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FIBRIN MICROTHREADS FOR STEM CELL DELIVERY IN CARDIAC APPLICATIONS

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1. hMSCs
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Authorship

Section	Author	Editor
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1.0 Introduction	ALL	ALL
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2.2 Myocardial Infarction and Heart Failure	LD	HH
2.3 Cell-Based Cardiac Repair	HH	JR
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3.2 Project Assumptions	HH,JR	LD
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9.0 Future Recommendations	HH	JR

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Abstract

Cell delivery is a promising treatment for cardiac repair; however it is limited by low cellular engraftment rates. With this limitation, we propose the use of fibrin microthreads as a mechanism for localized cellular delivery. The purpose of this project was to design a system to increase cell density on biological microthreads for use in cell assisted cardiovascular regeneration. The final design consisted of a dynamic seeding method paired with bundled microthreads, yielding 648±234cells/mm; indicative of a confluent cellular monolayer.

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Part I. Proposal

1.0 Introduction

In a 2006 report by the American Heart Association, it was estimated that 1,200,000 Americans each year will suffer from a new or recurrent heart attack, resulting in approximately 450,000 deaths [1]. Heart attacks, or myocardial infarctions (MI), may be caused by tissue death due to ischemia. When tissue becomes ischemic, cardiomyocyte cell death occurs causing scar tissue formation. As a result, the mechanical properties of the heart are compromised, which may lead to erratic beating in the heart and a decrease in global heart function. This anomalous mechanical function can lead to poor blood flow and potential heart failure.

In the field of cardiovascular regeneration, researchers are continuously working on new technologies and improving old ones. Recently, cell based cardiac repair has been proposed as a possible treatment for MI. In particular human mesenchymal stem cells (hMSCs) have generated a great deal of excitement. However, although research is persistent, there are still many limitations. Examples of such limitations in the field include the quantity of cells delivered to the infarcted region and the engraftment rate of those cells into the surrounding tissue. In general, low engraftment rates (6-12%) [2] can be attributed to non-localized cell delivery and cell leakage. Although significant advances using cells have been made in regeneration of the heart and blood vessels, these limitations require further investigation.

The purpose of this project was to design a system to increase cell density on biological microthreads for use in cellular assisted cardiovascular regeneration. These microthreads can be used to deliver stem cells that may allow for the regeneration of ischemic tissue. These microthreads should maximize the quantity of cells that can be delivered given dimensional constraints of the device.

To increase the quantity of cells attached to biological microthreads an initial investigation of current literature was completed. This literature review compiled a background on the problem of myocardial infarction and heart failure followed by an analysis of cell based cardiac repair as a method of regenerating the heart post-MI. Within the category of cell based cardiac repair, several varieties of cells were investigated for cardiac applications; however hMSCs were found to be the most plausible cell type for this project. Following the selection of hMSCs as the cell type, cellular delivery options were considered. It was determined that biological microthreads were the best platform for cellular delivery as they can be employed to localize cellular delivery to the site of ischemia, potentially solving the low engraftment rate limitation [2-4]. The selection of fibrin microthreads over other biological microthreads was made because fibrin is a natural biomaterial that is biocompatible, bioresorbable, and essential in normal wound healing [5]. Finally, various techniques to increase cellular attachment, such as physical surface alterations, surface adhesion molecules, and cell seeding techniques, were explored.

In order to devise the best method to increase stem cell density on biological microthreads, an iterative design process followed the literature review. As a part of the design process, constraints, objectives, and functions that the device must follow were consolidated. In addition, various techniques to increase cell density on biological microthreads such as physical surface alterations, surface adhesion molecules, and cell seeding techniques were compared via morphological charts and evaluation matrices for their ability to fulfill the client statement.

Based on the design analysis, experiments were conducted showing that increased hMSC density on fibrin microthreads could be obtained by altering the seeding technique and physically altering the surface. Increased hMSC density on the fibrin microthreads was obtained with a dynamic (rotational) seeding, as compared to the current standard of static (droplet) seeding. Additionally, physical surface alterations, which were accomplished by bundling fibrin microthreads, also led to increased hMSC density on the fibrin microthreads. After further experiments, it was found that the optimum number of

fibrin microthreads per bundle was ten while the optimum seeding duration was 24 hours. When bundles of ten fibrin microthreads were rotationally seeded for 24 hours, a confluent monolayer of hMSCs could be obtained on the microthreads. To prove that fibrin microthreads could be used as a cellular delivery device, hMSC seeded bundles of ten microthreads were sutured fully through the heart, revealing that cells were still present on the microthreads following suturing. Results of this experiment were noteworthy because it indicated that hMSCs were not sheared off of the fibrin microthreads upon entry into the epicardium of the heart. In conclusion, rotationally hMSC seeded bundles of ten fibrin microthreads are an exciting and novel technique for localized cellular delivery and may allow for cardiac regeneration in the future.

2.0 Literature Review

2.1 The Heart

The heart is a dual pump situated in the thoracic cavity where it is responsible for pumping blood to both the systemic and pulmonary circulations. As a part of the circulatory system, the heart works in conjunction with blood vessels to pump blood throughout the entire body. Blood itself serves as a transport medium while the vessels are the passageways through which blood travels to different organ systems.[6] In systemic circulation, the oxygenated blood travels throughout the body depositing oxygen to power cellular processes.

The heart is a vital organ that must function properly in order to sustain life. Should adverse events arise within the heart, such as arrhythmias, blocked arteries, or ischemia, serious health complications can occur.

2.2 Myocardial Infarction and Heart Failure

It is estimated that each year 1,200,000 Americans suffer from a MI, contributing to 450,000 deaths each year from heart failure [1]. Heart failure due to MI can occur when approximately 25% of the ventricle's cardiomyocytes die [7]. In the United States, the estimated direct cost for heart failure in 2006 was $29.6 billion[1]. Increasingly complex medical therapy has decreased morbidity and mortality associated with heart failure. However, on any given day, about 4,000 people are waiting for a heart transplant, but with donor shortages there are only enough donor hearts to provide about 2,000 transplants each year [8]. Because of the alarming number of deaths each year due to heart failure, researchers are developing new methods of cardiac regeneration. Although research for new and improved treatments is persistent, there are still many limitations in cardiovascular regeneration, primarily due to the inability of adult cardiomycocytes to adequately proliferate and generate new myocardial tissue to repair the heart.

2.3 Cell-Based Cardiac Repair

Current treatment options for heart failure are limited. Traditional approaches include the use of medication and surgery to increase blood flow to the heart [1]. In response to the emergent issue of cardiovascular diseases in the United States, alternative methods to treating diseases such as MI and heart failure have been developed. Recently, the area of cell based cardiac repair has been given a great deal of attention. Although cell based cardiac repair was not initially accepted, the evidence for cell based therapies has shown that implanted cells could create new tissue and improve function of the failing heart [9].

In the last few decades, a number of different cell based cardiac repair therapies have been developed. This area of research first began with the use of skeletal myoblasts. Original expectations for skeletal myoblasts were that the cells would transdifferentiate into cardiomyocytes; however it is clear that myoblasts remain committed to form mature skeletal muscle in the heart [10]. Following the use of skeletal myoblasts, other cell lineages were explored such as endothelial progenitor cells (EPCs), embryonic stem cells, as well as adult stem cells. Although EPCs and embryonic stem cells have advantageous qualities in cardiac bioengineering applications, each possess drawbacks. For example, EPCs are difficult to harvest and their homing capacity is often affected upon expansion [11]. On the other hand, the use of embryonic stem cells poses ethical issues.

The use of adult stem cells for cardiac regeneration is advantageous due to their inherent cellular plasticity. Adult stem cells can be found in all mature humans. It is a cell that is undifferentiated, meaning that it has not yet generated structures or manufactured proteins characteristic of a specialized cell type [12, 13]. These cells can be found among differentiated cells in a tissue or organ, can renew themselves, and can differentiate to yield the major specialized cell types of the tissue or organ, such as neural [14], bone, cartilage, fat, muscle, marrow stroma [15, 16]digestive tract [12], and cardiomyocytes [17]. Their primary endogenous function is to maintain and repair the tissue in which they reside [18-20].

2.4 Human Mesenchymal Stem Cells

Among various adult stem cells, human mesenchymal stem cells are the best candidate for cellular delivery to cardiac tissue. They can be easily obtained from the femoral bones of patients for clinical applications [21] and then isolated, propagated in culture, and frozen for preservation without losing their capacity to form a variety of cell types, including cardiomyocytes [17]. Until recently, cardiac myocytes were considered to be terminally differentiated. Recent evidence suggests that cardiac myocytes have the potential to enter the cell cycle [22, 23] and that stem cells may be useful in increasing myocyte mass through either differentiation into myocytes [24] or by inducing native myocytes to proliferate [25].

In addition to increasing myocyte mass, studies involving hMSCs have indicated other beneficial effects of hMSCs in cardiac applications. In animal models, introduction of mesenchymal stem cells have been used to demonstrate that stem cells delivered post-MI improve angiogenesis [26] and myocardial wall movement [27]. One study demonstrated that marrow-derived stromal cells increased perfusion in a mouse hindlimb ischemia model [26]. Another study showed that hMSCs may also play a role in angiogenesis in diseased hearts; for example, transplanted hMSCs have increased capillary density in a rat model of dilated cardiomyopathy [28].

2.5 Delivery Options

One barrier to achieving the potential benefits of hMSCs as agents for cardiac repair, is the delivery technique. In an attempt to restore cardiovascular function post MI in the human heart, vast quantities of hMSCs need to be transported to the infarcted area of the heart tissue. The average left ventricle contains approximately 4 billion cardiomyocytes. Heart failure due to MI can occur when approximately 25% of the ventricle, or one billion, cardiomyocytes die. Current technology cannot achieve the goal of complete regeneration, however substantial physiological benefit, or repair, can be derived from the delivery and engraftment of cells into the infarcted heart [7].

Due to the necessity of engrafting a large enough quantity of hMSCs into the heart, different cell delivery options were investigated. A common technique for cellular

delivery to the heart is injection. There are several methods of injection; including intramyocardial injection, intracoronary injection, and intravenous injection. Intramyocardial injection is not a viable technique because it can result in a loss of cells to the injection site, as well as lead to hypoxia and stress, thereby resulting in a significant quantity of cell death [29]. Similar to intramyocardial injection, intracoronary injection is not beneficial because there is a risk of myonecrosis, or tissue death, if cell quantity and infusion characteristics are not precisely determined. In addition, intracoronary injection is also correlated to a decrease in the coronary blood flow in the heart [3]. A third approach used to inject hMSCs is intravenous infusion. Although this appears to be the simplest and least invasive tool for cell delivery, it poses problems with the homing of the cells to the proper organ [3]. The concept of the cells homing to non-cardiac organs and the lack of cell engraftment deems this means of delivery inappropriate for this particular project.

Targeting of cell delivery is essential for localized myocardium repair. If stem cells are to have a maximal effect, they must be concentrated at the site of injury. It has been reported that, in general, hMSCs are attracted to sites of injury [30-33] and specifically to sites of infarction [34, 35]. Although hMSCs are attracted to sites of infarction, the mode of delivery is essential because the success of cellular based regeneration is dependent on the quantity of cells delivered and engrafted to the area of damage [24]. If cell delivery is not localized or if cells leak from the delivery site, such as through the injection of cells, low engraftment rates (3-12%) will result [2-4]. Therefore, improved engraftment rates can be accomplished through the direct application of cells to the myocardial infarction [24], such as through suturing the cells into the heart on biological microthreads.

2.6 Microthreads

To deliver the hMSCs to the infarcted region of the myocardium, a proper platform must be selected. Previous research has shown that biological microthreads can be used as a cellular platform [5], as they have the ability to be woven or braided in order to create a larger and stronger base. Additionally, biological microthreads also often mimic the

structure of natural tissue, are biocompatible, biodegradable and mechanically strong [5]. Finally, biological microthreads are known to aid in cell orientation and migration, which is a key aspect in tissue regeneration [5]. There are various types of biological microthreads, including collagen and fibrin. However, PhD candidate Jacque Guyette suggested the use of fibrin microthreads, as opposed to collagen microthreads, based on a higher hMSC affinity for fibrin microthreads. This affinity can likely be attributed to the properties of the wound healing process and hMSCs' affinity for sites of injury [30-33]; fibrin is found in the initial stages of wound healing, whereas collagen is found in the late stages of wound healing [36].

2.6.1 Fibrin Microthreads

Fibrin has the potential to be used as a scaffold to deliver cells to wound sites. The ischemic damage caused by myocardial infarction and heart failure can be modeled as a wound. The first step in the wound healing process is comprised of the formation of a fibrin, plasma-derived protein matrix, working concurrently with other extracellular matrix (ECM) proteins and cytokines [37]. Fibrin is an appealing natural biomaterial because it is biocompatible, bioresorbable, and essential in normal wound healing. In vitro studies indicate that fibrin can support the growth, migration, and differentiation of mesenchymal stem cells [38]. Fibrin biomaterials are able to form microenvironments that mimic physiologic conditions that are essential for cells.

In addition to the wound healing properties of fibrin, it has been demonstrated that the strength of fibrin microthreads over fibrin gels allows for assembly into bundles of fibrin microthreads that promote cell attachment and proliferation in a highly aligned scaffold for tissue regeneration [5]. Hydrated microthreads are roughly cylindrical and averaged 100 μm in diameter. These fibrin microthreads can be assembled into larger scale tissue constructs to direct cell alignment and migration for tissue regeneration. Typically when cells are applied to the fibrin microthread bundles, they align longitudinally with the long axes of all the microthreads and in the grooves between adjacent microthreads [5].

Furthermore, fibrin microthreads have improved structural and mechanical properties relative to other fibrin matrices, such as fibrin hydrogels. Maintaining the mechanical integrity of the microthreads throughout cell seeding and cell culture is critical for microthread transportation, arrangement into larger structures, and ease of implantation.

2.7 Increasing Cell Density, Cell Attachment, and Cell Quantity

A current limitation of fibrin microthreads as a platform for cellular delivery is the low density of hMSCs attached to the microthreads. To increase the attachment of hMSCs to fibrin microthreads, a variety of methods, including physical surface alterations, cellular adhesion molecules, and seeding technique, were reviewed.

2.7.1 Physical Surface Alterations

Physical surface alterations are able to influence cellular density [39]. The distribution, adhesion, proliferation and differentiation of cells are highly regulated depending partially on the cellular orientation and surface contact [40]. Cellular density can be manipulated when cells align on a surface and fill in the gaps and grooves [5]. Such parameters may be of particular importance in cell types that are multipotent, such as hMSCs. Through the creation of microgrooves on the surface of implanted devices, the activity of the cell can be monitored and regulated. Not only do the microgrooves control the spatial organization of cells, but also affect the activities of cells, leading to the expression of different genes, which may lead to increased cellular attachment [39].

Microgrooves in the scaffold are a potential method for altering the surface and improving cell adhesion. In one study, imprints were made in chitosan–collagen–gelatin blended membranes and the morphology and growth of hMSCs were observed [39]. In this study, the growth of hMSCs was affected by the spacing size between adjacent ridges. The proliferation of the hMSCs on 200 μm patterned membranes was more than four times of that on 20 μm patterned membranes [39]. This result supports the idea that the growth of hMSCs is limited by the size of the microgroove.

Fibrin microthreads can be bundled to make intricate structures with microgrooves between the microthreads. Hydrated fibrin microthreads average 100 μm in diameter [5].

The microthread diameter is a function of the extrusion tube diameter and can be increased to optimize microgroove size and distance between the microthreads. Therefore, if bundled, fibrin microthreads may provide a topography that induces attachment. Through the bundling of these microthreads into organized structures, cell orientation and attachment can be manipulated.

2.7.2 Chemical Surface Alterations: Cell Adhesion Molecules

Cellular attachment at the surface can be achieved in a number of different ways, including the use of adhesion molecules. A review of literature revealed that a number of different molecules may be used to increase cell adhesion. These molecules include a variety of proteins and peptides. A list of possible cell adhesion molecules can be viewed in **Appendix A: Summary of Cell Adhesion Molecules**.

With further research, it was concluded that fibronectin and RGD peptides were feasible for use as a cellular adhesion molecule when applied to fibrin microthreads [41, 42]. As an ECM protein, fibronectin participates in a number of cellular processes including tissue repair, wound healing, and cell migration/adhesion. Additionally, RGD peptides have been investigated as a cellular adhesion molecule in a number of different scaffolding approaches. The outcome of such studies suggests that in low concentrations RGD peptides are actively able to stimulate mesenchymal stem cell attachment to a scaffold [42].

2.7.3 Cell Seeding Techniques

Another method to improve cellular attachment on fibrin microthreads is to establish a superior cell seeding technique, as compared to those currently used in Professor Gaudette's lab. The initial step in adhering cells to a platform, is cell seeding. Cell seeding is fundamental in all tissue engineering applications that incorporate cells onto a platform prior to culture or implantation [43]. Through the improvement of a cell seeding method, cell density can be increased. Advantageous features for any seeding technique include minimized cell injury, high seeding efficiency, reduced seeding time, and

reproducibility. Cells can be destroyed through adverse effects, such as apoptosis or cell lysing. In order for in vitro cell growth to take place within the scaffolds, the seeding technique must minimize these adverse effects [44]. Additionally, the efficiency of the seeding technique influences the cell distribution and consequently affects the ability to achieve homogeneity in the cultured tissues. Efficiency is critical to cell seeding for multiple reasons. Any significant reduction in the total time required for cell seeding could also lead to substantial reductions in expense. Further, long seeding periods can result in adverse effects in which cells might expire or age beyond their useful state, which, in turn, affects the cells' adhesive, proliferation, and differentiation processes [38].

There are a myriad of methods for seeding cells. These methods can ultimately be divided into two types of seeding; static or dynamic. Static seeding is performed by depositing a concentrated cell suspension directly onto the platform. Currently, fibrin microthreads are seeded using several static methods, including the droplet and migration techniques. In droplet seeding, droplets of cell suspension are placed along the length of the microthreads. For migration seeding, microthreads are placed on polymer pedestals, allowing the cells to migrate off of the pedestal and onto the microthread. Dynamic seeding procedures involve stirring or agitation of cells in suspension together with the fibrin microthreads. Previously described devices developed for dynamic seeding take advantage of different, single driving forces such as filtrations [45], rotations [46], electric field [47], magnetic field [48], or vacuum [49]. The use of a seeding device is often difficult since mechanical forces are usually involved in seeding procedures and can be responsible for shear-mediated membrane lysis or triggering of apoptotic pathways [50]. However, studies have shown that the dynamic seeding methods render a more uniform cell distribution within scaffolds, obtaining larger cell yields when compared to static seeding [51].

3.0 Project Approach

3.1 Project Hypothesis

3.1.1 Hypothesis #1

Dynamic cell seeding will increase hMSC density on fibrin microthreads in a reduced time compared to static seeding.

***Rationale*:** Personal communication with Masters of Science candidate Megan Murphy demonstrated that current methods for seeding of cells onto fibrin microthreads are static seeding techniques. The current method of static seeding is droplet seeding. In this method, the fibrin microthreads are adhered to a washer and a drop of cell suspension (500,000 cells/mL), is placed on the washer. Gravity allows for the cells to attach to the fibrin microthread. The limitation of the droplet method is that many of the cells do not attach to the fibrin microthread while gravity induces the cells to fall to the bottom of the culture plate. This method provides an attachment of 37±19 hMSCs/mm on a hydrated bundle of three fibrin microthreads for a 24 hour seeding duration.

Studies have shown that the dynamic seeding methods render a more uniform cell distribution within scaffolds with larger cell yields when compared to static seeding [51, 52]. Therefore, because of the inefficiencies of the current methods, an improved cell seeding technique should allow for increased cell density in a reduced time period.

***Specific Aim*:** Design a dynamic seeding method that increases cell density in a reduced time period as compared to static seeding.

3.1.2 Hypothesis #2

Physical surface alterations, namely bundling fibrin microthreads, will enhance the attachment of hMSCs on fibrin microthreads.

***Rationale*:** Microgrooves have the ability to control the spatial organization of cells because the distribution and adhesion of cells are highly regulated [40]. As demonstrated previously, cells align on bundles of microthreads longitudinally, and fill in the gaps and grooves between microthreads [5]. Therefore, if bundled, fibrin microthreads may provide a topography that induces increased hMSC attachment.

***Specific Aim*:** Compare the number of hMSCs attached to individual fibrin microthreads and bundled fibrin microthreads with comparable diameters.

3.1.2 Hypothesis #3

The application of adhesion molecules, namely fibronectin, to the surface of fibrin microthreads will increase cellular affinity for the microthreads, and thereby improve cell density and attachment.

Rationale: The interaction of cells with solid substrates is important for their attachment. Cells can attach, spread, and migrate on a variety of extracellular glycoproteins including fibronectin, laminin, vitronectin, and collagen. These interactions occur through specific cell surface receptors [53]. As an ECM protein, fibronectin participates in a number of cellular processes including tissue repair, wound healing, cellular migration, and cellular attachment [41]. With its role in cell adhesion, many studies have been focused on using fibronectin as a means to increase cell attachment on synthetic scaffolds [42]. Results from these studies suggests that fibronectin is able to induce significant motogenic activity in hMSCs [54]. Therefore, the application of fibronectin onto the surface of fibrin microthreads could assist in the attachment and migration of cells.

***Specific Aim*:** To adsorb the adhesion molecule, fibronection, to the surface of fibrin microthreads to increase cell density.

3.2 Project Assumptions

To develop a manageable model of our design, several primary project assumptions were established:

- The use of hMSCs in an infarcted area will produce advantageous results in myocardial regeneration
- Fibrin is the best possible choice as a cellular platform for microthreads

3.3 Project Goals

As previously stated, the ultimate goal of the project was to develop a method to increase cell density on fibrin microthreads. To accomplish this task, we proposed the following specific aims:

- Design and develop methods that will result in increased cellular attachment to fibrin microthreads
  - Enhance cell-seeding technique on fibrin microthreads
    - Design equipment that will support the developed cell-seeding technique
  - Explore bundling of fibrin microthreads as a technique to increase cell density on microthreads
  - Determine if utilizing fibronectin, as an adhesion molecule, will increase cell density on microthreads
- Develop a method to quantify cell density on microthreads

4.0 Design

4.1 Objective, Functions and Specifications

Objectives

Based on meetings with the clients and users of the final product, paired with a review of relevant literature, design objectives were produced for the device. These objectives were based on how the stakeholders (designers, clients, and users) 'want' the device to perform. An evaluation of these objectives and their relative importance to the stakeholders can be found in the pairwise comparison chart (Table 13 and Table 14) and objective tree (Figure 19) in **Appendix B: Objectives**. These objectives are categorized below:

Cell density and cell quantity

- Increase cell density from that received with the current static (droplet) seeding technique (37 hMSCs/mm in 24 hours)
- Decrease the time to achieve increased density compared with current technique
- Maximize cell quantity on microthreads
- Control quantity of cells delivered

Cell attachment

- Strength of cell attachment; cells cannot be sheared off when being implanted
- Number of cells attached

Mechanical integrity

- Maintain mechanical integrity of microthreads to allow them to function as sutures

Cell alignment

- Cell alignment in tissue
- Cell alignment on microthreads

Client convenience

- Ease of use for client
- Compatibility with other cell types or lineages
- Easy and cheap to mass manufacture

The first category was the main objective, or goal, of this project. Prior to the selection of fibrin microthreads as a feasible scaffold for tissue regeneration, the cell density on the microthreads must be increased. To increase the density of the cells and maintain the viability of microthreads as a scaffold platform, the speed at which the increased density was attained must be maximized. Maximization of the speed of increased cell density minimizes the amount of time the cells can remodel the fibrin. Remodeling of the fibrin by the cells is detrimental because it decreases the mechanical integrity of the fibrin microthreads. Concomitant with increasing cell density and speed, it was necessary to control the quantity of cells delivered to the heart. By controlling the quantity of cells delivered on the microthreads, the process is made repeatable, and thereby marketable as a scaffold device.

The second objective, although similar to the first, stipulates that, not only should the density be increased, but the cells that are on the microthreads need to adhere. It was central to increasing cell density that the cells attached to the microthreads. The strength of cell attachment implied the ability to transport and manipulate the device without the microthreads detaching. The number of cells attached denotes that once the fibrin microthread has reached its maximum cell capacity, all of the cells are attached.

Following cell density and attachment, maintaining the mechanical integrity of the microthreads was vital. The cells must be seeded rapidly, without detaching; otherwise the mechanical integrity of the system will be compromised. Therefore, the microthreads needed to be compiled into a cellular delivery device, where cells could be seeded densely onto the microthreads (prior to fibrin remodeling), so as to ensure no loss in mechanical integrity. For this compilation to occur, the microthreads must be transported without any additional loss of mechanical integrity.

The third category related to the alignment of cells, both on the microthreads and in the tissue. This was a minor objective, because by bundling the microthreads, the cells are

inherently aligned on the microthreads. Yet, the alignment of the cells upon delivery to cardiac tissue may increase global heart function, due to increased scar compliance [27].

These objectives would not be constructive unless the fifth objective was met. The final device must be easy to use by the client, and therefore easily deployed in the heart. The methods devised for increasing cell density and attachment must be feasible with the available technology and budget constraints. Additionally, ease of use to the client would enhance the project; if compatibility with other cell types and lineages was maintained, and the device was easy, cheap, and scalable for manufacturing.

Functions

While an objective is something that the device *should* accomplish, a function is something that the device *must* do. An elaborate listing of functions can be found in the morphological chart (Table 15) and function enumeration in **Appendix C: Functions**. The following list is a brief overview of what the functions that the microthread must do in order to accomplish our project goals and objectives.

- Must be biocompatible
- Maintain mechanical integrity of the microthread
- Must be transportable
- Prevent cell lysing and apoptosis
- Produce limited by-products
- Must be scaleable for implantation into animal and human tissues
- Must be deliverable to the organ or tissue of interest
- Increase cell density

Although the design process begins with the production of the microthreads, it was first necessary to consider safety. If the system to increase density on the microthreads was successful, it would not be significant if it was not safe for patient use. Once the microthreads were made, they must be transportable. During the transportation process, they cannot break or tear, as they must be fully intact. Once the microthreads have been

transported to the hood, for cell seeding, they must be sterile for cell culture. With the cells on the microthreads, not only must the microthreads provide a hospitable environment for the cells to survive, but the mechanical integrity must be maintained. This is vitally important to the success of the project as fibrin is remodeled quickly.

Constraints

- Must be biocompatible
    - Must be composed of nontoxic material
    - Must have nontoxic degradation products
- Must have adequate mechanical integrity and be delivered to the heart tissue
- Must be cost effective for both producers and the consumers
    - Cannot cost more than our allotted budget
- Must be finished by April 2008

These constraints were devised to immediately filter out any design alternatives that were not feasible. The device must be biocompatible as it will be inserted into the body; an immune response is not desirable. The device will be placed into the heart and therefore must have adequate mechanical integrity to be implanted in the heart. The costs must remain within the designated cost budget and also must be completed before the due date in April 2008.

4.2 Specifications

Following the clear definition of the project objectives, functions and constraints; design specifications were developed to detail the specific performance standards that the device must fulfill. These specifications were developed to help assess the efficacy of the device after the completion of experimental analysis. The overall specification for the number of hMSCs on fibrin microthreads that should be obtained are given in Table 1.

Specification	Description
Cell Density	Increased cell quantity in a shortened seeding duration from that achieved with static seeding on individual microthreads (37 cells/mm in 24 hours).

Table 1. Specifications.

As defined by our initial project statement, the goal of this project was to increase cell density on fibrin microthreads. To apply the maximum number of cells per microthread, a confluent monolayer should be achieved. Calculations for obtaining this specification are located in **Appendix D: Calculations for Specifications**.

To put this specification into better perspective, one must examine the amount of cells necessary to deliver to a region of infarction. Heart failure can occur when an infarct kills approximately 25% of the left ventricle, or 1 billion cardiomyocytes [7]. To replace this many cells would take a large quantity of fibrin microthreads). Current technology cannot achieve the goal of complete regeneration, however substantial physiological benefit, or repair, can be derived from the engraftment of cells into the infarcted heart [55]. It is important to note that the success of cell engraftment is not quantified in the majority of the human studies; thus the results are interpreted as a qualitative correlation of functional changes.

Although extensive research has not been done involving hMSC engraftment in the human heart, studies have investigated engraftment rates in animal models. Research has indicated that noticeable improvement to cardiac function occurs when 230,000 or more hMSCs are engrafted into the rat heart [24,2]. This implies that a minimum of 230,000 hMSCs must be delivered to the heart, assuming a 100% engraftment rate. With our specification that there must be an individually confluent layer of 1,017 cells per one mm of length on a hydrated bundle of 10 fibrin microthreads. This suggests that we must have a minimum of $\frac{230{,}000\,cells}{1017\,\frac{cells}{mm}} = 226mm = 22.6cm$ of a hydrated bundle of 10 fibrin microthreads sutured into a rat heart.

Delivery method	Number of cells delivered	Percent Engrafted	Total number of engrafted cells	Animal model
Collagen scaffold [24]	1 million	23%	230,000	Rat
IC (intracoronary) infusion [3]	50 million	6%	3 million	Porcine
EC (endocardial) injection [3]	50 million	3%	1.5 million	porcine
IM injection [2]	4 million	6-12%	240,000-480,000	Rat

Table 2. Engraftment rates from previous animal studies.

Note: This is not the exact number of viable cells delivered; as calculating the exact percentage of cells delivered is complicated by an unknown rate of hMSC proliferation and loss.

4.3 Client Statement

Therefore, based on the aforementioned objectives, functions, constraints, and specifications, the client statement was re-evaluated.

4.3.1 Initial Client Statement

Design a system to increase cell density on biological microthreads for use in adult stem cell-assisted cardiovascular regeneration.

4.3.2 Revised Client Statement

Design a system to increase cell density on biological microthreads for use in cellular assisted cardiovascular regeneration. These biological microthreads must be biocompatible, using natural biomaterials that do not elicit an adverse tissue response. Microthreads must be able to support human mesenchymal stem cells (hMSCs), which should assist in increasing global heart function in the infarcted heart. In order to allow for the regeneration of ischemic tissue, optimal cell density and cell quantity must be achieved. Based on literature references and preliminary data, a bundle of 10 fibrin microthreads, assuming individual confluency, must be able to support 370-1017 cells per millimeter. This confluent monolayer of cells must be achieved in the shortest

duration possible, as fibrin microthreads are rapidly remodeled by cells, typically within one week. In addition to maintaining the mechanical integrity of the fibrin microthreads via rapid seeding technique, the strength of cell attachment must be strong enough to withstand the shear of suturing through the heart. To make this an industrially applicable technique, the biological microthreads must be easily delivered using standard surgical techniques.

4.4 Developing Design Alternatives

This section analyzed the process followed to develop basic design concepts, create design alternatives, and select a final design. The initial stages of this process begun by identifying various means to fulfill the functions which then combined the means into design alternatives. The design alternatives were then critiqued based on their ability to fulfill our objectives and constraints. Finally, the most compatible design was selected.

4.4.1 Morphological Chart and Evaluation Matrices

The morphological chart, shown in **Appendix C: Functions** Table 15 lists all the functions along with the possible means to carry out each function that were produced via brainstorming (**Appendix E: Design Alternatives**). To form design alternatives, combinations of rational and feasible means were paired. The following list details a number of the possible alternatives:

- Dynamic seeding (rotation seeding), cell adhesion molecule, bundled microthreads
- Static seeding (droplet method), cell adhesion molecule
- Dynamic seeding (rotation seeding), surface modification (microgrooves)
- Static Seeding (droplet method), surface modification

These alternatives were organized into a numerical evaluation matrix and weighted according to how well they fulfilled the constraints and objectives. A list of detailed

metrics can be seen in **Appendix E: Design Alternatives**. An evaluation matrix was compiled to evaluate each candidate design. An excerpt of this table can be seen in Table 3. Each objective was broken down and defined on a scale of 0-2, with 0 being the least promising and 2 being the most promising in fulfilling the objective. For example, the objective 'number of cells attached' was given a score of 2 if the design had very high potential for increasing the number of cells attached to the fibrin microthread, and a score of 0 was given if the design would not feasibly increase the number of cells attached to the fibrin microthread.

Objectives & Constraints ↓	Design →	Weight	Design 1	Design 1	Design 1	Design 1
Constraint 1		Y/N				
Objective 1		0.133				
Total Score						

Table 3. Sample evaluation matrix to evaluate design alternatives.

The top row lists the design alternatives, and the left column lists the design objectives (O) and constraints (C). The second column shows the objective weights that were established earlier from the weighted objective tree. The designs were evaluated by scoring them against the metrics (0, 1, or 2) and then multiplying by its corresponding objective weight. Constraints were given a score of Y or N based on the designs ability to fulfill the constraint (Y: Yes) or inability to fulfill the constraint (N: No). The final score for the design was obtained by adding the weighted metric score for each objective. The top design candidates can be seen in Table 4. The full list of evaluated designs and total scores, along with a description of each design and justification of scoring, can be found in **Appendix E: Design Alternatives**.

Design Alternatives	**Total Score**
Dynamic seeding (rotation seeding), cell adhesion molecule	1.33
Static seeding (droplet method), cell adhesion molecule	.701
Dynamic seeding (rotation seeding), surface modification (microgrooves)	.979
Static Seeding (droplet method), surface modification	.824

Table 4. Evaluation of design alternatives.

After the basic methods to increase the attachment of hMSCs to fibrin microthreads were established, several candidate options for surface alterations, cell adhesion molecules, and cell seeding techniques were evaluated against the design objectives using another set of metrics. Detailed calculations for these metrics can be seen in **Appendix E: Design Alternatives**. Each objective was placed in a scale of 0-2, following the same process as listed above with 0 being the least favorable and 2 being the most favorable in fulfilling the objective. After these metrics were established, an evaluation matrix was made to evaluate each of the candidates under the three methods for increasing cell density. An example of this table can be seen in Table 5. Additionally, Table 6 provides the scores associated with each candidate for seeding technique, surface alterations, and surface adhesion molecules.

Objectives →	Objective 1	Objective 2	Objective 3	Total
Objective Weights →	.133			
Method 1	X (.133)			

Table 5. Sample evaluation matrix evaluating each of the candidate methods for increasing cell density.

Candidate Methods to Increase Cell Density	**Subcategories**	**Total Score**
Dynamic seeding technique		1.67
	Rotational seeding	*1.67*1.44=2.40*
	Stirred seeding	*1.67*0.68=1.14*
	Agitation	*1.67*0.41=0.68*
Physical surface alteration		1.09
	Bundling of microthreads	*1.09*1.34=1.45*
	Patterned microgrooves on microthreads	*1.09*0.73=0.79*
	Electrospray onto microthreads	*1.09*0.256=0.28*
Cell adhesion molecules		0.99
	Fibronectin	*0.99*1.34=1.32*
	RGD peptides	*0.99*0.73=0.72*
	Collagen	*0.99*0.71=.070*
	Laminin	*0.99*0.46=0.45*

Table 6. Ranking of each candidate method to increase cell density on microthreads.

Part II. Methods and Results

5.0 Methodology

This chapter encompasses the methods for producing and testing the selected materials and methods to increase cell attachment to the fibrin microthreads. The production of the microthreads, methods for seeding, and methods for quantifying the attachment of hMSCs to fibrin microthreads are discussed.

5.1 Cell Culture

A majority of the experiments conducted in correlation to the completion of this project required the use of cultured cells. Experiments were performed using passage 14-19 hMSCs (Lonza, Allendale, NJ) cultured in Dulbecco's modified Eagle's Medium (DMEM; Mediatech, Herndon, VA) enhanced by 10% fetal bovine serum (FBS; Mediatech, Herndon, VA) and 1% penicillin streptomycin (Mediatech, Herndon, VA).

To maintain sterility, all cell culture experiments were completed aseptically in a biological safety hood. An exact cell culture protocol can be viewed in **Appendix F: Cell Culture Protocol**. Cells used in each experiment were incubated at 5% CO2 and 37°C in tissue culture treated flasks.

5.2 Fibrin Microthread Production

Fibrin microthreads were self-assembled from solutions of fibrinogen and thrombin using a coextrusion process employing a blending applicator tip (Micromedics, St. Paul, MN), which consisted of a dual bore (25 ½ gauge; BD, Franklin Lakes, NJ) needle attached to PE (polyethylene) tubing (BD, Franklin Lakes, NJ). Two diameters of PE tubing (ID 0.38 mm / ID 0.58mm) were utilized to create different diameter microthreads. In this system, fibrinogen and thrombin solutions were warmed to 37°C and placed into separate 1 mL syringes (BD, Franklin Lakes, NJ). The fibrinogen solution was prepared fibrinogen from bovine plasma (Sigma, St. Louis, MO) that was dissolved in HEPES buffered saline

(HBS; 20 mM HEPES, 0.9% NaCl; Sigma, St. Louis, MO) at 70 mg/mL. The other solution was prepared thrombin from bovine plasma (Sigma, St. Louis, MO) that was stored frozen as a stock solution at a concentration of 40 U/mL in HBS and then was diluted from the stock to a final concentration of 6 U/mL in 40 mM $CaCl_2$ solution.

The blending applicators Luer lock to the two 1-ml syringes through individual bores. The individual bores, enabled the simultaneous extrusion of the contents of two separate syringes filled with fibrinogen and thrombin. With the aid of a syringe pump (Sage Instruments, Cambridge, MA), the two solutions were simultaneously extruded through the PE tubing at a pump speed of 0.125 ml/min into a bath of 10 mM HEPES, pH 7.4 maintained at room temperature. After allowing the coextruded thick diameter fibers (coextruded with 0.58 mm PE tubing) to self-assemble for 15 minutes, microthreads were removed from the bath, air-dried under the tension of their own weight, and stored at room temperature until use. The thin-diameter fibers, which were coextruded with 0.38 mm PE tubing, were allowed to self-assemble for 15 minutes and the microthreads were removed from the bath and bundled into groups of three, five, or ten microthreads when damp. The bundled microthreads were then air-dried under the tension of their own weight, and stored at room temperature until use.

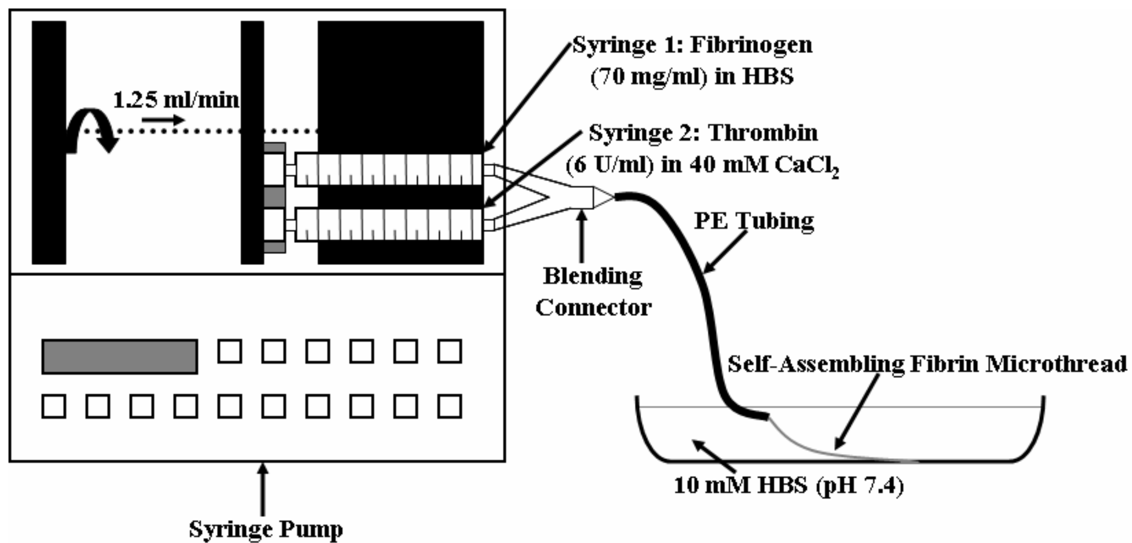

Figure 1. Schematic drawing of coextrusion system for producing self-assembled fibrin microthreads. Image adapted from a schematic by Cornwell, 2007.

To sterilize the fibrin microthreads, they were placed into a 1-well tissue culture plate (BD, Franklin Lakes, NJ). The microthreads were then rehydrated in sterile Dulbecco's phosphate buffered saline (DPBS; Mediatech, Herndon, VA) for 15 minutes, sterilized with isopropyl alcohol (Sigma, St. Louis, MO) for 1 hour, and then rinsed three times in sterile DPBS for 15 minutes.

5.3 Preliminary Experiments

Initial experiments were conducted comparing the number of cells adhered to fibrin microthreads using dynamic versus static seeding techniques. Additionally, these preliminary experiments evaluated the number of cells adhered to bundles of three thin diameter (hydrated diameter: 140±56 μm) fibrin microthreads versus individual thick diameter (hydrated bundled diameter: 123±27 μm).

5.3.1 Static Seeding: Droplet Method

The current seeding technique developed to seed hMSCs onto fibrin microthreads is droplet seeding, which is a form of static seeding. Static seeding using the droplet method follows the previously mentioned techniques for fibrin thread production. Once

sterilized, thick fibrin microthreads and bundles of three fibrin microthreads were cut to 2.5 cm in length and were glued to 3.0 cm aluminum washers with sterile silicone adhesive (Silastic Silicone Type A; Dow Corning, Midland, MI). Concurrently, Thermanox^{TM} coverslips (Nalge Nunc International, Rochester, NY), which had been sterilized for 1 hour in isopropyl alcohol, were glued with the same silicone adhesive to the middle of each well of two 35 mm wells of a 6-well tissue culture plate (BD, Franklin Lakes, NJ) to serve as defined cell-seeding areas. The microthreads on washers were placed on top of the Thermanox^{TM} coverslip in the 35 mm well and the silicone adhesive sets for 24 hours. Following standard procedure for passaging, hMSCs were released from monolayer with trypsin, centrifuged, and re-suspended at a concentration of 500,000 cells/mL in media (10% FBS and 1% Penicillin Streptomycin in DMEM). Using a 1-mL syringe and a 27 ½ gauge needle, 100 µL of hMSC suspension were added to each well, over the microthreads and onto the Thermanox^{TM} coverslip (Figure 2). The 6-well tissue culture plates were then placed into 37° C, 5% CO_2 incubators. At 24 hours, washers with microthreads were removed from the 35 mm wells and the microthreads were then stained with Hoechst dye (according to 5.4.2 Hoechst Nuclear Stain). The microthread bundles were then viewed on a glass slide under a fluorescent microscope. Cell counts were taken, and attachment was assessed relative to the number of seeded cells per millimeter of fibrin microthread. Once the cells were stained and imaged with Hoechst dye, the microthreads were placed in 96 well plates (BD, Franklin Lakes, NJ) which contained 100 µl of media. One additional column of media was added as a control, as well as one column with media and sterilized microthreads without cells. 20 µl of MTS (Promega, Madison, WI) was added and allowed to sit for 4 hours and then the absorbance was read at 490 nm (according to 5.4.1 MTS Assay).

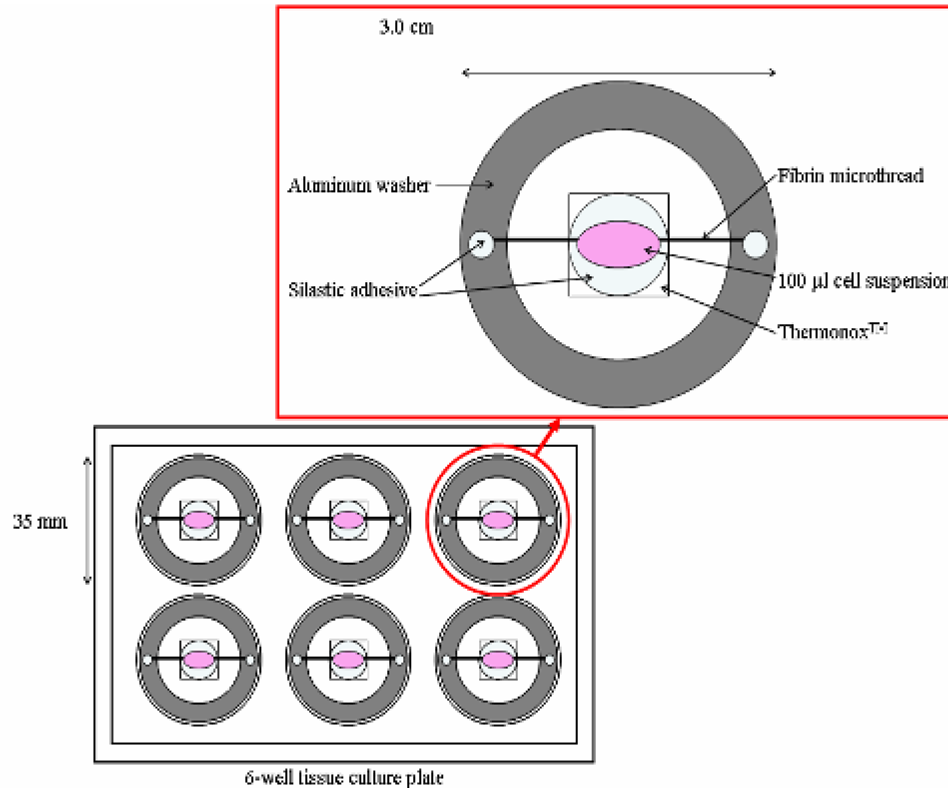

Figure 2. Schematic of droplet seeding.

Image adapted from a schematic by J. Guyette.

5.3.2 Dynamic Seeding: Rotational Method

To improve the density of hMSCs attached to fibrin microthreads, as compared to the static (droplet) seeding technique, a new dynamic seeding technique was developed, utilizing a rotational seeding system. Following fibrin microthread production and sterilization, the microthreads were threaded through 5cm of Silastic tubing (1.98 mm inner diameter (ID) x 3.18 mm outer diameter (OD); Dow Corning, Midland, MI) using a guide wire to pass the microthreads through the tubes. Following standard procedure for passaging, hMSCs were released from monolayer with trypsin, centrifuged, and re-suspended at a concentration of 500,000 cells/mL in media (10% FBS and 1% Penicillin Streptomycin in DMEM). 1 µl of cell suspension was then added to Eppendorf tubing to facilitate the injection process. Each piece of Silastic tubing was then injected with 100 µm of cell suspension (Figure 3) using a 1-ml syringe and a 27 ½ gauge needle. A 22 gauge needle (BD, Franklin Lakes, NJ) was used to release the pressure when injecting the solution. The labeled tubes were then attached to a rotational seeding device (4 RPM;

Figure 4) within a incubator set at 5% CO_2 and 37°C. The tubes were left to rotate for 24 hours, removed from the rotational seeding device and then analyzed using the Hoechst nuclear stain and MTS viability assay.

Figure 3. Cell suspension being injected into Silastic tubing.

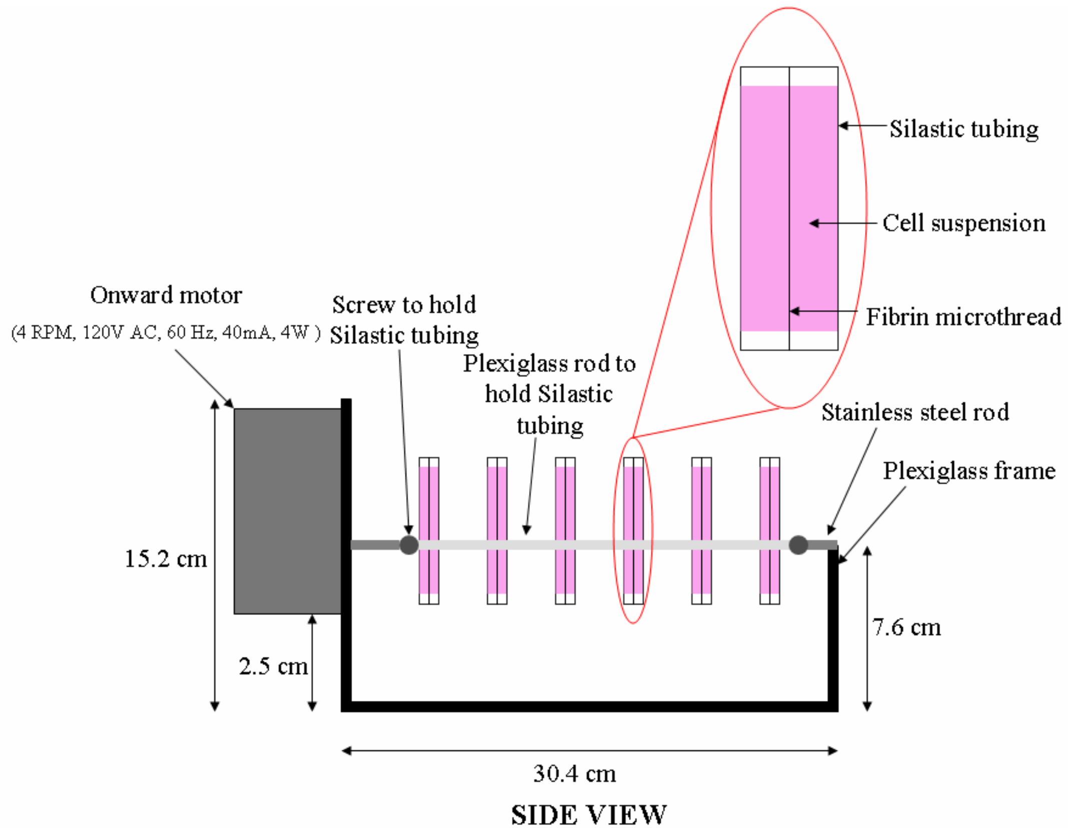

Figure 4. Rotational seeding device.

5.4 Cell Quantification

Two methods were initially used to quantify the number of cells present on fibrin microthreads. The MTS assay was used in preliminary experiments, however, due to the lack of sensitivity of the MTS, the primary technique used for cell quantification in succeeding experiments was individually counting cell nuclei after the application of Hoechst dye.

5.4.1 MTS Assay

The MTS assay is used to measure the number of live cells in culture. Mitochondria break down a tetrazolium compound to form a purple formazen dye. The concentration of this dye is measured and related directly to the cell's metabolic activity.

Following cell seeding, fibrin microthreads were placed in individual wells of a 96-well plate. 100 µl of media was placed on top of the microthreads, along with 20 µl of MTS solution. Concurrently, a 96-well plate was set up according to Table 7 to be used as a standard curve for obtaining cell quantities per fibrin microthread. The plates were then incubated for up to 4 hours and the absorbance was read at 490nm.

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

Table 7. MTS standard curve for cell concentrations.

5.4.2 Hoechst Nuclear Stain

Another method to quantify the number of cells present on fibrin microthreads was to use a nuclear stain, such as Hoechst dye (Sigma, St. Louis, MO), and then view cells under an optical microscope. Hoechst dye fluorescently labels the cells by binding to DNA.

This florescent marker excites at 350 nm and emits blue light at 460 nm, An example can be seen in Figure 5.

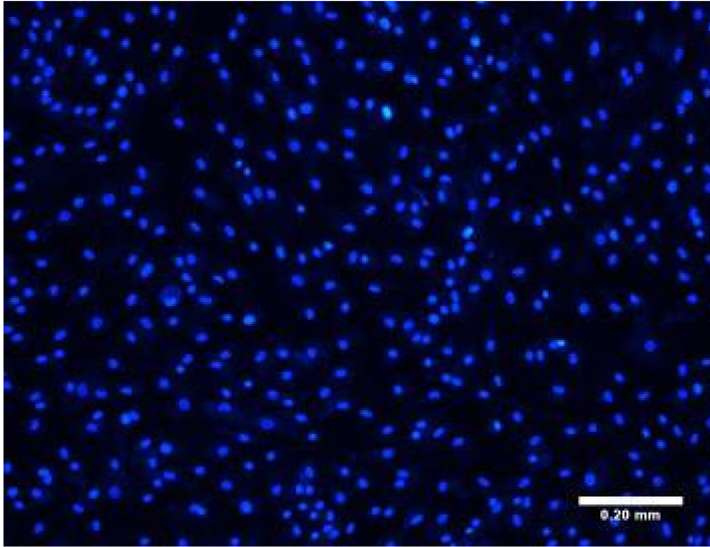

Figure 5. Hoechst stained hMSCs.

The dye was used after experiments to determine the amount of cells adhered to the fibrin platform. Before applying the dye, the microthreads were washed for 15 minutes in DPBS and then fixed for 15 minutes using 4% paraformaldehyde. Once the cells were fixed, the Hoechst dye was made in a working solution by diluting it 1:6000 in DPBS. The dye was then applied to the microthreads for 15 minutes, washed in DPBS for 15 minutes. Finally, the microthreads were mounted to the glass slide with an aqueous mounting media.

5.5 Experimental Methods

Preliminary results indicated that dynamic seeding (rotational seeding) was a more effective seeding technique than static seeding (droplet seeding) and that bundling thin fibrin microthreads encouraged a larger number of cells to adhere than individual thick fibrin microthreads. Due to these results, the ensuing experiments were conducted using a rotational seeding technique and bundled fibrin microthreads with only one parameter

altered in each experiment. The experiments conducted, following the preliminary results, were as follows:

5.5.1 Varying the number of fibrin microthreads in each bundle

Individual fibrin microthreads were extruded, using the technique previously described (5.2 Fibrin Production), with 0.38 mm ID PE tubing. Briefly, these individual fibrin microthreads were bundled into groups of 3, 5, or 10, sterilized, and then placed in sterile Silastic tubing (ID 1.98 mm x OD 3.18 mm). Cultured hMSCs were then injected into the Silastic tubing containing the bundled fibrin microthreads and rotationally seeded (5.3.2 Dynamic Seeding: Rotational Method) for 24 hours.

5.5.2 Varying the seeding duration

Individual fibrin microthreads were extruded, using the technique previously described (5.2 Fibrin Production), with 0.38 mm ID PE tubing. Briefly, these individual fibrin microthreads were bundled into groups of 10, sterilized, and then placed in sterile Silastic tubing (ID 1.98 mm x OD 3.18 mm). Cultured hMSCs were then injected into the Silastic tubing containing the bundled fibrin microthreads and rotationally seeded (5.3.2 Dynamics Seeding: Rotational Method) for 12 hours, 24 hours, or 48 hours.

5.5.3 Varying the seeding tube diameter

Individual fibrin microthreads were extruded, using the technique previously described (5.2 Fibrin Production), with 0.38 mm ID PE tubing. Briefly, these individual fibrin microthreads were bundled into groups of 10, sterilized, and then placed in sterile Silastic tubing (ID 1.98 mm x OD 3.18 mm or ID 0.51 mm x OD 0.94 mm). Cultured hMSCs were then injected into the Silastic tubing containing the bundled fibrin microthreads and rotationally seeded (5.3.2 Dynamics Seeding: Rotational Method) for 24 hours.

5.5.4 Passively adsorbing an adhesion protein (fibronectin) to the fibrin microthreads

Individual fibrin microthreads were extruded, using the technique previously described (5.2 Fibrin Production), with 0.38 mm ID PE tubing. Briefly, these individual fibrin microthreads were bundled into groups of 10, sterilized, and then placed in sterile Silastic tubing (ID 1.98 mm x OD 3.18 mm). Then, the microthreads were washed with DPBS and 100µl of fibronectin (50 µg/ml) (Sigma, St. Louis, MO) was injected into the Silastic tubing and placed at 4°C to allow for passive adsorption into the fibrin microthread bundles. After 24 hours, the fibronectin was removed from the Silastic tubing. Cultured hMSCs were then injected into the Silastic tubing containing the bundled fibrin microthreads and rotationally seeded (5.3.2 Dynamics Seeding: Rotational Method) for 24 hours.

5.5.5 Proof of concept: delivering hMSCs to cardiac tissue via suturing

In order to test the feasibility of our project, fibrin microthreads were sutured through the epicardium of a heart from a 200 lb pig. Bundles of ten fibrin microthreads were rotationally seeded for 24 hours in thin diameter tubing (Silastic tubing, ID 0.51 mm x OD 0.94 mm). The first group was control fibrin microthreads that were Hoechst dyed, immediately following seeding. The second group of fibrin microthreads were sutured into the epicardium of the pig heart and pulled out of the tissue and then Hoechst dyed. An additional group was sutured into the cardiac tissue and then cryosectioned and the slides were then H & E stained.

6.0 Results

6.1 Preliminary Results

To obtain an increased density of hMSCs on fibrin microthreads, initial experiments were conducted to investigate the effect of seeding technique and the effects of surface alterations, on hMSC attachment to fibrin microthreads. The microthreads were either statically seeded, using the droplet method, or dynamically seeded, using a rotational seeding technique. Additionally, these microthreads were either individual thick diameter threads (average diameter: 123±27 μm) or bundles of three thin fibrin microthreads (average bundled diameter: 140±56 μm). There is no statistically significant difference in these diameters when compared using a two-tailed, unequal variance t-test (p=0.1596).

The results of this preliminary experiment can be seen in Figure 6 and Table 8. Results were obtained by counting the Hoechst-stained hMSCs under a fluorescence microscope. Although use of the MTS assay was investigated as an analysis technique to quantify the number of cells per microthread, the MTS results were not considered valid, as the MTS did not appear to have enough resolution or sensitivity to acquire an accurate cell count, as can be seen in **Appendix G: MTS Sensitivity and Results**. Therefore, using a two-tailed unequal variance t-test, preliminary results of Hoechst-stained hMSCs indicated that the bundles of three dynamically seeded fibrin microthreads (295±115 cells/mm) were statistically significant (p=0.007) compared to the group with the next largest average cells per millimeter, which was dynamically seeded thick microthreads (57±40 cells/ mm). The cells tended to clump into large groups along the length of the fibrin microthreads, and did not provide a confluent monolayer, which can be seen in Figure 7. Other experimental groups provided even lower cell counts with the thick microthreads statically seeded at 37±19 cells/ mm and thin bundles of three microthreads statically seeded at 40± 24 cells/ mm.

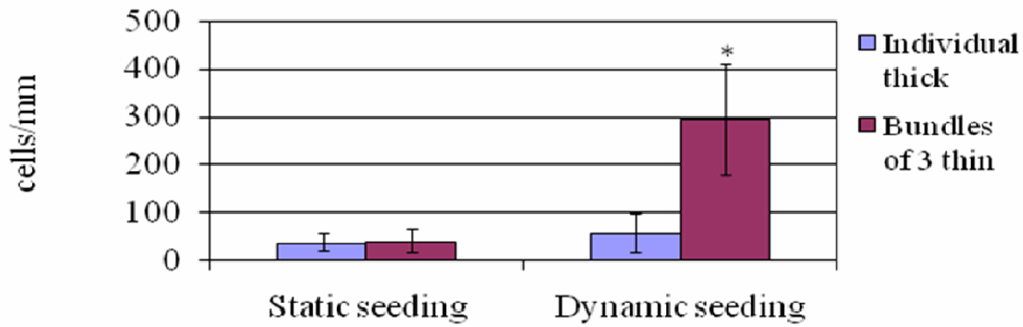

Figure 6. Improved hMSC attachment on 24 hour rotationally seeded bundles of three fibrin microthreads.

	Static seeding with thick fibrin microthreads	**Static seeding with bundles of 3 thin fibrin microthreads**	**Dynamic seeding with thick fibrin microthreads**	**Dynamic seeding with bundles of 3 thin fibrin microthreads**
Overall average (cells/mm)	37	40	57	295
Standard error (cells/mm)	19	24	40	115

Table 8. Seeding technique and microthread bundling average and standard error of the quantity of cells per millimeter of microthread.

a) b)

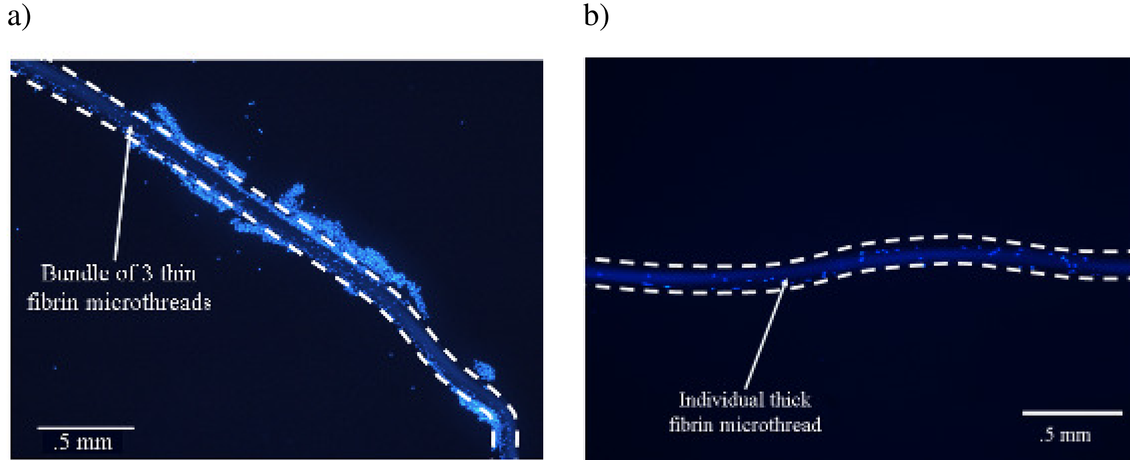

Figure 7. a) Bundle of microthreads dynamically seeded with groups of hMSCs along the length of the microthread. b) Statically seeded thick fibrin microthread with fewer attached hMSCs.

6.2 Rotator Design

Based on these results, a rotational seeding device was designed (Figure 8 and Figure 9).

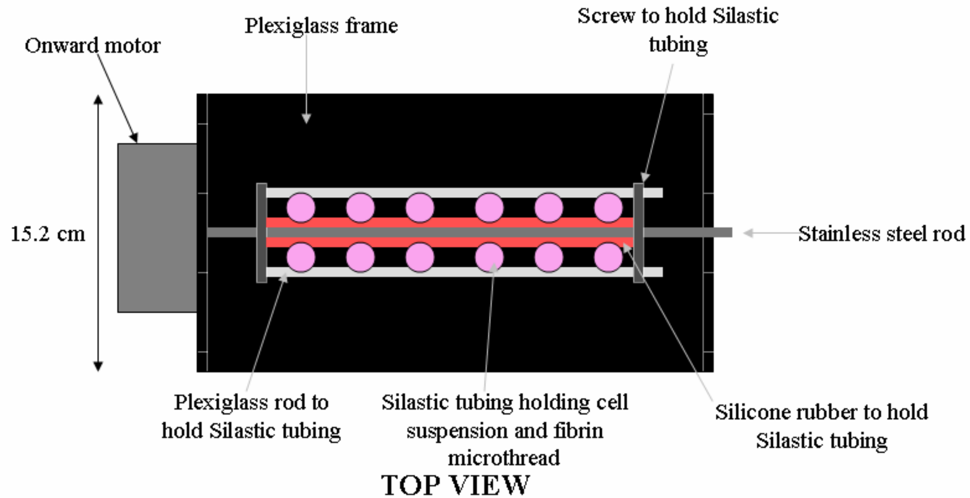

Figure 8. Schematic drawing of the rotational seeding device.

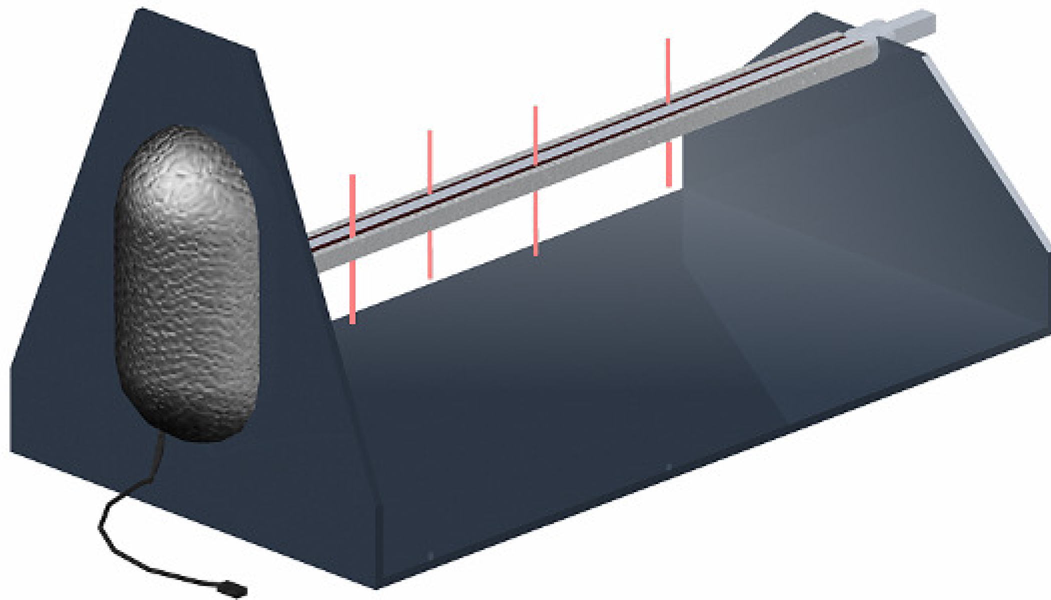

Figure 9. CAD drawing of the rotational seeding device.

6.3 Results

Following the preliminary results, which indicated that dynamic (rotational) seeding resulted in improved hMSC attachment on fibrin microthreads, a rotational seeding device was designed (Figure 8 and Figure 9). This rotational seeding device was used for seeding the hMSCs onto the fibrin microthreads in the following experiments. Additionally, the following results only use bundled microthreads, after preliminary results indicated that bundles of three thin fibrin microthreads had increased hMSC attachment, as compared to an individual fibrin microthread of approximately equivalent diameter.

6.3.1 Comparison of number of fibrin microthreads per bundle: groups of 3, 5 and 10

Bundles of 3, 5, and 10 thin fibrin microthreads (individually extruded with 0.38 mm ID PE tubing and then bundled) were rotationally seeded for 24 hours and then compared for increased cells per millimeter of fibrin microthread. Using a two-tailed unequal variance

t-test, results indicated that the bundles of ten fibrin microthreads (345±225 cells/mm) were not statistically significant (p=0.197) compared to the group with the next largest average cells per millimeter, which was bundles of 5 fibrin microthreads (103±71 cells/mm). The bundle of three fibrin microthreads provided even lower cell counts (46±25 cells/mm). These results can be viewed below Figure 10 and Table 9. Additionally, a confluent monolayer of hMSCs was achieved on the fibrin microthread, as compared to preliminary results which showed clumps of cells along the length of the fibrin microthread.

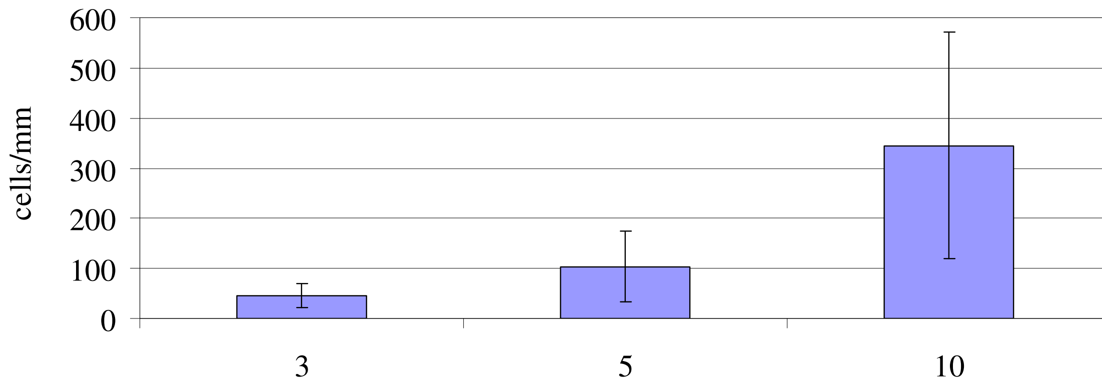

Figure 10. Graph showing improved hMSC attachment on bundles of ten fibrin microthreads rotationally seeded for 24 hours.

	Bundles of 3 fibrin microthreads	Bundles of 5 fibrin microthreads	Bundles of 10 fibrin microthreads
Overall average (cells/mm)	46	103	345
Standard error (cells/mm)	25	71	225

Table 9. Average and standard error, comparing the cells per millimeter of microthread for bundles of 3, 5, and 10 fibrin microthreads.

6.3.2 Comparison of seeding duration of fibrin microthreads: 12, 24, or 48 hours

Bundles of ten fibrin microthreads were rotationally seeded for 12 hours, 24 hours, or 48 hours then compared for increased cells per millimeter of fibrin microthread. Using a two-tailed unequal variance t-test, results indicated that the bundles of ten fibrin microthreads (648±234 cells/ mm) were found to be significantly increased ($p=0.0002$) as compared to both the 12 hour seeding duration and the 48 hour seeding duration. A confluent monolayer of hMSCs was achieved along the length of the fibrin microthread as can be seen in Figure 11. Bundles of ten fibrin microthreads rotationally seeded for 12 hours had 233±117 cells/ mm and those seeded for 48 hours had 208±115 cells/ mm. For the bundles of ten fibrin microthreads seeded for 48 hours the media diffused or leaked out of the tubing that was holding the rotationally seeded fibrin microthread. The results of this experiment can be viewed below in Figure 12 and Table 10.

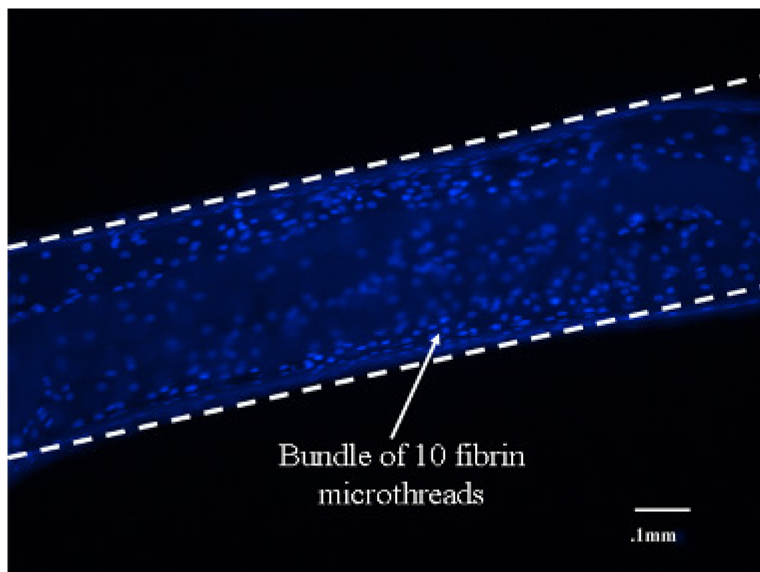

Figure 11 . A confluent monolayer of hMSCs on a bundle of ten fibrin microthreads rotationally seeded for 24 hours.

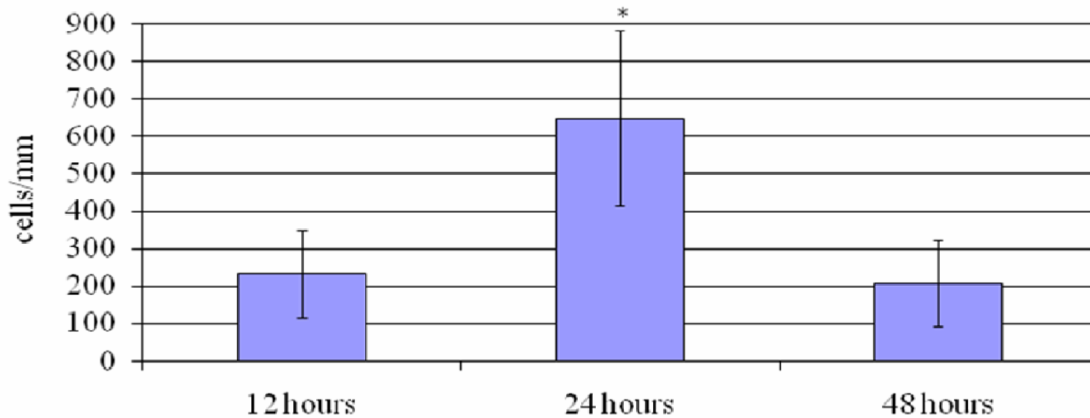

Figure 12. Graph showing improved hMSC attachment on bundles of ten fibrin microthreads rotationally seeded for 24 hours.

	12 hours	24 hours	48 hours
Overall average (cells/mm)	233	648	208
Standard error (cells/mm)	117	234	115

Table 10. Average and standard error, comparing the number of cells permillimeter of fibrin microthread for rotational seeding durations of 12 hours, 24 hours and 48 hours.

6.3.3 Comparison of seeding tube diameter: thin or thick Silastic tubing

Bundles of ten fibrin microthreads were rotationally seeded for 24 hours in thin diameter tubing (Silastic tubing, ID 0.51 mm x OD 0.94 mm) or thick diameter tubing (Silastic tubing, ID 1.98 mm x OD 3.18 mm) with the same quantity of cells in each tube (50,000 cells per tube; 10 µl of 5,000,000 cells/ml in the thin diameter tubing and 100 µl of 500,000 cells/ml in the thick diameter tubing) and then compared for increased cells per millimeter of fibrin microthread. Thick diameter tubing had been used in all prior experiments.

Using a two-tailed unequal variance t-test, results indicated that the bundles of ten fibrin microthreads in thick diameter tubing (515±245 cells/ mm) resulted in statistically significantly (p=0.003) more hMSCs per millimeter of microthread as compared to

bundles of ten fibrin microthreads in thin diameter tubing (96±41 cells/ mm). For the bundles of ten fibrin microthreads seeded for in thin diameter tubing, the media diffused or leaked out of the tubing that was holding the rotationally seeded fibrin microthreads. The results of this experiment can be viewed below in Figure 13 and Table 11.

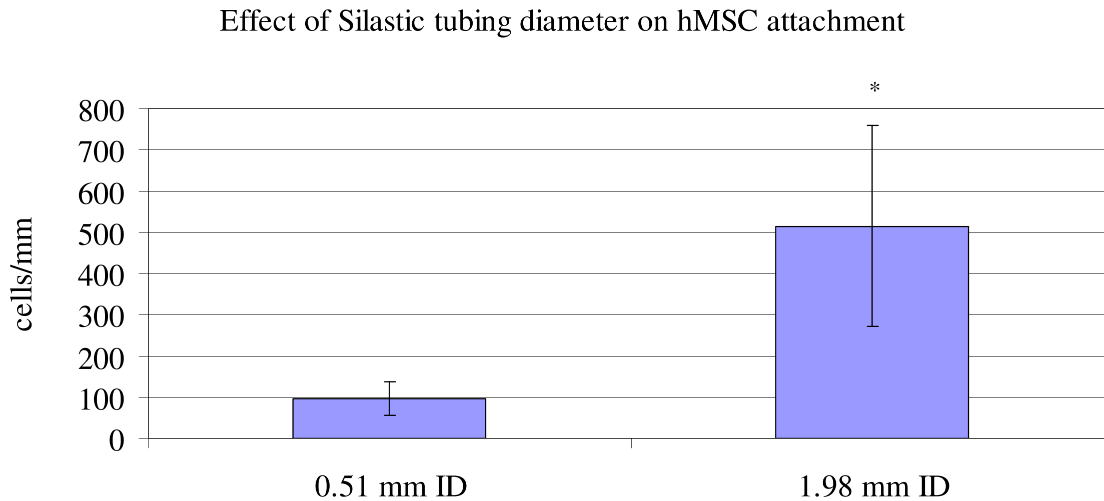

Figure 13. Graph showing no improvement of hMSC attachment on bundles of ten fibrin microthreads rotationally seeded for 24 hours in thin diameter tubing.

	Thin (Silastic tubing, ID 0.51 mm x 0.94 mm)	Thick (Silastic tubing, ID 1.98 mm x 3.18 mm)
Overall average (cells/mm)	96	515
Standard error (cells/mm)	41	245

Table 11. Average and standard error, comparing the number of quantity of cells per millimeter of fibrin microthread for thin and thick diameter tubing.

6.3.4 Proof of concept: suturing rotationally seeded, hMSC covered fibrin microthreads into a pig heart

Bundles of ten fibrin microthreads were rotationally seeded for 24 hours in Silastic tubing (ID 1.98 mm x OD 3.14 mm). The first group was control fibrin microthreads that were Hoechst dyed. The second group of fibrin microthreads were sutured into the epicardium of the pig heart and pulled out of the tissue and then Hoechst dyed. A cell seeded fibrin microthread sutured through the epicardium of a pig heart can be seen in Figure 14. Additionally, the fibrin microthread in the porcine cardiac tissue was cryosectioned and can be viewed in Figure 15. A comparison of the control threads and the threads sutured fully through the heart can be seen in Figure 16. After being sutured fully through the porcine cardiac tissue, hMSCs remained on the bundles of ten fibrin microthreads.

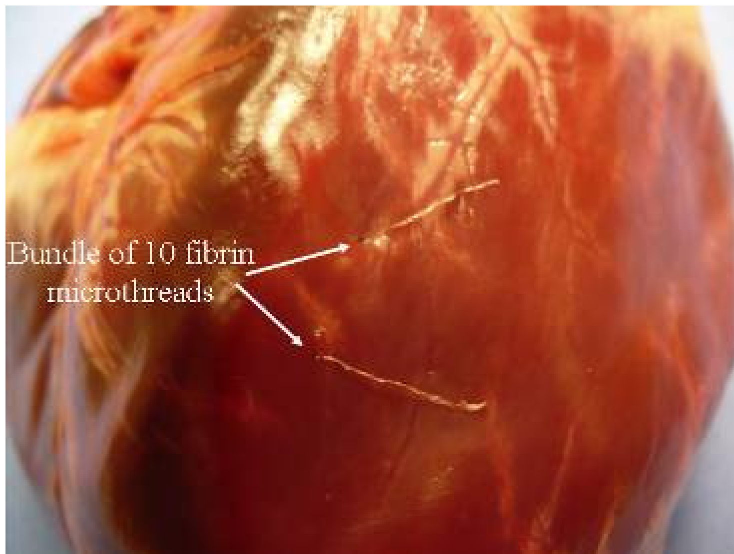

Figure 14. A bundle of 10 fibrin microthreads, seeded for 24 hours with hMSCs has enough mechanical integrity (i.e. the hMSCs have not remodeled the fibrin) to be sutured into the epicardium of a pig heart without breaking.

Figure 15. Cryosectioned porcine cardiac tissue with a bundle of 10 fibrin microthreads, stained with H & E.

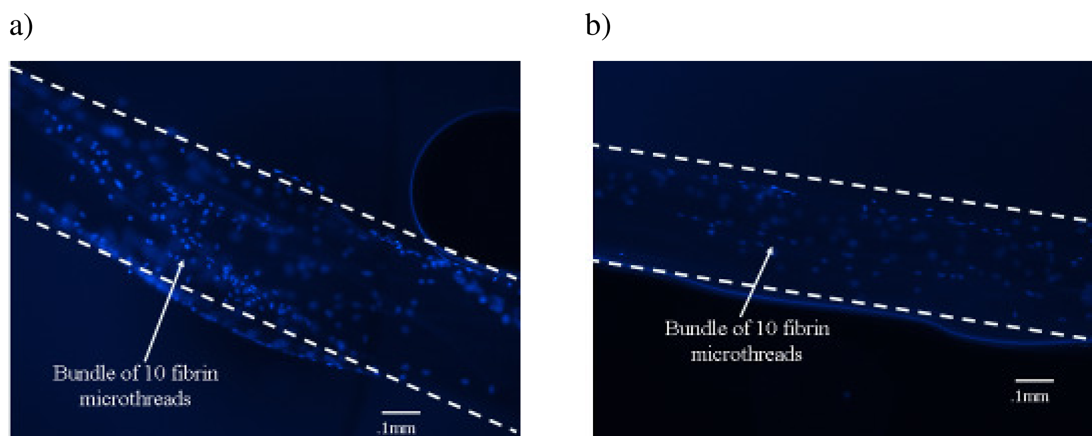

Figure 16. a) Bundle of microthreads dynamically seeded for 24 hours and stained with Hoechst dye (control). b) Bundle of microthreads dynamically seeded for 24 hours, sutured into the epicardium of a pig heart, pulled out, and stained with Hoechst dye.

7.0 Analysis and Discussion

7.1 Preliminary Results

The preliminary results indicated that dynamic seeding, using a rotational method, resulted in improved hMSC attachment as compared to static seeding, using a droplet

method. Additionally, it was found that bundles of three thin fibrin microthreads had improved attachment when compared to individual thick fibrin microthreads. The intent behind bundling the fibrin microthreads was to increase the available surface area for the hMSCs, while maintaining approximately the same diameter as the thick fibrin microthreads. The bundles of three thin fibrin microthreads had an average bundled diameter of 140±56 µm and the individual thick diameter threads had an average diameter of 123±27 µm. There was no statistically significant difference in these diameters when compared using a two-tailed, unequal variance t-test ($p=0.1596$).
The main benefit of dynamic seeding over static seeding is that shorter time durations were required to seed the same quantity of hMSCs on the fibrin microthreads. Furthermore, bundling hMSCs allows for a larger quantity of hMSCs to be seeded, as compared to an individual fibrin microthread of equivalent diameter. The advantage of having a large quantity of hMSCs seeded in a shorter time duration is that the time for the hMSCs to remodel the fibrin microthread is minimized, thereby maintaining the fibrin microthread integrity. The integrity of the fibrin microthread is critical because the microthreads must be sutured into the heart without fracturing. Additionally, a shorter seeding duration is clinically advantageous in that the microthreads could be potentially seeded immediately before a surgery.

An additional benefit of rotationally seeding hMSCs in Silastic tubing is that a longer section of fibrin microthread can be seeded. A 100 µl droplet of cell suspension spans 1-2 cm of the microthread in the droplet technique; since the fibrin microthread is placed in Silastic tubing for the rotational technique, 100 µl of the cell suspension is spans along 3-4 cm of the microthread.

7.1.1 Rotator design

Based on initial results a rotational seeding device (Figure 8 and Figure 9) was constructed. This device was specifically designed to rotate at a slow speed (4 RPM), as previous investigations of dynamic seeding techniques have found that the use of a seeding device is difficult since mechanical forces can be responsible for shear-mediated

membrane lysis or triggering of apoptotic pathways [50],[56]. Additionally, a special arm was created to secure the Silastic tubing for rotation. This special arm consisted of a rubber piece and a plexiglass piece. The Silastic tubing was placed vertically between the rubber and plexiglass and secured with screws, so that the tubing was secure during rotation. The entire device was compact enough to fit into an incubator

7.2 Results

After receiving the preliminary results, subsequent experiments were conducted with a rotational seeding technique and bundles of thin fibrin microthreads. Experimental variables were analyzed independently, to investigate further methods for increasing hMSC attachment on fibrin microthreads.

Fibrin microthreads were bundled into groups of 3, 5, and 10 and the attachment of hMSCs per millimeter of microthread was compared. The highest average number of hMSCs per millimeter was achieved by the bundles of 10 fibrin microthreads. The result was not statistically significant, however, bundles of 10 fibrin microthreads were selected for use in future experiments. A bundle of 10 fibrin microthreads (average bundled diameter: 0.36 mm) was similar to the size of sutures typically used in the heart (synthetic 5-0 suture, diameter: 0.15 mm). Additionally, 10 bundled fibrin microthreads were stronger than bundles of 3 or 5 fibrin microthreads, making them more capable to withstand the mechanical forces when suturing into the heart.

Following the selection of bundles of ten fibrin microthreads, the seeding duration was evaluated. It was found that seeding for 24 hours was optimum. At 12 hours of rotational seeding, the hMSCs were not as densely seeded on the fibrin microthreads as they were after 24 hours. After 48 hours of rotational seeding the media had either leaked or diffused out of the Silastic tubing, leaving the threads dry. Therefore there were fewer hMSCs attached to the fibrin microthreads after 48 hours, as compared to 24 hours.

In order to further increase the density of hMSCs on the fibrin microthreads, smaller diameter tubing was used for rotational seeding. This was done with the intent that condensing the hMSCs around the microthreads would increase the cell attachment, and could be potentially used as a protective sheath when suturing the microthreads. However, there was a statistically significant decrease in cell density when 0.51 mm ID Silastic tubing was used as compared to the 1.98 mm ID Silastic tubing, which had been used in the previous experiments. The decrease in cell density could have been caused by inadequate cell concentration (i.e. the cell concentration was too high for the smaller diameter Silastic tubing) or by media evaporation from the tubing.

The final method investigated was the addition of an adhesion protein to the fibrin microthread. Fibronectin was utilized as an alternative method to increase surface adhesion, however after being passively adsorbed for 24 hours at a concentration of 50 ug/ml, fibronectin did not enhance the quantity of cells on the fibrin microthreads. This could be for several reasons, including, but not limited to, the following:

- The fibronectin may have preferentially adsorbed to the tubing and not to the fibrin microthreads
- The fibronectin may have desorbed from the fibrin microthread during seeding

It is unlikely that the hMSCs did not adhere to the fibronectin, as previous studies indicate that hMSCs have increased attachment on fibronectin, as compared to an uncoated surface or to other adhesion proteins [41]. Therefore, the method of applying adhesion molecules to the fibrin microthreads needs further investigation to be formulated into an effective technique.

Following the selection of bundles of ten fibrin microthreads rotationally seeded for 24 hours in 1.98 mm ID Silastic tubing, the microthreads were evaluated for their ability to be sutured into cardiac tissue. It was shown that, overall, there were fewer cells on the fibrin microthreads after they were sutured through the heart. However, these images show proof of concept that hMSC-seeded fibrin microthreads can be utilized for cellular

delivery to cardiac tissue and that the hMSCs are not sheared off upon suture entry into the tissue. Due to the loss in the quantity of cells per millimeter of microthread which can be seen qualitatively in Figure 16, therefore in the future it is proposed that a protective encapsulating sheath be used for the cell seeding and suturing the microthread into the heart, as seen below in Figure 17 and Figure 18.

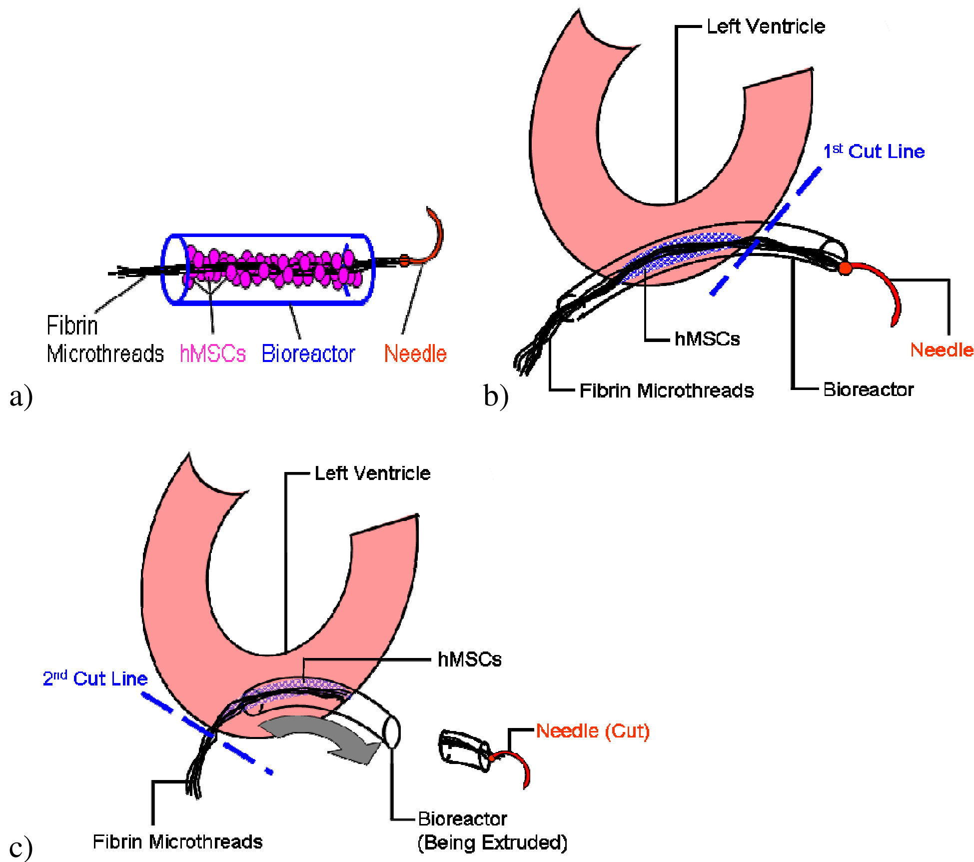

Figure 17. Proposed mechanism for cellular delivery using fibrin microthreads. a) Seeding the microthreads in tubing. b) Using the tubing as a protective cellular delivery sheath. c) Removing the sheath; leaving the cell seeded fibrin microthread in the heart.

Image courtesy of J. Guyette.

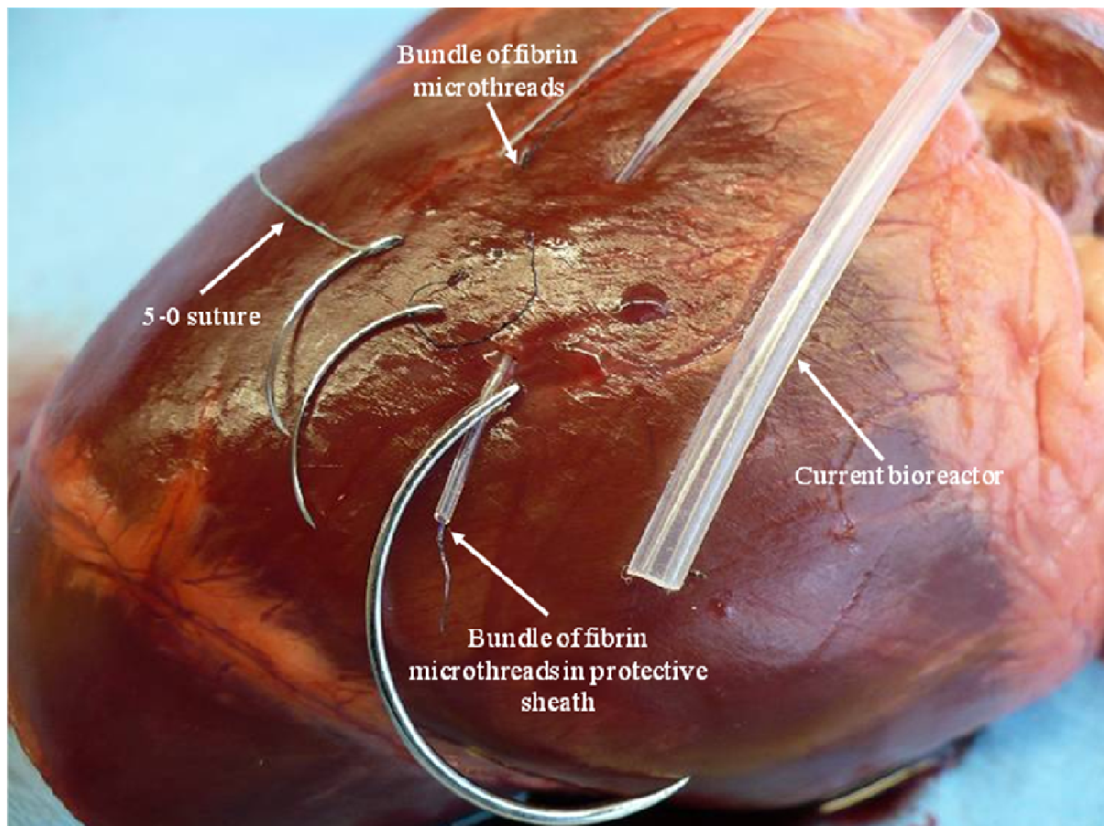

Figure 18. Comparison of a 5-0 suture that is typically used in cardiac applications, a bundle of fibrin microthreads, a bundle of fibrin microthreads in the proposed protective sheath (also to be used as the bioreactor), and the current bioreactor.

Based on the specifications found in Table 1, the maximum number of hMSCs that could conceivably be attached to the fibrin microthreads is between 370-1017 hMSCs per millimeter of a bundle of 10 microthreads. The lower specification value, 370 hMSCs per millimeter, is achieved when it is assumed that the bundle of 10 microthreads is approximately a cylinder (average diameter: 363 µm) and that individual, large diameter cylinder is then seeded. The higher specification value, 1017 hMSCs per millimeter, is achieved when you assume that individual microthreads (average diameter: 99.7 µm) are cylindrical in shape and are seeded with a confluent monolayer and then bundled.

The average of all experiments conducted with bundles of ten fibrin microthreads dynamically seeded for 24 hours in 1.98 mm ID Silastic tubing, resulted in 648±234cells/mm. This result falls within the design specifications found in Table 1 and

indicates that a nearly confluent monolayer of hMSCs on bundles of ten fibrin microthreads has been achieved.

The specification upper specification limit of 1017 hMSCs per millimeter was not achieved because the individual fibrin microthreads were not seeded with hMSCs and then bundled. Instead, ten fibrin microthreads were bundled and then seeded with hMSCs. It can be seen in Figure 15 that microgrooves are present due to bundling the fibrin microthreads; however ten individual microthreads cannot be seen. It may be that when the fibrin microthreads are being seeded with hMSCs in media for 24 hours they begin to form one cylindrical thread. The partially microgrooved surface observed in Figure 15 validates that the number of cells achieved per millimeter (648 cells/mm) falls in a middle range within the specifications for a confluent monolayer (370-1017 cells/mm). It should be noted that it is possible that bundling the microthreads may harbor more cells than can be achieved by a confluent monolayer. Additionally, the results revealed that more than one layer of cells were attached to the microthread.

8.0 Conclusions

The following conclusions summarize our efforts towards creating a deliverable product in the field of cardiovascular regeneration.

The use of fibrin microthreads as a method to deliver hMSCs is an innovative approach in regenerative medicine which has not been explored until recently. The current standard in seeding hMSCs onto the fibrin microthreads utilizes a static method in which cell suspension is dropped onto a microthread suspended across a stainless steel washer. Although this technique is able to attain a considerable amount of cells on the microthreads, the process requires an extensive period of time (up to 5 days to achieve maximal cell density), over which the microthread begins to degrade. The first step in improving this process was to test a new seeding process.

Based on our results, we concluded that dynamic seeding provides a better method of attaching the hMSCs to the microthreads. In line with our original hypotheses, preliminary results indicated that dynamically seeding hMSCs onto fibrin microthreads provided a superior method of hMSC attachment than static seeding. In conjunction with the analysis of dynamic seeding, the effect of surface alterations, achieved via bundling the microthreads, was investigated. Once again, preliminary results suggested that bundling microthreads increased cell density. Each of these experiments was performed in a 24 hour time period, allowing the threads to maintain mechanical integrity. Upon analysis of results, it was concluded that bundled microthreads dynamically seeded for 24 hours were able to increase cell density as compared to static seeding, seen in Figure 7. The duration of time at which the microthreads were rotated was also varied; however, microthreads rotated for 24 hours significantly increased cell attachment to the microthreads as depicted in Figure 11. Other parameters, such as tubing diameter and the effect of cell adhesion molecules on cell density were explored, resulting in low cell counts and therefore were not used in this project.

In conclusion, by dynamically rotating bundled microthreads for 24 hours, an average cell count of 648 cells/mm of microthread was achieved. This significant number suggests that despite their small size, enough cells can be delivered to the tissue of interest to provide a hospitable environment for regeneration. Results of this study suggest that the 648 cells/mm of bundled microthreads that have been rotationally seeded can be delivered to a rat heart in approximately 22.6 cm of microthreads assuming 100% engraftment. Although future testing is required, fibrin microthreads are a exciting and novel method for cardiac tissue regeneration.

9.0 Future Recommendations

Although the work that was completed in this project produced significant results, there are a number of additional pathways that can be explored to elicit even greater results that may ultimately lead to clinical trials for the treatment of MI.

When beginning this project, the brainstorming process cultivated a number of ideas for use in increasing cell density on fibrin threads. These ideas included the use of cell adhesion molecules, directly coextruding hMSCs into the fibrin microthreads, and superficial surface alterations. Cell adhesion molecules have been used in previous research as a method to increase cell attachment to fibrin scaffolds. A number of cell adhesion molecules had been explored for such applications, including fibronectin, victronectin, and RGD peptides. Although preliminary testing was completed by passively adsorbing fibronectin on the microthreads, results were discouraging, as fewer hMSCs were attached to the microthreads with passively adsorbed fibronectin. However, use of a different cell adhesion molecule, such as vitronectin or RGD peptides may produce better results

A further idea to increase the quantity of hMSCs attached to fibrin microthreads is to coextrude the hMSCs within the fibrin microthreads. As outlined in

5.2 Fibrin Microthread Production, fibrin microthreads are produced through the coextrusion of fibrinogen and thrombin with the aid of a syringe. This is a non-sterile process and thus requires further sterilization of the microthreads before they are threaded through the Silastic tubing. While coextrudeding the hMSCs when producing the microthreads is an attractive technique for a number of reasons, there are a number of inherent limitations with such a process. Limitations include creating a method to coextrude the hMSCs and fibrin in a sterile manner. This would require the purchase of additional equipment, including the possible purchase of a syringe pump.

Another area that requires advancement for future research is the method of quantifying the number of cells per millimeter of microthread. When quantifying the number of cells on microthreads, the cells were counted by hand with the aid of the computer program Image J. This is a subjective technique that can be improved. The use of an assay such as MTS and Alamar Blue was explored, with disappointing results, as can be seen in **Appendix G: MTS Sensitivity and Results.** Results of such assay suggested that a more sensitive assay, or a different method altogether was necessary.

In addition, although Hoechst dye was used to quantify the number of cells on the microthread, this method does not provide information about cell viability. Therefore, in the future, groups should explore the use of a live/dead assay to confirm that the cells being delivered to the tissue of interest are live, fully functional cells. Furthermore, a proliferation assay can also be used to determine whether the cells continue to divide, grow, and expand within the tissue. A cell-cycle marker such as Ki-67 may be a useful in determining such a parameter. Furthermore, the viability and differentiation of the hMSCs needs to be characterized before implantation in clinical trials.

Within the methods touched upon in this project, there are a number of improvements that can be made including the possibility of a different dynamic seeding technique,

smaller diameter tubing, varied cell concentrations, and different cell types. As compared to static seeding, the use of the dynamic rotational method proved to be a successful method in this project, proving feasibility in increasing hMSC density on microthreads. However, other methods of dynamic seeding, other than the rotational technique employed in this study, may be used to further increase cell density and attachment. Additionally, different diameter tubes may be used in such applications. In particular, the use of smaller diameter tubing (<0.51mm ID) would be especially important because such tubing may be used in suturing the microthreads into the tissue of interest, acting as a mode of protection (of both cells and microthread) during the suturing process. The tubing used in this project (1.98mm ID Silastic tubing) was too large to be sutured into the heart. However, with smaller diameter tubing, the microthreads may be kept in the tube with the cell suspension, removing the tubing only after suturing is complete. This is advantageous for a number of reasons, including protection of the microthread. Also, it eliminates having to remove the thread from the tubing before suturing, which may shear cells off of the microthreads. However, there are limitations to such a process. This particular avenue was explored; resulting in low cell counts.

Finally, upon optimization of hMSC density on or within fibrin microthreads, it is recommended that in vivo tests be conducted. These trials should investigate the effects of hMSCs delivered to the heart on fibrin microthreads, specifically the efficiency and localization of cell engraftment within the heart. Different avenues should be observed, including, but not limited to, engraftment rate of hMSCs in cardiac tissue, differentiation of hMSCs, and improvements in global heart function.

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Appendix A: Summary of Cell Adhesion Molecules

Substance	What is it?	Experimental Results
Fibronectin	Exists in two forms: • Insoluble glycoprotein dimer (ECM) • Soluble disulphide linked dimer (Plasma) [37] Involved in many cellular processes: • Tissue repair • Wound healing • Cell migration and/or adhesion	Has shown most potential as a cellular adhesion molecule [41]; feasible for use in project
RGD peptides	• Tripeptide found in extracellular matrix proteins • Interact with integrin receptor sites to initiate cell signaling • Modulator of cell adhesion	RGD peptides stimulate MSC attachment and spreading [42]; used in low concentrations; used in place of fibronectin; feasible for use in project
Collagen	• Collagen molecules form a wide range of structures within the human body [46] • Fibrous tissue found in most connective tissues	Promotes cell adhesion [57]; not favorable for use as a microthread for hMSC applications [58]
Vitronectin	• Abundant plasma protein • Regulates coagulation, fibrinolysis, complement activation, and cell adhesion [59]	Promotes cell adhesion [59]; not favorable for use as a microthread for hMSC applications [58]
Laminin	• Glycoprotein with 3 peptide chains • Varied functions: • Cell adhesion • Cell growth and differentiation • Cell migration	Mixed results obtained in scientific research [60] ; lack of reproducible results

Table 12. Summary of cell adhesion molecules.

Appendix B: Objectives

Objectives (O):
O1 - Cell alignment in tissue
O2 - Cell alignment on biological microthreads
O3 - Increase cell density
O4 - Increase speed of 'more density'
O5 - Strength of cell attachment
O6 - Number of cells attached
O7 - Maximize cell quantity on biological microthreads
O8 - Control quantity of cells delivered
O9 - Promote proliferation of seeded cells
O10 - Maintain mechanical integrity of biological microthreads
O11 - Ease of use for client
O12 - Compatibility with other cell types or lineages
O13 - Easy and cheap to mass manufacture

Objectives ↓ →	O1	O2	O3	O4	O5	O6	O7	O8	O9	O10	O11	O12	O13	Total
O1	X	0	0	0	0	0	0	½	0	0	1	1	1	3.5
O2	1	X	0	1	0	0	0	1	½	0	1	1	1	6.5
O3	1	1	X	½	0	½	1	1	½	1	1	1	1	9.5
O4	1	0	½	X	0	1	½	1	½	1	1	1	1	8.5
O5	1	1	1	1	X	1	½	1	1	1	1	1	1	11.5
O6	1	1	½	0	0	X	1	1	½	1	1	1	1	9
O7	1	1	0	½	½	0	X	1	½	1	1	1	1	8.5
O8	½	0	0	0	0	0	0	X	0	0	1	1	1	3.5
O9	1	½	½	½	0	½	½	1	X	1	1	1	1	8.5
O10	1	1	0	0	0	0	0	1	0	X	1	1	1	6
O11	0	0	0	0	0	0	0	0	0	0	X	1	1	2
O12	0	0	0	0	0	0	0	0	0	0	0	X	1	1
O13	0	0	0	0	0	0	0	0	0	0	0	0	X	0

Table 13. Global, or overall, project objective pairwise comparison chart.

Global Principal Objectives

1. Increase cell density
2. Maintain mechanical integrity of biological microthreads

Individual Principal Objectives

- G. Gaudette
  1. Maintain mechanical integrity of biological microthreads
  2. Increase cell density
  3. Maximize cell quantity on biological microthreads/control quantity of cells delivered
- M. Rolle
  1. Number of cells attached
  2. Maximize cell quantity on biological microthreads/increase speed of 'more density'
  3. Increase cell density
- M. Murphy
  1. Maintain mechanical integrity of biological microthreads
  2. Control quantity of cells delivered/strength of cell attachment
- MQP
  1. Strength of cell attachment
  2. Increase cell density
  3. Number of cells attached

Objective	**G. Gaudette**	**M. Rolle**	**M. Murphy**	**MQP**	**Averaged Total**	**Weighted Objective**
Cell alignment in tissue	0.5	1.5	0	3.5	1.4	1.4 + 1= 2.4/78.1= .0307
Cell alignment on biological microthreads	1.5	0	3.5	6.5	2.9	2.9 +1= 3.9/78.1= .0499
Increase cell density	11	10	7	9.5	9.4	9.4 +1= 10.4/78.1= .133
Increase speed of 'more density'	5	10.5	5.5	8.5	7.4	7.4 +1= 8.4/78.1= .108
Strength of cell attachment	7	4.5	9.5	11.5	8.1	8.1 +1= 9.1/78.1= .117
Number of cells Attached	8	11	6.5	9	8.6	8.6 +1= 9.6/78.1= .123
Maximize cell quantity on biological microthreads	9	10.5	6.5	8.5	8.6	8.6 +1= 9.6/78.1= .123
Control quantity of cells delivered	9	7	9.5	3.5	7.3	7.3 +1= 8.3/78.1= .106
Promote proliferation of the seeded cells	6	2	3.5	8.5	5.0	5.0 +1= 6.0/78.1= .0768
Maintain mechanical integrity of biological microthreads	12	7.5	10	6	8.9	8.9 +1= 9.9/78.1= .127
Ease of use for client	5	4.5	8.5	2	5.0	5.0 +1= 6.0/78.1= .0768
Compatibility with other cell types or lineages	3	4.5	2	1	2.6	2.6 +1= 3.6/78.1= .0461
Easy and cheap to mass manufacture	1	4.5	6	0	2.9	2.9 +1= 3.9/78.1= .0499
				Sum	**78.1**	**1**

Table 14. Averages from all stakeholder's pairwise comparison charts

Figure 19. Objective tree.

Appendix C: Functions

FUNCTION	POSSIBLE MEANS			
Prevent cell lysing and apoptosis	*Maintain nutrient rich environment around biological microthreads*	*Maintain platform as amicable cellular environment*	*Precondition cells to harsh ischemic environments*	*Biological scaffold*
Biocompatible	*Use of patients own stem cells to minimize immune response*	*Limited inflammatory response*	*Non-toxic materials*	
Limited Byproducts	*Avoid use of harmful solvents*	*Limit degradation of cell platform*	*Avoid necrosis prior to implantation*	
Cell platform needs to maintain mechanical integrity (need to be able to be transferred from cell culture to delivery to the heart)	*Increase speed of cell migration via adhesion molecules*	*Improve cell seeding technique*	*Limit fibrin degradation through inhibitors*	
Need to be transportable	*Develop transportation device*	*Possible use of aseptic cell culture dish*	*Float biological microthreads in serum*	
Need a sterile environment	*Aseptic cell culture techniques*	*Use of biological safety hood*	*Use of disinfectant*	
Produce mass amount of biological microthreads	*Optimize biological microthread extrusion technique*	*Hand extrude biological microthreads*		
Must be deliverable to the organ or tissue of interest	*Through sutures*	*Scaffolds*	*Direct injection*	
Increase Cell Density	*Bundling of biological microthreads*	*Inhibit fibrin degradation*	*Optimize cell seeding technique*	*Cell adhesion molecules*

Table 15. Morphological chart.

Function Enumeration

- Biological microthread Creation
  - Maintain mechanical integrity (must produce one continuous biological microthread when drawing through solution)
  - Must provide hospitable environment for hMSCs
  - Must be scaleable (ability to produce mass quantity of biological microthreads)
- Biological microthread Transportation to Incubation
  - Maintain mechanical integrity (cannot break or tear during transport)
  - Must provide a sterile environment
- Cell Seeding
  - Must attach/adhere cells

    - Must have a sterile environment for seeding
    - Prevent cell lysing and apoptosis during seeding
- Cell Culture
    - Must maintain mechanical integrity (so that cells do not entirely remodel the biological microthreads)
    - Cells must proliferate while in cell culture
    - Cell culture must provide a sterile environment
    - Must limit deleterious byproducts
    - Prevent cell lysing and apoptosis
- Biological microthread and Cell Transportation to Experimental Analysis
    - Maintain mechanical integrity (biological microthreads cannot break or tear during transport)
    - Must have sterile transportation vehicle
- Experimental Analysis
    - Must have a sterile environment for experiments
    - Must limit deleterious byproducts
    - Prevent cell lysing and apoptosis
- Biological microthread and Cell Transportation
    - Maintain mechanical integrity
    - Maintain sterile environment
- Biological microthread and Cell Implantation
    - Must provide cells to area of interest
    - Must be adaptable for inimally invasive for implantation
    - Must maintain cell viability upon implantation (prevent cell lysing and apoptosis)

- ***Design Safety
    - Biocompatibility must be maintained

Appendix D: Calculations for Specifications

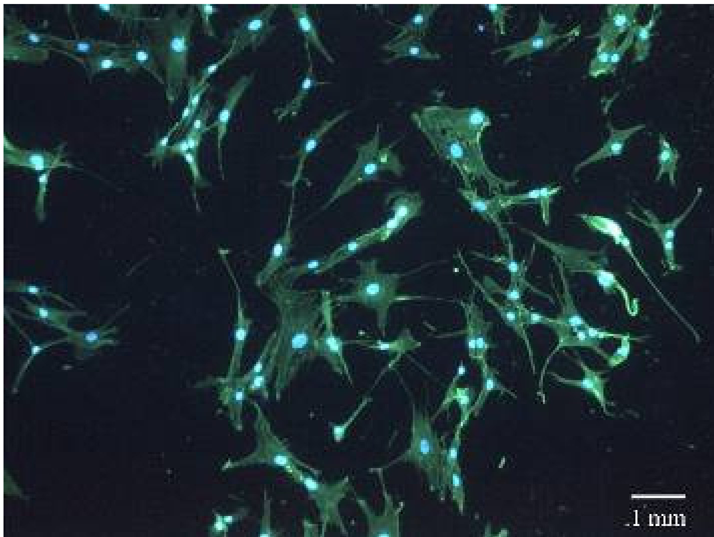


Figure 20. 10X image of hMSCs stained with Phalloidin (binds to filamentous actin and is green in the image) and Hoechst dye (binds to the nucleus and is blue in the image).
Image courtesy of M. Murphy.

Based off of Figure 20, the average cell area of a hMSC is 3079.23 μm^2. Data for these area measurements can be found in Table 16. Additionally, Table 17, Table 18, and Table 19 contain diameter measurements of hydrated bundles of 3, 5, and 10 fibrin microthreads. The average diameters of hydrated bundle of 3, 5, and 10 fibrin microthreads is 140 µm, 218 µm, and 363 µm, respectively.

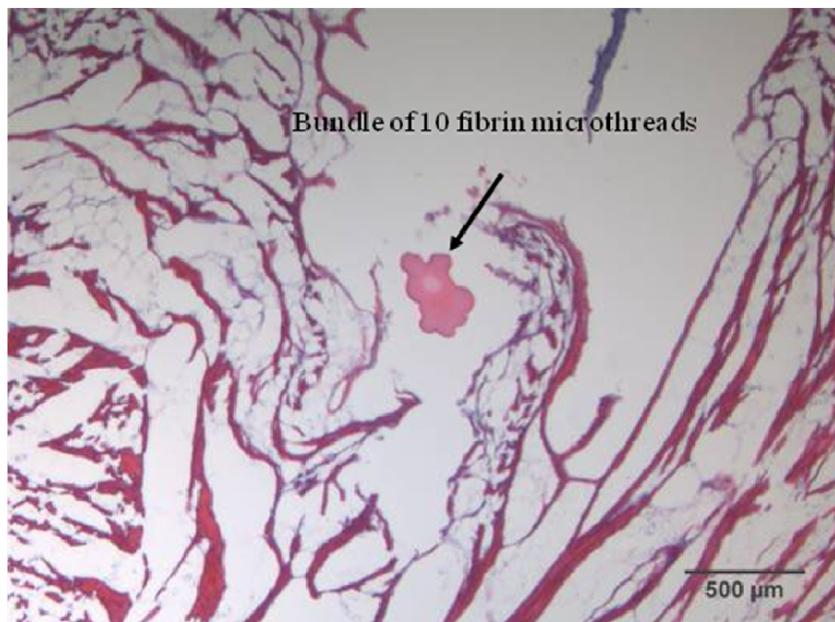


Figure 21. Cryosectioned porcine cardiac tissue with a hydrated bundle of 10 fibrin microthreads.

Method 1 for obtaining the number of cells per area of microthread of a confluent cell monolayer

Assuming that a hydrated bundle of 10 fibrin microthreads is approximately a cylinder, the surface area of 1 mm of this bundle is:

$$Surface\ Area\ of\ a\ Cylinder = 2 \cdot \pi \cdot r \cdot h = 2 \cdot \pi \cdot \frac{363 \mu m}{2} \cdot 1000 \mu m = 1.14 \cdot 10^6 \mu m^2$$ of

surface area for 1 mm (1000 µm) of a hydrated bundle of 10 fibrin microthreads

One millimeter of a hydrated bundle of 10 fibrin microthreads can hold a confluent monolayer of:

$$\frac{1.14 \cdot 10^6 \mu m^2}{3079.23 \frac{\mu m^2}{cell}} = 370 cells$$

Method 2 for obtaining the number of cells per area of microthread of a confluent cell monolayer

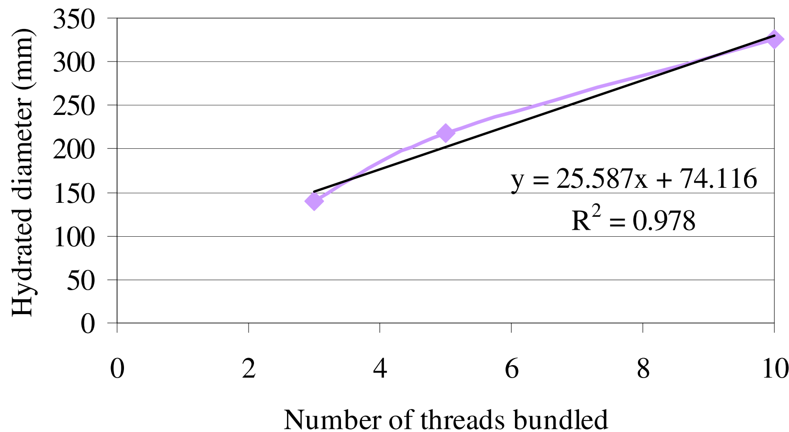


Figure 22. Average hydrated diameter for bundles of fibrin microthreads versus the number of threads in the bundle.

Based off of Figure 22, it can be extrapolated that one individual fibrin microthread has a diameter of 0.99703 mm. If the threads were individually seeded and then combined into a bundle of 10 fibrin microthreads a larger number of cells could be seeded, due to the increased surface area. Assuming that 1 hydrated fibrin microthreads is approximately a cylinder, the surface area of 1 mm of fibrin microthread is:

$$Surface\ Area\ of\ a\ Cylinder = 2 \cdot \pi \cdot r \cdot h = 2 \cdot \pi \cdot \frac{99.703\ \mu m}{} \cdot 1000\ \mu m = 6.26 \cdot 10^5 \mu m^2$$

of surface area for one hydrated fibrin microthread that is 1mm in length

One millimeter of one hydrated fibrin microthread can hold a confluent monolayer of:

$$\frac{3.13 \cdot 10^5 \mu m^2}{3079.23 \frac{mm^2}{cell}} = 101.7 cells$$

Therefore, if one millimeter of 10 of these individually seeded hydrated fibrin microthreads were bundled into a group, there would be:

$$10 \text{ fibrin microthreads} \cdot 101.7 \frac{cells}{mm^2} = 1017 cells$$

Based on these calculations, a confluent monolayer, one millimeter in length, of cells on a hydrated bundle of 10 fibrin microthreads can hold between 370 cells (when bundled and then seeded) and 1017 cells (when individually seeded and then bundled).

Raw Data for hMSC Area and Fibrin Microthread Bundled Diameter

Number of hMSCs counted	**Cell area (um^2)**
1	3212.37
2	2578.91
3	2787.90
4	2448.55
5	2518.21
6	1751.65
7	7165.57
8	1541.22
9	2747.72
10	2393.34
11	2578.07
12	2916.81
13	3895.83
14	1184.53
15	1975.66
16	1979.20
17	2652.91
18	7385.25
19	6158.95
19	2052.98
21	3728.71
22	3668.92
23	2752.05
24	1826.34
Average	**3079.23**
Standard Deviation	**1630.44**

Table 16. Average hMSC area, based on Figure 20.

The diameters given are averages, based on measuring the diameter in three locations along the length of the thread for each Experiment Image.

Experiment Image	Diameter (µm)
1-19-08_Rotated Bundles of 10_Thread 1 Image 1_Hoechst_10X_370.8ms	236
1-19-07_Thread 2_Image 1_Bundled 10 Rotated_Hoechst dye_10X_250.9ms:Blue	227
1-19-08_Rotated Bundles of 10_Thread 2 Image 1_Hoechst_10X_370.8ms	298
2-1-08_Rotated Bundle of 10_Thread 1 Image 1_Hoechst_10X_196.6ms.tif	320
2-1-08_Rotated Bundle of 10_Thread 1 Image 2_Hoechst_10X_50.4ms.tif	291
2-1-08_Rotated Bundle of 10_Thread 1 Image 3_Hoechst_10X_50.4ms.tif	354
2-1-08_Rotated Bundle of 10_Thread 1 Image 4_Hoechst_10X_50.4ms	363
2-1-08_Rotated Bundle of 10_Thread 2 Image 1_Hoechst_10X_50.4ms.tif	339
2-1-08_Rotated Bundle of 10_Thread 2 Image 2_Hoechst_10X_50.4ms.tif	399
2-8-08_Rotated Bundle @ 12hr_Thread 1 Image 1_Hoechst_10X_127.0ms	297
2-8-08_Rotated Bundle @ 12hr_Thread 1 Image 2_Hoechst_10X_127.0ms	295
2-8-08_Rotated Bundle @ 12hr_Thread 1 Image 3_Hoechst_10X_127.0ms	321
2-8-08_Rotated Bundle @ 12hr_Thread 1 Image 4_Hoechst_10X_127.0ms	352
2-18-08_Rotated Lg Dia. Bundle 12 HOURS_ Thread 1 Image 1_Hoechst_10X_147.0ms (2-18-0~1)	359
2-18-08_Rotated Lg Dia. Bundle 12 HOURS_ Thread 1 Image 2_Hoechst_10X_147.0ms (2-18-0~2)	368
2-18-08_Rotated Lg Dia. Bundle 12 HOURS_ Thread 1 Image 3_Hoechst_10X_147.0ms (2-18-0~3)	406
2-18-08_Rotated Lg Dia. Bundle 12 HOURS_ Thread 1 Image 4_Hoechst_10X_147.0ms (2-18-0~4)	348
2-18-08_Rotated Lg Dia. Bundle 12 HOURS_ Thread 2 Image 1_Hoechst_10X_61.2ms (2-18-0~1)	246
2-18-08_Rotated Lg Dia. Bundle 12 HOURS_ Thread 2 Image 2_Hoechst_10X_61.2ms (2-18-0~2)	357
2-18-08_Rotated Lg Dia. Bundle 12 HOURS_ Thread 3 Image 1_Hoechst_10X_61.2ms (2-18-0~1)	490
2-18-08_Rotated Lg Dia. Bundle 12 HOURS_ Thread 3 Image 2_Hoechst_10X_61.2ms (2-18-0~2)	451
2-18-08_Rotated Lg Dia. Bundle 12 HOURS_ Thread 3 Image 3_Hoechst_10X_61.2ms (2-18-0~3)	404
2-18-08_Rotated Lg Dia. Bundle 12 HOURS_ Thread 3 Image 4_Hoechst_10X_61.2ms (2-18-0~4)	343
2-18-08_Rotated Lg Dia. Bundle 12 HOURS_ Thread 3 Image 5_Hoechst_10X_61.2ms (2-8720~1)	438
2-18-08_Rotated Lg Dia. Bundle 12 HOURS_ Thread 3 Image 6_Hoechst_10X_61.2ms (2-8726~1)	457
2-25-08_Rotated 12 Hr_ Thread 1 Image 1_Hoechst_10X_99.6ms	410
2-25-08_Rotated 12 Hr_ Thread 1 Image 2_Hoechst_10X_99.6ms	348
2-25-08_Rotated 12 Hr_ Thread 1 Image 3_Hoechst_10X_99.6ms	343
2-25-08_Rotated 12 Hr_ Thread 1 Image 4_Hoechst_10X_99.6ms	363
2-25-08_Rotated 12 Hr_ Thread 1 Image 5_Hoechst_10X_99.6ms	421
2-25-08_Rotated 12 Hr_ Thread 1 Image 6_Hoechst_10X_99.6ms	361
2-25-08_Rotated 12 Hr_ Thread 1 Image 7_Hoechst_10X_99.6ms	353
2-25-08_Rotated 12 Hr_ Thread 1 Image 8_Hoechst_10X_99.6ms	406

2-25-08_Rotated 12 Hr_ Thread 1 Image 9_Hoechst_10X_99.6ms	410
2-25-08_Rotated 12 Hr_ Thread 2 Image 1_Hoechst_10X_99.6ms	307
2-25-08_Rotated 12 Hr_ Thread 2 Image 2_Hoechst_10X_99.6ms	337
2-25-08_Rotated 12 Hr_ Thread 2 Image 3_Hoechst_10X_99.6ms	404
2-25-08_Rotated 12 Hr_ Thread 2 Image 4_Hoechst_10X_99.6ms	415
2-25-08_Rotated 12 Hr_ Thread 2 Image 5_Hoechst_10X_99.6ms	384
2-25-08_Rotated 12 Hr_ Thread 3 Image 1_Hoechst_10X_99.6ms	335
2-25-08_Rotated 12 Hr_ Thread 3 Image 2_Hoechst_10X_99.6ms	302
2-25-08_Rotated 12 Hr_ Thread 4 Image 1_Hoechst_10X_99.6ms	444
2-25-08_Rotated 12 Hr_ Thread 4 Image 2_Hoechst_10X_99.6ms	457
2-25-08_Rotated 12 Hr_ Thread 4 Image 3_Hoechst_10X_99.6ms	450
2-25-08_Rotated 12 Hr_ Thread 4 Image 4_Hoechst_10X_99.6ms	431
2-25-08_Rotated 12 Hr_ Thread 4 Image 5_Hoechst_10X_99.6ms	510
2-25-08_Rotated 12 Hr_ Thread 4 Image 6_Hoechst_10X_99.6msi	420
2-25-08_Rotated 12 Hr_ Thread 5 Image 1_Hoechst_10X_99.6ms	452
2-25-08_Rotated 12 Hr_ Thread 5 Image 2_Hoechst_10X_99.6ms	455
2-25-08_Rotated 12 Hr_ Thread 5 Image 3_Hoechst_10X_99.6ms	346
2-18-08_Rotated Lg Dia. Bundle 24 HOURS_ Thread 1 Image 1_Hoechst_10X_50.4ms (2-18-0~1)	825
2-18-08_Rotated Lg Dia. Bundle 24 HOURS_ Thread 1 Image 2_Hoechst_10X_50.4ms (2-18-0~2)	636
2-18-08_Rotated Lg Dia. Bundle 24 HOURS_ Thread 1 Image 3_Hoechst_10X_50.4ms (2-18-0~3)	515
2-18-08_Rotated Lg Dia. Bundle 24 HOURS_ Thread 1 Image 4_Hoechst_10X_50.4ms (2-18-0~4)	478
2-18-08_Rotated Lg Dia. Bundle 24 HOURS_ Thread 2 Image 1_Hoechst_10X_50.4ms (2-18-0~1)	578
2-18-08_Rotated Lg Dia. Bundle 24 HOURS_ Thread 2 Image 2_Hoechst_10X_50.4ms (2-18-0~2)	508
2-18-08_Rotated Lg Dia. Bundle 24 HOURS_ Thread 2 Image 3_Hoechst_10X_50.4ms (2-18-0~3)	446
2-18-08_Rotated Lg Dia. Bundle 24 HOURS_ Thread 2 Image 4_Hoechst_10X_50.4ms (2-18-0~4)	463
2-18-08_Rotated Lg Dia. Bundle 24 HOURS_ Thread 2 Image 5_COUNTING PICTURE_Hoechst_10X_26.7ms (2-419F~1)	448
2-18-08_Rotated Lg Dia. Bundle 24 HOURS_ Thread 3 Image 1_Hoechst_10X_50.4ms (2-441B~1)	447
2-18-08_Rotated Lg Dia. Bundle 24 HOURS_ Thread 3 Image 2_Hoechst_10X_50.4ms (2-519B~1)	460
2-4195~1	486
2-5199~1	434
2-18-0~1 (2-18-08_Rotated Lg Dia. Bundle 24 HOURS_ Thread 3 Image 1_Hoechst_10X)	377
2-18-0~2 (2-18-08_Rotated Lg Dia. Bundle 24 HOURS_ Thread 3 Image 2_Hoechst_10X)	388
2-18-0~3 (2-18-08_Rotated Lg Dia. Bundle 24 HOURS_ Thread 3 Image 3_Hoechst_10X)	316
2-25-08_Rotated 24 Hr_ Thread 1 Image 1_Hoechst_10X_99.6ms	415
2-25-08_Rotated 24 Hr_ Thread 1 Image 2_Hoechst_10X_99.6ms	408
2-25-08_Rotated 24 Hr_ Thread 1 Image 3_Hoechst_10X_99.6ms	444
2-25-08_Rotated 24 Hr_ Thread 1 Image 4_Hoechst_10X_99.6ms	259
2-25-08_Rotated 24 Hr_ Thread 1 Image 5_Hoechst_10X_99.6ms	285

2-25-08_Rotated 24 Hr_ Thread 1 Image 6_Hoechst_10X_99.6ms	227
2-25-08_Rotated 24 Hr_ Thread 2 Image 1_Hoechst_10X_99.6ms	320
2-25-08_Rotated 24 Hr_ Thread 2 Image 2_Hoechst_10X_99.6ms	347
2-25-08_Rotated 24 Hr_ Thread 2 Image 3_Hoechst_10X_99.6ms	372
2-25-08_Rotated 24 Hr_ Thread 2 Image 4_Hoechst_10X_99.6ms	320
2-25-08_Rotated 24 Hr_ Thread 2 Image 5_Hoechst_10X_99.6ms	329
2-25-08_Rotated 24 Hr_ Thread 2 Image 6_Hoechst_10X_99.6ms	359
2-25-08_Rotated 24 Hr_ Thread 3 Image 1_Hoechst_10X_99.6ms	309
2-25-08_Rotated 24 Hr_ Thread 3 Image 2_Hoechst_10X_99.6ms	352
2-25-08_Rotated 24 Hr_ Thread 3 Image 3_Hoechst_10X_99.6ms	342
2-25-08_Rotated 24 Hr_ Thread 3 Image 4_Hoechst_10X_99.6ms	349
2-25-08_Rotated 24 Hr_ Thread 3 Image 5_Hoechst_10X_99.6ms	286
2-25-08_Rotated 24 Hr_ Thread 3 Image 6_Hoechst_10X_99.6ms	319
2-25-08_Rotated 24 Hr_ Thread 4 Image 1_Hoechst_10X_99.6ms	338
2-25-08_Rotated 24 Hr_ Thread 4 Image 2_Hoechst_10X_99.6ms	288
2-25-08_Rotated 24 Hr_ Thread 4 Image 3_Hoechst_10X_99.6ms	399
2-25-08_Rotated 24 Hr_ Thread 4 Image 4_Hoechst_10X_99.6ms	311
2-25-08_Rotated 24 Hr_ Thread 5 Image 1_Hoechst_10X_99.6ms	286
2-25-08_Rotated 24 Hr_ Thread 5 Image 2_Hoechst_10X_99.6ms	377
2-25-08_Rotated 24 Hr_ Thread 5 Image 3_Hoechst_10X_99.6ms	323
2-25-08_Rotated 24 Hr_ Thread 5 Image 4_Hoechst_10X_99.6ms	317
2-25-08_Rotated 24 Hr_ Thread 5 Image 5_Hoechst_10X_99.6ms	305
2-8-08_Rotated Bundle @ 48hr_Thread 1 Image 1_Hoechst_10X_127.0ms	309
2-8-08_Rotated Bundle @ 48hr_Thread 1 Image 2_Hoechst_10X_127.0ms	272
2-18-08_Rotated Lg Dia. Bundle 48 HOURS_ Thread 1 Image 1_Hoechst_10X_50.3ms (2-18-0~1)	341
2-18-08_Rotated Lg Dia. Bundle 48 HOURS_ Thread 1 Image 2_Hoechst_10X_50.3ms (2-18-0~2)	292
2-18-08_Rotated Lg Dia. Bundle 48 HOURS_ Thread 2 Image 1_Hoechst_10X_50.3ms (2-18-0~1)	291
2-18-08_Rotated Lg Dia. Bundle 48 HOURS_ Thread 2 Image 2_Hoechst_10X_50.3ms (2-18-0~2)	444
2-18-08_Rotated Lg Dia. Bundle 48 HOURS_ Thread 2 Image 3_Hoechst_10X_50.3ms (2-18-0~3)	377
2-25-08_Rotated 48 Hr_ Thread 1 Image 2 _Hoechst_10X_127.0ms	491
2-25-08_Rotated 48 Hr_ Thread 1 Image 3 _Hoechst_10X_127.0ms	450
2-25-08_Rotated 48 Hr_ Thread 1 Image _Hoechst_10X_127.0ms	485
2-25-08_Rotated 48 Hr_ Thread 2 Image 1 _Hoechst_10X_127.0ms	350
2-25-08_Rotated 48 Hr_ Thread 2 Image 2 _Hoechst_10X_127.0ms	375
2-25-08_Rotated 48 Hr_ Thread 2 Image 3 _Hoechst_10X_127.0ms	375
2-25-08_Rotated 48 Hr_ Thread 2 Image 4 _Hoechst_10X_127.0ms	360
2-25-08_Rotated 48 Hr_ Thread 3 Image 1 _Hoechst_10X_127.0ms	264
2-25-08_Rotated 48 Hr_ Thread 3 Image 2 _Hoechst_10X_127.0ms	390
2-25-08_Rotated 48 Hr_ Thread 3 Image 3 _Hoechst_10X_127.0ms	307
2-25-08_Rotated 48 Hr_ Thread 3 Image 4 _Hoechst_10X_127.0ms	291
2-25-08_Rotated 48 Hr_ Thread 3 Image 5 _Hoechst_10X_127.0ms	326
2-25-08_Rotated 48 Hr_ Thread 3 Image 6 _Hoechst_10X_127.0ms	315
2-25-08_Rotated 48 Hr_ Thread 4 Image 1 _Hoechst_10X_127.0ms	331

2-25-08_Rotated 48 Hr_ Thread 4 Image 2 _Hoechst_10X_127.0ms	297
2-25-08_Rotated 48 Hr_ Thread 4 Image 3 _Hoechst_10X_127.0ms	311
2-25-08_Rotated 48 Hr_ Thread 4 Image 4 _Hoechst_10X_127.0ms	495
2-25-08_Rotated 48 Hr_ Thread 4 Image 5 _Hoechst_10X_127.0ms	321
2-25-08_Rotated 48 Hr_ Thread 5 Image 1 _Hoechst_10X_127.0ms	292
2-25-08_Rotated 48 Hr_ Thread 5 Image 2 _Hoechst_10X_127.0ms	294
2-25-08_Rotated 48 Hr_ Thread 5 Image 3 _Hoechst_10X_127.0ms	448
2-25-08_Rotated 48 Hr_ Thread 5 Image 4_Hoechst_10X_127.0ms	303
2-11-08_Rotated LARGE DIA. Bundle 24 hr_Thread 1 Image 1_Hoechst_10X_305.0ms	334
2-11-08_Rotated LARGE DIA. Bundle 24 hr_Thread 1 Image 2_Hoechst_10X_196.6ms	418
2-11-08_Rotated LARGE DIA. Bundle 24 hr_Thread 1 Image 3_Hoechst_10X_45.7ms	506
2-11-08_Rotated LARGE DIA. Bundle 24 hr_Thread 1 Image 4_Hoechst_10X_81.9ms	477
2-11-08_Rotated LARGE DIA. Bundle 24 hr_Thread 2 Image 1_Hoechst_10X_50.4ms	338
2-11-08_Rotated LARGE DIA. Bundle 24 hr_Thread 2 Image 2_Hoechst_10X_50.4ms	321
2-11-08_Rotated LARGE DIA. Bundle 24 hr_Thread 2 Image 3_Hoechst_10X_50.4ms	392
2-11-08_Rotated LARGE DIA. Bundle 24 hr_Thread 2 Image 4_Hoechst_10X_50.4ms	412
2-11-08_Rotated LARGE DIA. Bundle 24 hr_Thread 2 Image 5_Hoechst_10X_50.4ms	347
2-11-08_Rotated LARGE DIA. Bundle 24 hr_Thread 2 Image 6_Hoechst_10X_50.4ms	431
2-11-08_Rotated LARGE DIA. Bundle 24 hr_Thread 2 Image 7_Hoechst_10X_50.4ms	436
2-11-08_Rotated LARGE DIA. Bundle 24 hr_Thread 2 Image 8_Hoechst_10X_50.4ms	361
2-11-08_Rotated LARGE DIA. Bundle 24 hr_Thread 2 Image 9_Hoechst_10X_11.8ms	266
2-11-08_Rotated LARGE DIA. Bundle 24 hr_Thread 3 Image10_Hoechst_10X_50.4ms	295
2-11-08_Rotated LARGE DIA. Bundle 24 hr_Thread 3 Image 1_Hoechst_10X_50.4ms	232
2-11-08_Rotated LARGE DIA. Bundle 24 hr_Thread 3 Image 2_Hoechst_10X_50.4ms	277
2-11-08_Rotated LARGE DIA. Bundle 24 hr_Thread 3 Image 3_Hoechst_10X_50.4ms	396
2-11-08_Rotated LARGE DIA. Bundle 24 hr_Thread 3 Image 4_Hoechst_10X_50.4ms	353
2-11-08_Rotated LARGE DIA. Bundle 24 hr_Thread 3 Image 5_Hoechst_10X_50.4ms	388
2-11-08_Rotated LARGE DIA. Bundle 24 hr_Thread 3 Image 6_Hoechst_10X_50.4ms	387
2-11-08_Rotated LARGE DIA. Bundle 24 hr_Thread 3 Image 7_Hoechst_10X_50.4ms	368
2-11-08_Rotated LARGE DIA. Bundle 24 hr_Thread 3 Image 8_Hoechst_10X_50.4ms	508
2-11-08_Rotated LARGE DIA. Bundle 24 hr_Thread 3 Image 9_Hoechst_10X_50.4ms	326
2-11-08_Rotated SMALL DIA. Bundle 24 hr_Thread 1 Image 1_Hoechst_10X_37.4ms	246
2-11-08_Rotated SMALL DIA. Bundle 24 hr_Thread 1 Image 2_Hoechst_10X_12.9ms	125
2-11-08_Rotated SMALL DIA. Bundle 24 hr_Thread 1 Image 3_Hoechst_10X_12.9ms	221
2-11-08_Rotated SMALL DIA. Bundle 24 hr_Thread 2 Image 1_Hoechst_10X_23.1ms	226
2-11-08_Rotated SMALL DIA. Bundle 24 hr_Thread 3 Image 1_Hoechst_10X_23.1ms	211
2-11-08_Rotated SMALL DIA. Bundle 24 hr_Thread 3 Image 2_Hoechst_10X_23.1ms	225
2-11-08_Rotated SMALL DIA. Bundle 24 hr_Thread 3 Image 3_Hoechst_10X_23.1ms	252
2-11-08_Rotated SMALL DIA. Bundle 24 hr_Thread 3 Image 4_Hoechst_10X_23.1ms	277
2-18-08_Rotated Sm. Dia. Bundle 24 HOURS_ Thread 1 Image 1_Hoechst_10X_30.8ms (2-18-0~1)	266
2-18-08_Rotated Sm. Dia. Bundle 24 HOURS_ Thread 1 Image 2_Hoechst_10X_30.8ms (2-18-0~2)	304
2-18-08_Rotated Sm. Dia. Bundle 24 HOURS_ Thread 1 Image 3_Hoechst_10X_30.8ms (2-18-0~3)	373
2-18-08_Rotated Sm. Dia. Bundle 24 HOURS_ Thread 2 Image 1_Hoechst_10X_30.8ms (2-18-0~1)	238

2-18-08_Rotated Sm. Dia. Bundle 24 HOURS_ Thread 2 Image 2_Hoechst_10X_30.8ms (2-18-0~2)	351
2-18-08_Rotated Sm. Dia. Bundle 24 HOURS_ Thread 2 Image 3_Hoechst_10X_30.8ms (2-18-0~3)	349
2-18-08_Rotated Sm. Dia. Bundle 24 HOURS_ Thread 2 Image 4_Hoechst_10X_30.8ms (2-18-0~4)	325
2-18-08_Rotated Sm. Dia. Bundle 24 HOURS_ Thread 1 Image 2_Hoechst_10X_30.8ms-1 (2-18-0~1)	280
2-18-08_Rotated Sm. Dia. Bundle 24 HOURS_ Thread 1 Image 3_Hoechst_10X_30.8ms-1(2-18-0~2)	303
Average	**363**
Standard Deviation	**87**

Table 17. Average diameter for hydrated bundles of ten fibrin microthreads.

Experiment Image	**Diameter (μm)**
1-19-07_Thread 1_Image 1_Bundled 5 Rotated_Hoechst dye_10X_429.2ms	175
1-19-07_Thread 1_Image 2_Bundled 5 Rotated_Hoechst dye_10X_429.2ms	187
1-19-07_Thread 1_Image 3_Bundled 5 Rotated_Hoechst dye_10X_429.2ms	186
1-19-08_Rotated Bundles of 5_Thread 1 Image 1_Hoechst_10X_370.8ms	204
1-19-08_Rotated Bundles of 5_Thread 1 Image 2_Hoechst_10X_370.8ms	197
1-19-08_Rotated Bundles of 5_Thread 1 Image 3_Hoechst_10X_370.8ms	183
1-19-08_Rotated Bundles of 5_Thread 1 Image 4_Hoechst_10X_370.8ms	159
1-19-08_Rotated Bundles of 5_Thread 2 Image 1_Hoechst_10X_370.8ms	258
1-19-08_Rotated Bundles of 5_Thread 2 Image 2_Hoechst_10X_370.8ms	263
1-19-08_Rotated Bundles of 5_Thread 2 Image 3_Hoechst_10X_370.8ms	233
1-19-07_Thread 3_Image 3_Bundled 5 Rotated_Hoechst dye_10X_305.0ms	233
2-1-08_Rotated Bundle of 5_Thread 1 Image 1_Hoechst_10X_196.6ms.tif	179
2-1-08_Rotated Bundle of 5_Thread 1 Image 2_Hoechst_10X_196.6ms	371
2-1-08_Rotated Bundle of 5_Thread 2 Image 1_Hoechst_10X_196.6ms.tif	277
2-1-08_Rotated Bundle of 5_Thread 2 Image 2_Hoechst_10X_196.6ms.tif	208
2-1-08_Rotated Bundle of 5_Thread 3 Image 2_Hoechst_10X_196.6ms.tif	169
Average	**218**
Standard Deviation	**54**

Table 18. Average diameter for hydrated bundles of five fibrin microthreads.

Experiment Image	Diameter (µm)
11-28-07_Droplet_thread 4_5X_250.9ms (10X)	106
11-28-07_Droplet_thread 4_10X_161.9ms (20X)	106
11-28-07_Droplet_thread 6_5X_250.9ms (10X)	174
11-28-07_Droplet_thread 6_10X_139.9ms (20X)	169
12-12-07_Droplet_Bundled_thread 1_5X_983.9ms (10X)	114
12-12-07_Droplet_Bundled_thread 2_5X_983.9ms (10X)	171
12-12-07_Droplet_Bundled_thread 2_10X_770.9ms (20X)	144
11-28-07_Rotator_thread 4 Area 1_5X_699.3ms	90
11-28-07_Rotator_thread 4 Area 1_10X_32.3ms	108
11-28-07_Rotator_thread 4 Area 1_10X_139.9ms	96
11-28-07_Rotator_thread 4 Area 2_5X_408.9ms	105
11-28-07_Rotator_thread 4 Area 3_10X_86.0ms	96
11-28-07_Rotator_thread 4 Area 3_10X_187.3ms (5X)	87
11-28-07_Rotator_thread 5_5X_86.0ms	98
11-28-07_Rotator_thread 5_10X_86.0ms	107
11-28-07_Rotator_thread 6 Area 1_5X_86.0ms	148
11-28-07_Rotator_thread 6 Area 1_10X_86.0ms	149
11-28-07_Rotator_thread 6 Area 2 (Left)_10X_86.0ms	141
11-28-07_Rotator_thread 6 Area 2 (Right)_10X_86.0ms	188
11-28-07_Rotator_thread 6 Area 2_5X_86.0ms	183
12-12-07_Rotation_Bundled_thread 1_5X_1.5s	100
12-12-07_Rotation_Bundled_thread 2_5X_937.1ms	88
12-12-07_Rotation_Bundled_thread 1_10X_473.1ms	102
12-12-07_Rotation_Bundled_thread 2_10X_473.1ms	103
1-19-08_Rotated Bundles of 3_Thread 1 Image 1_Hoechst_10X_370.8ms-1	113
1-19-08_Rotated Bundles of 3_Thread 2 Image 1_Hoechst_10X_370.8ms	117
1-19-08_Rotated Bundles of 3_Thread 3 Image 1_Hoechst_10X_370.8ms:Blue	211
2-1-08_Rotated Bundle of 3_Thread 1 Image 1_Hoechst_10X_276.6ms.tif	149
2-1-08_Rotated Bundle of 3_Thread 1 Image 2_Hoechst_10X_276.6ms.tif	259
2-1-08_Rotated Bundle of 3_Thread 2 Image 1_Hoechst_10X_196.6ms	342
2-1-08_Rotated Bundle of 3_Thread 3 Image 1_Hoechst_10X_196.6ms	170
Average	**140**
Standard Deviation	**56**

Table 19. Average diameter for hydrated bundles of three fibrin microthreads.

Appendix E: Design Alternatives

Potential designs

D1 – Dynamic seeding (rotation seeding), cell adhesion molecule, bundled biological microthreads
D2 – Static seeding (droplet method), cell adhesion molecule
D3 – Dynamic seeding (rotation seeding), surface modification (microgrooves)
D4 – Static Seeding (droplet method), surface modification

Objectives (O)

The objectives selected for comparing design alerntatives were the top seven objectives from Table 14.
O1 – Increase cell density
O2 – Maintain mechanical Integrity of biological microthreads
O3 – Strength of cell attachment
O4 – Number of cells attached
O5 – Maximize cell quantity on biological microthreads
O6 – Control quantity of cells delivered
O7 – Increase speed of 'more density'

Objectives & Constraints ↓	Design →	Weight	D1	D2	D3	D4
C: Maintain Mechanical Integrity		Y/N	Y	Y	Y	Y
C: Cost effective		Y/N	Y	Y	Y	Y
O1		.133	2(.133)=.266	1(.133)=.133	2(.133)=.266	1(.133)=.133
O2		.108	1(.108)=.108	2(.108)=.216	0(.108)=.0	2(.108)=.216
O3		.117	2(.117)=.234	0(.117)=0	2(.117)=.234	0(.117)=0
O4		.123	2(.123)=.246	1(.123)=.123	1(.123)=.123	1(.123)=.123
O5		.123	2(.123)=.246	1(.123)=.123	1(.123)=.123	2(.123)=.246
O6		.106	1(.106)=.106	1(.106)=.106	1(.106)=.106	1(.106)=.106
O7		.127	1(.127)=.127	0(.127)=0	1(.127)=.127	0(.127)=0
Total			**1.333**	**.701**	**.979**	**.824**

Table 20. Design alternative comparison based off of weighted objectives from Table 14.

Objectives →	**O1**	**O2**	**O3**	**O4**	**O5**	**O6**	**O7**	**Total**
Objective Weights →	*.133*	*.108*	*.117*	*.123*	*.123*	*.106*	*.127*	
Cell Adhesion Molecules	2(.133)= .266	0(.108)= 0	1(.117)= .117	2(.123)= .246	1(.123)= .123	1(.106)= .106	1(.127)= .127	.985
Dynamic Seeding Technique	2(.133)= .266	2(.108)= .216	2(.117)= .234	2(.123)= .246	2(.123)= .246	2(.106)= .212	2(.127)= .254	1.67
Physical Surface Alteration	2(.133)= .266	1(.108)= .108	1(.117)= .117	2(.123)= .246	2(.123)= .246	1(.106)= .106	0(.127)= 0	1.09

Table 21. Candidate methods to increase cell density on fibrin microthreads.

Objectives →	**O1**	**O2**	**O3**	**O4**	**O5**	**O6**	**O7**	**Total**
Objective Weights →	*.133*	*.108*	*.117*	*.123*	*.123*	*.106*	*.127*	
Stirred	1(.133)= .133	0(.108)= 0	1(.117)= .117	1(.123)= .123	1(.123)= .123	0(.106)= 0	1(.127)= .127	.683
Centrifugation	1(.133)= .133	0(.108)= 0	0(.117)= 0	0(.123)= 0	1(.123)= .123	0(.106)= 0	2(.127)= .154	.410
Rotation	2(.133)= .266	1(.108)= .108	2(.117)= .234	2(.123)= .246	2(.123)= .246	2(.106)= .212	1(.127)= .127	1.44

Table 22. Candidate dynamic seeding techniques to increase cell density on fibrin microthreads.

Objectives →	**O1**	**O2**	**O3**	**O4**	**O5**	**O6**	**O7**	**Total**
Objective Weights →	*.133*	*.108*	*.117*	*.123*	*.123*	*.106*	*.127*	
Bundling of microthreads	2(.133)= .266	1(108)= .108	2(.117)= .234	1(.123)= .123	2(.123)= .246	1(.106)= .106	2(.127)= .254	1.34
Electrospray onto microthreads	1(.133)= .133	0(.108)= 0	0(.117)= 0	1(.123)= .123	0(.123)= 0	0(.106)= 0	0(.127)= 0	.256
Patterned microgrooves on microthreads	1(.133)= .133	0(.108)= 0	1(.117)= .117	1(.123)= .123	1(.123)= .123	1(.106)= .106	0(.127)= .127	.729

Table 23. Candidate physical surface alterations to increase cell density on fibrin microthreads.

Objectives →	**O1**	**O2**	**O3**	**O4**	**O5**	**O6**	**O7**	**Total**
Objective Weights →	*.133*	*.108*	*.117*	*.123*	*.123*	*.106*	*.127*	
Fibronectin	2(.133)= .266	1(108)= .108	2(.117)= .234	1(.123)= .123	2(.123)= .246	1(.106)= .106	2(.127)= .254	1.34
RGD peptides	1(.133)= .133	0(.108)= 0	1(.117)= .117	1(.123)= .123	1(.123)= .123	1(.106)= .106	0(.127)= .127	.729
Collagen	1(.133)= .133	1(.108)= .108	1(.117)= .117	1(.123)= .123	0(.123)= 0	1(.106)= .106	1(.127)= .127	.714
Laminin	0(.133)= 0	2(.108)= .216	1(.117)= .117	1(.123)= .123	0(.123)= 0	0(.106)= 0	0(.127)= 0	.456

Table 24. Candidate cell adhesion molecules to increase cell density on fibrin microthreads.

Appendix F: Cell Culture Protocol

Thawing hMSCs

Materials

1. DMEM (Dulbecco's Modified Eagle's Medium), 10% FBS, 1% P/S
with all supplements (serum = FBS, L-glutamine, antibiotics = P/S), 37C.
Stored at 4C (DMEM) & -5 to -20C (FBS, P/S).

2. Pipets: 25mL, 10mL, 5mL Serological Pipets.

3. Miscellaneous items: Sterile culture flasks, 15mL conical tubes, 70% Isopropyl Alcohol (IPA), Pipet Aid, Lab marker for labeling.

Procedure

1. Spray inside surface of hood with 70% IPA. Spray all exterior surfaces of containers to be brought into hood with 70% IPA. Set up all necessary items in hood.

2. Remove stored cryovial(s) containing cells from Liquid Nitrogen cryotank/Dry ice. *Optional:* Wipe cryovial(s) with 70% IPA and in sterile field, brefly twist cap a quarter turn to relieve pressure, then retighten.

3. Thaw cells rapidly by immediately immersing vial(s) into 37° C water bath. Do not submerge them completely and watch them closely. Gently agitate for approx. 2 min (no longer than 3 min). *Note*: Most cell death occurs between –50° C and 0° C when thawing.

4. When fully thawed (all ice crystals melted), remove vial(s) immediately, wipe dry then spray outside of vial(s) thoroughly with 70% IPA before bringing cells into the hood. Transfer thawed cell suspension into 15mL tube containing 5mL pre-warmed media to dilute.

5. Centrifuge cells at 500-600rpm for 5 minutes to remove any residual DMSO.
While cells are being spun down, set up new flasks and add appropriate amount of DMEM to each: ~2-5mL for T25, ~12-15mL for T75. Temperature equilibrate to 37° C.

6. Decant supe; Resuspend cell pellet in minimum volume of fresh pre-warmed media. Perform CELL COUNTING. seed cells by transferring the appropriate amount of cell suspension into new culture flask(s) with fresh medium. *Note*: Amount of suspension transferred will depend on the density at which cells were frozen and desired cell density for new seed.

7. Place cells in incubator, and replace with equal volume of fresh medium after 24 hrs to remove any (floating) dead cells. Observe cells daily for growth (confluency reached by ~1 week) and freedom from contamination. Media to be changed every 3-4 days.

8. Clean up hood and spray down surface with 70% IPA. Close it and turn on UV light.

Cell Subculture

Materials

1. DMEM (Dulbecco's Modified Eagle's Medium), 10% FBS, 1% P/S
with all supplements (serum = FBS, L-glutamine, antibiotics = P/S), 37C.
Stored at 4C (DMEM) & -5 to -20C (FBS, P/S).

2. DPBS, 37C. Stored at RT.

3. 0.25% Trypsin, 37C. Stored at -20C. (Not to be left in water bath for extended period of time.)

4. Pipets: 25mL, 10mL, 5mL Serological Pipets, 5mL aspirating Pasteur Pipets.

5. Miscellaneous items: Sterile culture flasks for seeding, 70% Isopropyl Alcohol (IPA), Pipet Aid, Lab marker for labeling.

Procedure

1. Spray inside surface of hood with 70% IPA. Spray all exterior surfaces of containers to be brought into hood with 70% IPA. Set up all necessary items in hood.

2. Remove all media from culture dish/flask.

3. Add sterile DPBS to flask for ~1min wash: ~2mL for T25, ~5mL for T75.
Rinse entire bottom surface by gently rocking flask. *Remember:* Handle flasks vertically as to not allow media to enter neck of dish, specially designed for CO2 exchange.

4. Remove (Aspirate) DPBS.

5. Add Trypsin: ~2mL for T25, ~5mL for T75, gently to bottom edge/corner of flask (as to not shock/dislodge cells). Again, rock flask gently to ensure full coating of bottom surface. Check cells under microscope to make sure they are detaching from flask and have "rounded-up" morphology. (Trypsin is a protease that acts to degrade protein.)

6. Set up new flasks and add appropriate amount of fresh DMEM to each: ~2-5mL for T25, ~12-15mL for T75.
Note: No need to aspirate trypsin since DMEM will inactivate its proteolytic action.

7. When all cells appear round, add DMEM: ~2mL for T25, ~5mL for T75 and thoroughly wash flask to gather up all cells from the bottom of the flask by gently triturating up and down while tilting the flask.

8. Add appropriate volume (depending on % confluency) of cell suspension to fresh flasks. Gently rock/swirl flask to spread out cells.

9. Place cells in incubator and observe daily for growth (toward confluency) and freedom from contamination. Media to be changed every 3-4 days.

10. Clean up hood and spray down surface with 70% IPA. Close it and turn on UV light

Cell Counting

Materials
1. DMEM (Dulbecco's Modified Eagle's Medium), 10% FBS, 1% P/S
with all supplements (serum = FBS, L-glutamine, antibiotics = P/S), 37C.
Stored at 4C (DMEM) & -5 to -20C (FBS, P/S).

2. DPBS, 37C. Stored at RT.

3. 0.25% Trypsin, 37C. Stored at -20C. (Not to be left in water bath for extended period of time.)

4. Pipets: 25mL, 10mL, 5mL Serological Pipets, 5mL aspirating Pasteur Pipets.

5. Miscellaneous items: Trypan Blue dye, hemacytometer + coverslip, 70% EtOH, Pipet Aid, Eppendorf pipettor and associated tips, Lab marker for labeling.

Procedure
1. Follow SUBCULTURING hMSCs protocol for a T75 flask of cells.

2. After trypsinizing hMSCs, place DMEM (~5mL) + Trypsin (~5mL) + cells into 15mL centrifuge tube.

3. Centrifuge at 600rpm for 5min @ RT.

4. Decant supernatant; Resuspend pellet in ~1mL DMEM.

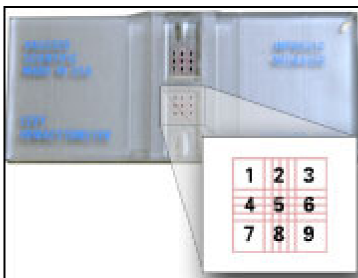
5. Prepare a 1:10 dilution (thus dilution factor = 10) of cell suspension to be counted as follows:

Place 50 µL Trypan Blue + 40 µL non-sterile DPBS + 10 µL in a small Eppendorf tube. Triturate gently as to increase accuracy of count.
Note: Trypan Blue is toxic and a potential carcinogen so extra care should be taken with its use.

6. Prepare the hemacytometer by placing a clean coverslip onto its center grid section (mirror-like polished surface with wells). Both should be cleaned with ethanol prior to use.

7. Carefully load a small amount (~10µL) of cell suspension into the wells underneath and on each end of the coverslip.
Note: A hemacytometer is a specialized glass slide with a 3x3 grid pattern etched upon it whose volume is known. When covered by a coverslip, cells spread out due to capillary action.

8. Using a microscope, cells are counted within each of squares of the hemacytometer grid to obtain a measure of cell concentration as follows:



Count all viable cells in each of the 4 corner fields adjacent to the center square (i.e. squares that lie along a diagonal, here 1, 3, 7 & 9) for each side of hemacytometer for a total of 8 fields. Adopt a rule for counting cells that fall on grid lines to eliminate duplicate counts (i.e. count cells on left or top lines of a square, but not those on bottom or right lines).
Note: Dead cells appear blue as stained by Trypan Blue and should be excluded from the count, while viable cells appear bright and do not take up the dye unless exposed to it for an extended period of time after which they may absorb it and appear non-viable.

9. Use the following equations with numbers attained from count to calculate cell concentrations.

Final count or actual cell density in cells/mL → Eqn: $C_1V_1 = C_2V_2$

average count per field
 # viable cells / mL = [# viable cells / total # fields] X dilution factor X 10^4

total # viable cells = # viable cells / mL X original vol from which sample removed
= C_1 X V_1

final resuspension volume *(# mL of cells to add)* = total # cells / target cell density or []
$V_2 = C_1V_1$ / C_2

% viability = total # viable cells / total # cells X 100
Note: Must perform dead count for total # cells.

Appendix G: MTS Sensitivity and Results

As can be seen below in Figure 23, the MTS assay was not sensitive enough for low cell counts which were found on the fibrin microthreads. This can be seen through the large standard deviation and negative cell concentration for droplet seeding with individual thick fibrin microthreads.

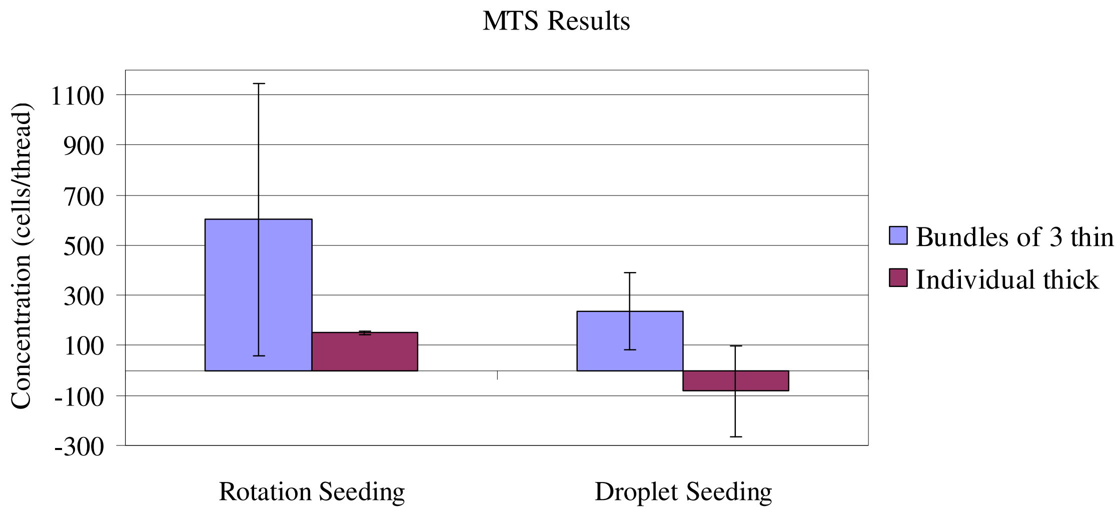


Figure 23. MTS results not sensitive to low cell counts on fibrin microthreads

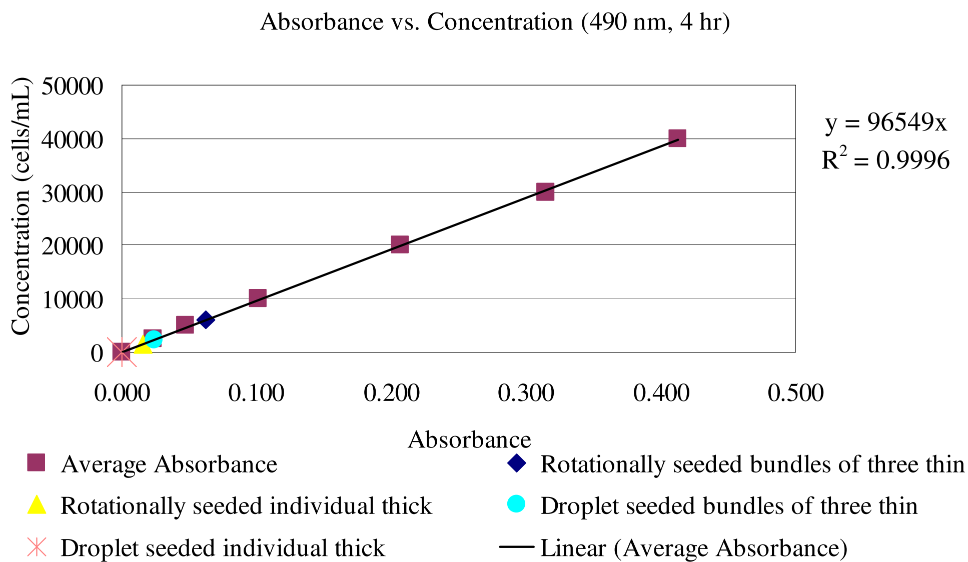


Figure 24. Concentration vs. absorbance for MTS results

Appendix H: Glossary and Acronyms

Acronyms
MI: Myocardial infarction
hMSC: Human mesenchymal stem cell
ECM: Extracellular matrix
Fn: Fibronectin
RGD: Arginine-glycine-aspartate
FBS: Fetal bovine serum
DPBS: Dulbecco's phosphate buffered saline
PE: Polyethylene
ID: Inner diameter
OD: Outer diameter

Glossary
Adhesion molecules- Proteins used to increase cell attachment to the surface of fibrin microthreads.

Adult stem cell- Undifferentiated cell that can renew itself and differentiate into the specialized cell types of a tissue or organ.

Bundled microthreads- Discrete fibrin microthreads can be bundled to make intricate structures with microgrooves between the fibrin threads.

Cardiac regeneration- Applying tissue engineering, stem cell therapy, medical devices and other techniques to repair damaged or diseased areas of the heart.

Cell-based cardiac repair- The use of cells as a method of treating cardiovascular diseases such as MI and heart failure.

Cell seeding- Method by which cells are transferred from a flask to the platform of interest.

Droplet seeding- A static cell seeding technique in which fibrin microthreads are adhered to a washer and a drop of cells is placed on the washer and gravity allows for the cells to attach to the fibrin microthread.

Dynamic seeding- Involves stirring or agitation of cells in suspension together with the scaffold.

Fibrin- A natural biomaterial that is biocompatible, bioresorbable, and essential in normal wound healing.

Fibrin microthread- Biological microthreads produced by coextruding solutions of 70 mg/mL fibrinogen and 6 U/mL thrombin through small diameter polyethylene tubing.

These biological microthreads have the potential for cell-mediated tissue ingrowth and regeneration.

Fibronectin- An ECM protein, that participates in a number of cellular processes including tissue repair, wound healing, and cell migration/adhesion

Heart- The organ that pumps the oxygenated blood and nutrients, via the aorta, throughout the body.

Heart failure- A condition that can result from any structural or functional cardiac disorder that impairs the ability of the heart to fill with or pump a sufficient amount of blood through the body.

Human mesenchymal stem cell- An adult stem cells found in bone marrow.

Ischemia- A restriction in blood and oxygen supply that can result in the damage of tissue.

Microthread suturing- A method in which fibrin microthreads can transplant stem cells into an infarcted area of the heart.

Myocardial infarction- Necrosis of a region of the myocardium; can potentially lead to heart failure.

Rotational seeding- A dynamic cell seeding technique in which fibrin microthreads contained in Silastic tubing are rotated vertically around a horizontal axis while being suspended in cell suspension.

Static seeding- Cell seeding performed by allowing a cell suspension to statically deposit cells on the platform of interest.