	x	1	1	0.5	1	1	**5.5**
Inexpensive	0	0	x	1	0	0	0	**1**
Storability	0	0	0	X	0	0	0	**0**
Degradable/easily removable	0	0.5	1	1	X	1	0	**3.5**
Multi-functional	1	0	1	1	0	X	0	**3**
Mechanical integrity/durability	1	0	1	1	1	1	X	**5**

Appendix H: User's Pairwise Comparison Chart

Pairwise Comparison Chart for Main Objectives								
	User-friendly	**Compatible**	**Inexpensive**	**Storability**	**Degradable/easily removable**	**Multi-functional**	**Mechanical integrity/durability**	**Total**
User-friendly	X	0.5	1	1	0.5	0	0.5	**3.5**
Compatible	0.5	X	1	1	0.5	1	1	**4.5**
Inexpensive	0	0	X	1	0	0	0	**1**
Storability	0	0	0	X	0	0	0	**0**
Degradable/easily removable	0.5	0.5	1	1	X	1	0.5	**3.5**
Multi-functional	1	0	1	1	0	X	0.5	**3.5**
Mechanical integrity/durability	0.5	0	1	1	0.5	0.5	X	**3.5**

Appendix I: Post-Seeding Technique

1) Remove thread bundle
2) Place thread bundle in 250mL of dionized water for 5minutes
3) Place in trypsin (800µL in 1.7mL tube) for 30minutes
    a. Every 5minutes, shake tube to ensure detachment
4) Add 800µL DMEM (10% FBS, 1%P/S) to inactivate trypsin
5) Centrifuge at 10,000RPM for 10minutes
6) Aspirate supernatant with 100µL pipette (leave 10-20µL in tube)
7) Add 10µL of cell suspension to trypan blue solution and count cells
    a. Confirm how much suspension remains to calculate the number of cells on the thread (use 10µL pipette and pipette 5mL at a time until the suspension is left)
    b. Count all cells (including blue ones!)

Appendix J: Mechanical Testing Protocol

1. Switch on Instron machine.
2. Open the Bluehill 2 Software.
3. Create new method icon.
    a. Specimen→ geometry (tubular), OD (1/16in = 0.0625in = 1.5875), wall thickness (0.5875mm), length(3cm), final length (predict to 3.5cm), final OD (predict to 1.48), final wall thickness (predict to 0.48mm)
    b. Control → Ramp1→ control mode 1 → Tensile extension and Rate 1 → 1mm/min
    c. Control→ end of test → end of test 1→ criteria 1→ tensile extension and Value 1→ 5mm
    d. End of test → Calculations → Yield, maximum tensile extension
    e. Results 1 → statistics → Mean +SD
    f. Results 1 → Load at Yield (zero slope)
    g. Graph 1→ Graph title, sample number
    h. Export Results and Raw Data →Comma separated values
    i. Reports → choose saving settings
4. Perform test.
    a. Place sample in grips and tighten to secure.
    b. Bluehill → method → first in list is your method
    c. Bluehill → test → select your test → type sample file name (Example: "PTFE 1, 3/3/2010")
    d. Next
    e. Zero extension and load
    f. Select start
    g. Stop at breakage
    h. Return button
    i. Save as → keep file type
    j. Finish button → yes