3D Electrospun Tissue Scaffold:
Achieving Customized Fiber Alignment

Major Qualifying Project
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Authorship

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ABSTRACT

Engineering of 3D scaffolds for tissue repair requires the ability to align cells to mimic cell orientations of native tissues. Our study focused on utilizing electrospinning technology to develop 3D nanofiber scaffolds whose alignment can be customized to mimic the microarchitecture of a tissue. Current electrospinning techniques have limited control of fiber orientation. We engineered a collection plate that can be adapted to produce 3D biomaterial scaffolds for use with commercially available electrospinning units. The custom fiber aligned scaffolds were seeded with green fluorescent protein (GFP) expressing cells to show viability for cell seeding and alignment along the fiber direction. The major device design characteristics and results are presented in this report.
1. INTRODUCTION

One of the more common knee ailments that affect individuals of all activity levels is a meniscal tear. Though often resulting from sport-related incidents, this injury can occur from simple twisting and pressure-inducing movements of the knee. Meniscal surgeries are performed on roughly 1 million patients annually in the United States alone, generating a $4 billion market (Frizziero et al., 2012). The majority of the meniscus is avascular with the exception the peripheral regions, specifically 20-30% of the medial and 10-25% of the lateral meniscus, meaning that all other regions are unable to repair self-sufficiently without vascular networks to facilitate nutrient perfusion. Therefore, there is a need for a novel tissue scaffold capable of fostering cell growth and proliferation in 3D environments to test the viability of a complete meniscus repair under in vitro conditions.

Several treatment options are available for meniscus repair based on the severity. Physical therapy is the initial and ideal choice of treatment for mild injuries. All other treatments are surgical, varying in the amount of intervention. Repair can be partial, subtotal, or total replacement of the cartilage. Partial surgery is the most common procedure. Surgeons create incisions in the meniscus and insert sutures as a fixation method. This ensures that adequate mobility is present, referencing a 2 to 5 mm translation medially and 9 to 11 mm laterally to model in vivo conditions (Baker, 2015). However treatment complications are commonplace, often negatively impacting the weight distribution in the knee post-surgery. These imbalances also directly cause bone-on-bone contact, known as osteoarthritis, which leads to cartilage degradation.

Anatomically, the lateral section covers the front of the knee while the medial lies in the middle of the meniscus.
Figure 1: Diagram detailing different areas and connections of the meniscus

*(Physical Healthcare, 2016)*

From a cross-sectional viewpoint, the outer region of the meniscus is filled with long oval shaped cells, shaped for communication, whereas the inner region is comprised of round cells in the extracellular matrix, specifically fibrochondrocytes, and chondrocytes (Markis *et al.*, 2012).

The injury rate demonstrates the need for a biocompatible, naturally constructed tissue-engineered scaffold both for *in vitro* research and for meniscal implantation. A properly engineered meniscus would inhibit imbalances in the knee from forming, while allowing for full functionality. Current meniscus repairs are not customizable. A standardized procedure to engineer menisci *in vitro* could lead to the development of customized menisci based on patient-specific MRIs. A commercially available meniscus, Menaflex, developed by ReGen Biologics
was originally approved by the FDA but has since been retracted. Another product, Actifit, developed by Orteq has not yet been cleared by the FDA for full replacements. Additionally, the major drawback of the current scaffold designs is the shared inability to provide sufficient vasculature to allow proper nutrient diffusion needed for the facilitation, growth and maintenance of the menisci cells and its matrix.

This project hones in on development of a universal tissue scaffold to further meniscus repair research, which can be tailored to individual patients in the future. The biomaterials chosen need to be compatible with the cellular microenvironment to allow for tissue formation. The scaffold must facilitate alignment of cells to imitate the micro-geometries and microarchitecture of the meniscal tissue to promote normal functioning of the tissue. In order for an engineered tissue to be successful, it is important to ensure proper alignment of cells in a three dimensional environment, which mimic the 3D micro architecture and micro-geometries similar to the tissue of origin. For a load bearing tissue such as the meniscus, mechanical stimulation and conditioning will be necessary before implantation so that the meniscus will behave effectively in the day to day activities of the patient.

A meniscus in the natural environment experiences enormous amounts of shear, tensile, and compressive forces. A tissue-engineered meniscus must withstand certain parameters to be considered implantable. These parameters are a shear stress of 120 KPa, tensile force of 100-300 MPa, and compressive forces ranging from 100-150 KPa (Markis et al., 2012).

To achieve these specifications, the project must meet established milestones. Research needs to be conducted on which biomaterials are to be utilized in the scaffold as well as how the scaffold will guide cells into an alignment that mimics nature. In this project, our team aims to design a biomorphic meniscal scaffold that will closely mimic the fiber and cell alignment whose
function should resemble the native tissue. The design process and design alternatives are presented in this report and discussed in detail.
2. LITERATURE REVIEW

An extensive literature review was done in order to determine the anatomy/functions of the meniscus, types of injuries, their treatment options and complications, and relevance. Tissue engineering strategies and the current state of the art are also reviewed in this chapter.

2.1. Function of the Meniscus

The primary functions of the meniscus consist of (1) load transmission, (2) shock absorption, and (3) lubrication, with some capacity to support knee stability. All of these functions must be considered and the detailed means must be analyzed within the scope of our project to ensure that our testing and validation methods are physiologically accurate and feasible.

In analyzing the mobile segment of the meniscus, we discovered that depending on the type of activity between 50-90% of the force endured by the knee joint is distributed by this area of the cartilaginous tissue. This distribution is primarily performed in the flexion phase where 90% of the load is transmitted, while with a 50% distribution is exhibited in knee extension, which is because much of the meniscus action is in response to compressive forces (Aagaard et al., 1999). As recognized in common meniscal injury types, tensile “hoop stress” strength is the result of the expose to weight bearing loads that, if compromised, leads to serious degrading tears. Split into two phases, this loading response consists of first an initial mechanical reaction where the proteoglycan-collagen network, along with interstitial fluids, compress to increase hydrostatic pressure. The second phase is referred to as fluid extrusion, where this interstitial fluid flows from the fiber matrix, thus decreasing the hydrostatic pressure and resulting in stress relaxation across the meniscus.
Additionally, as alluded to in the above function, the meniscus assists in joint lubrication. This is expressed when the joint experiences a load as the synovial (interstitial) fluid is released to ensure low frictional forces in the articular cartilage. And finally, the stability of a healthy knee is due in part to the meniscus by deepening the tibial socket, which inhibits femoral translation on the tibia by increasing contact with the femoral condyles (Dept. of Rehabilitation Services, 2007).

2.2. Types of Meniscal Injuries & Risk Levels

As the most prevalent pathology of the knee, the desire for meniscal tear treatments is a global need. There are five varieties of tears, each with ranging levels of risk.

Market Need

Approximately ~60% of people over the age of 65 will experience a form of degenerative meniscal tear at some point in their lifetime and of this population, 38% will exhibit complications from current treatment methods. A particularly challenging aspect is working with the avascular sections of the meniscus, which causes severe deficiencies in the production of effective regeneration and replacement of the damaged tissue. This means that almost 2 in 5 individuals that undergo treatment will not regain full mobility (Mordecai, 2014). Of the current treatments both in practice and in the pipeline, some show promising preliminary results, which will be discussed in detail later, but as of now, there is no “gold standard” for the treatment of meniscal tears. The current market for meniscus repairs, replacements and treatment is estimated at approximately $4 billion (Frizziero et al., 2012).
Horizontal

Horizontal cleavage tears are more representative of older individuals. These consist of tears extending outward from the free margin to the intrameniscal substance where myxoid degeneration can occur. By dividing the meniscus into superior and inferior flaps, horizontal tears result in instabilities across different regions. This type of tear is a more frequent injury pattern and is caused by twisting exercises and extreme flexion of the knee joint. As such, this type of tear is often associated with athletes competing in martial arts and basketball, in addition to the over 40 population.

Longitudinal

A vertical or longitudinal injury is characterized by a tear parallel to the circumferential fibers of the meniscus and is referred to as a bucket-handle tear if severe enough, which can either remain attached by the anterior or posterior segment of the meniscus. This tear can result in true locking of the knee due to physiological shifts in the intercondylar notch.

Radial

Radial tears occur outwards from the free to periphery regions of the meniscus, where it can diminish the meniscal capacity of hoop stress-distribution through transection of the cartilage tissue. Due to the severe consequences of such a tear a total meniscectomy surgery is required for repair.

Oblique

Oblique tears, also known as “parrot-beak tears”, are also fairly common which results in mechanically unstable flaps and requires re-sectioning of the meniscal tissue to avoid tear propagation. If not addressed these flaps can interfere with knee flexion and are associated with osteoarthritis.
Complex

Complete degenerative tears typically occur in older patients or from extreme overuse, associated with the onset of osteoarthritis. These tears occur in various regions of the meniscus based on the sections most deteriorated from repetitive motions, based on the individual patient’s activity levels and preferences.

2.3. Treatment & Complications

Patients with meniscal injuries have a variety of treatment options that are currently available to them depending on the severity/type of injury (Markis et al., 2012). The current options for treating meniscal tears include the following: physical therapy, partial meniscectomy, complete meniscectomy, surgical repair, or transplantation. The list below explains when each treatment option is used and what the complications are with each treatment option.

Physical Therapy

This treatment option is a non-invasive procedure that does not involve any form of surgery to treat the meniscal tear. Physical therapy is completed in conjunction with much resting and icing of the knee, along with applying compression to the knee and elevating it during the rest period. The downside to physical therapy is that it is only applicable as the main treatment option for minor meniscal tears. With physical therapy, the knee joint is strengthened back to its normal capacity; however, the small tear is sometimes never fully repaired if the tear is in an avascular region of the meniscus. Other complications include the pain and inflammation that is a product of this treatment option. This type of treatment is often only associated with tears in the vascular region of the meniscus known as the red region.
Partial Menisectomy

This treatment option is a surgical procedure performed on patients who have torn a portion of their meniscus in the avascular region, called the white region. This procedure is often chosen when the tear is classified as moderate or severe. A partial meniscectomy is when a portion of the meniscus is removed. Through surgical procedures, the surgeon will remove the damaged portion of the knee meniscus. Any procedure that removes a portion of the meniscus causes imbalances of loading within the knee and often leads to osteoarthritis. The load distribution is increased by an average of 235-335% for this type of surgery.

Complete Meniscectomy

This treatment option is also a surgical procedure that is most often performed on patients who have torn a portion of their meniscus known as the white zone. A complete removal of the meniscus is not as common as other procedures; however, it is used for severe or moderate tears to the meniscus to relieve pain and poor functionality. This procedure is an invasive procedure where the surgeon removes the entire meniscus. This treatment option has similar complications as the partial meniscectomy except these complications are amplified. The knee load distribution is increased by over 335% and the lack of a complete meniscus increases the chances of osteoarthritis due to no lubrication and cushioning between the tibia and femur. Postoperative complications can also include infection, chronic pain, or even bleeding or clotting within the joint.

Surgical Repair

Surgical repair is a treatment method used to repair the red region of the meniscus. The red region of the knee, being the vascular region of the knee, has a better ability to heal than the avascular regions of the knee. With tears or lesions in the vascular region of the meniscus,
surgeons can use sutures and stitching to repair the tear and allow for a natural healing process in the body. Unlike physical therapy, this type of treatment is performed on tears of the meniscus to the red region that are categorized as moderate to severe. Complications of this surgery are an extended healing time, inflammation and pain, as well as an increased need for re-surgery.

**Transplantation**

Knee meniscus transplantation is another treatment method for moderate to severe meniscal tears that occur in the white region of the meniscus. This procedure includes a complete meniscectomy of the damaged meniscus and then an implanted meniscus from a cadaver. A major challenge associated with this procedure is that there are very limited resources for the use of transplantation. Also, the transplants used for meniscal transplantation often have sizing issues and are not patient specific, which causes complications with load bearing. There is also a small risk of a patient receiving an infection due to the donor implant.

Through each of these different treatment options that are currently available for patients, there are specific complications associated with each treatment option as seen above. There are general complications with meniscal tears as well that are seen with all types of treatment options. With surgical operations on the knee to treat meniscal tears, 38% of people have complications with these surgeries and are in need of re-surgery. As previously mentioned, with the implant of a new meniscus or surgical procedures on the damaged meniscus, the functional load bearing properties of the knee are altered. With the absence of a meniscus or part of a meniscus the articular cartilage is worn down overtime leading to joint stiffness, high levels of pain, and the chance of having osteoarthritis. Lastly, all of these procedures have long recovery times that can last up to a year in time.
2.4. Meniscus Anatomy

The meniscus is a two-part component of the knee. There is a lateral meniscus and a medial meniscus that act as significant functional components of the knee. The image below gives a visual of how each portion of the meniscus is oriented in the knee joint (Figure 2):

![Meniscus shape/location](Figure 2)

*Figure 2: Meniscus shape/location*

*(Caronia et al., 2013)*

The lateral meniscus is more circular in shape but smaller in size. The medial meniscus is more of a c-shaped construct in the knee that is slightly larger than the lateral meniscus. The lateral meniscus provides more mobility within the knee and covers a larger portion of the tibial plateau. It also bears 100% of the load transmission in the knee when the knee is in full flexion. The lateral meniscus covers 75-93% of the tibial plateau laterally while the medial meniscus covers 51-74% of the tibial plateau medially (Śmigielski, 2015). The table below shows various dimensions of the knee meniscus:
Each portion of the meniscus is composed of two different regions, an inner region and an outer region. The inner region of the meniscus is the white region and is composed of mainly chondrocyte cells. The white region is avascular and aneural as it receives little to no blood supply. The white/inner region of the meniscus is composed of 70% collagen dry weight; 60% of it is collagen type II and 40% collagen type I. The outer region of the meniscus is the red region and is composed of fibroblast-like cells. This region is more vascular than the inner region of the meniscus and is 80% dry weight of collagen type I (Fox et al., 2015). The image below displays the different regions of the meniscus (Figure 3):

Table 1: Meniscal Dimensions

(Calvao et al., 2015)
The knee meniscus is composed of 72% water and 28% organic material. The organic material is composed of 75% collagen, 17% glycosaminoglycans (GAGs), 2% DNA, 1% adhesion glycoproteins, and 1% elastin. The knee meniscus performs many of its functions such as tension, shear, and compressive strength, load bearing and transmission, as well as shock absorption due to the alignment of fibers within the meniscus. The fiber alignment provides much of the structural integrity of the meniscus itself (Markis et al., 2012). The meniscus is a multi-layered construct within the knee that is composed of circumferential fibers (curved), and radial fibers (perpendicular to circumferential fibers). Throughout the inner and outer region of the meniscus are the circumferential fibers. The radial fibers are found throughout these regions as well. Mesh network fibers can be found more towards the surface of the meniscus. The two images below give a visual as to how the fibers are oriented in the meniscus (Figure 4):
2.5. Universal Relevance

To fully understand the possible expansion into broader research realms surrounding our 28-week project, we took a step back from the in-depth, technical research to evaluate where our study fits in the tissue engineering field. This analysis of relevance helped to give us perspective into the gravity of any advances in our field that may arise as a result of this project.

Informed Project Intent

The ultimate intent of our research on meniscal injuries is to discover successful methods of replacing damaged menisci with naturally grown, healthy meniscus constructs. This involves the utilization of biocompatible, bioactive scaffolding materials to act as the backbone for tissue growth of which they are products in development and undergoing approval processes for commercial use. But as our research on synthetic and artificial implants has shown, there is an inherent need for novel scaffolding techniques to develop long-lasting and safe replacement menisci. The drawbacks of these non-natural implant techniques are discussed later in detail but
the major takeaway is that these acellular scaffolds are unable to facilitate the necessary nutrient perfusion for both the regeneration and maintenance of the meniscal connective tissue.

**Project Expectations and Applicability**

The expectation of this project is to prove multiple cell alignments within the same tissue construct are possible, modeling the circumferential and radial fiber alignments in the native meniscus. The importance of this novel aspect cannot be understated, as no methods currently exist to produce custom fiber alignments that display the necessary mechanical properties for this implementation for a target tissue. By using cells to validate our techniques, our construct is expected to handle the nutrient perfusion that is critical for cell functioning and viability. Also in expanding past the unnatural environment of 2D cell cultures into the field of representative 3D cultures, we will be able to create a basis for 3D living tissue research. Through an understanding of the culture microenvironments and their direct influence on extracellular matrix component (ECM) production and the requisite cell properties, there is viability to create a universally advantageous tissue scaffold for many relevant scaffolding studies.

**2.6. Tissue Engineering Strategies**

In the context of this project, the tissue engineering approach envisaged here involves the development of a biomorphic scaffold that is able to provide relevant biochemical signals to chondrocyte cells to restore and regrow tissue functionality. It is important to have a system that supports cell life and allows the cells to keep their phenotype. The scaffold should allow cells to grow, align and differentiate in a way that mimics the native tissue. Biochemical factors may be added to aid in cell growth and functionality.
**Nutrient Perfusion**

Nutrients in the body can only diffuse up to 100-200 μm (Jain and Carmeliet, 2000). Because of this, most cells are located within this distance from a capillary. However, cartilage is an avascular tissue, meaning there is a lack of blood vessels. Necrosis can occur within hours with improper flow of nutrients in and waste out (Miller, 2014). Because of this, there needs to be a method of perfusing nutrients through the tissue.

Flow perfusion bioreactors are often used to get nutrients throughout the scaffold. Dynamic perfusion bioreactors can also stimulate the tissue (Jaasma et al., 2008). These systems are normally used with porous scaffolds. As long as the porosity is high enough, and the pores are interconnected, this provides a system that can allow for nutrient perfusion.

**Cellular Scaffold**

Cells are the living, active part of an engineered tissue. Chondrocytes are the cells that make up the meniscus, and are necessary for meniscus function.

*Chondrocytes*

Chondrocytes are essential for meniscal function as they produce, maintain, and remodel the cartilaginous ECM (Chung et al., 2008). Chondrocytes are terminally differentiated cells that do not proliferate. This means that progenitor cells will need to be cultured to the desired cell density before differentiation. The differentiation pathway starts with MSC’s which are differentiated into chondroblasts which can then be differentiated to chondrocytes.

For clinical purposes, normally autologous chondrocytes would be isolated, expanded, and seeded for engineered cartilage tissue (Chung et al., 2008). However, human progenitor cells are very limited in their abilities. The ATDC5 immortalized mouse chondrogenic cell line is an acceptable research model for cartilage tissue once differentiated (Tare et al., 2005). These cells
can be cultured and expanded to get the desired number of cells, then differentiated using insulin and ascorbic acid (Negishi et al., 2001).

ATDC5 Culturing

ATDC5 cells are typically cultured in an incubator at 37°C, 5% CO2, and 70% humidity. The culture media used with these cells is composed of Dulbecco’s Modified Eagle Medium (DMEM) without L-Glutamine, with pH indicator phenol red and 4.5 g/L glucose and sodium pyruvate in a 1:1 ratio with Ham’s F12. The media is also composed of 1X Glutamax, 1X Penicillin Streptomycin, and 5% fetal bovine serum (FBS).

Three Dimensional Tissue

The easiest way to culture cells is in a two dimensional environment. However, tissues in the body are three dimensional in geometry. In order to mimic the natural tissue better, there are ways of creating three-dimensional tissues, mainly with scaffolds and hydrogels.

Scaffolds

Scaffolds in tissue engineering provide the structure and shape for the cells to grow on. Scaffolds provide the mechanical strength until the cells and ECM grow and gain sufficient strength on their own. It is important for scaffolds to degrade at a rate that mirrors cellular/ECM growth so that isomorphic tissue regeneration can occur. Figure 5 below shows the idea of isomorphic tissue regeneration.
There are many ways of creating a scaffold. The main ways porous scaffolds can be created by freeze drying, solvent casting/porogen leaching; melt molding/porogen leaching, and gas foaming. However, these methods cannot have alignment in the pores, except for in freeze-drying. In this method, the pore alignment cannot be very closely controlled, and the pores are all in one direction.

Electrospinning is a technique used to create a fibrous scaffold, which is very attractive due to the extremely high surface area to volume ratio that it produces. A high surface area to volume ratio means that more cells can be seeded more densely on the scaffold. Electrospinning also allows for high control of the fibers diameter and some control of fiber arrangement. However, as highlighted later in this chapter, there is a large gap in knowledge and technology for fiber arrangement. There are no methods that can currently give a curved aligned fibrous scaffold.
Hydrogels

Hydrogels in the simplest terms are polymer gels in which water makes up the liquid component. Hydrogels have been used a lot recently for three dimensional cell suspension. The rate of crosslinking and final stiffness need to be considered when deciding on which hydrogel to use. The rate of crosslinking is how quickly the hydrogel can go from liquid to gel form. If this process takes too long, the cells, which are denser than the liquid, will sink causing an uneven distribution of cells. Generally, if the hydrogel crosslinking occurs within 2-3 minutes the cells will remain evenly distributed. The final stiffness is important for many reasons. First, this will provide the mechanical strength for the combination. Also, cells react to the environment around them. Many studies have been performed showing that the stiffness of the cellular environment affects cellular differentiation and phenotype.

Hydrogels do not normally have a nutrient perfusion pathway. This pathway must be created if gels thicker than 100-200 μm want to be created, which is more realistic to most tissues in the body. Hydrogels also do not offer any guidance to cellular arrangement. This is a major downside to hydrogels, as every tissue has a specific arrangement of cells and ECM.

2.7. Current State of the Art

Removing the damaged portion of the meniscus without replacing the tissue is the current accepted medical practice (Fox et al., 2015). To address this limitation, meniscus implants are being produced from tissue scaffolds. Without a standard scaffold available on the market, there are many varieties. Implants can be designed for total or partial meniscal replacements. The scaffolds are fabricated out of natural or synthetic materials (Zheng, Z. et al., 2015). Macromolecules from the natural extracellular matrix from the meniscus would provide an ideal
environment for tissue engineering. Such molecules would be collagen and glycosaminoglycans (GAGs).

There are many cartilage tissue scaffolds that are available commercially. Most scaffolds are similar in design. A major design consideration for a meniscal scaffold is that mature chondrocytes do not proliferate (Bahcecioglu, G. et al., 2014). Therefore, scaffolds must be made to allow for progenitor cells to be cultured to the correct density before differentiation is induced.

The overarching drawback of the current scaffolds is the lack of alignment in the fibers to guide cell growth. A scaffold for the meniscus needs a system that will replicate the collagen fibers and GAG deposits in the superficial and sub-chondral zones.

**Collagen Matrix**

One approach to creating an implant for replacement is using a collagen matrix (Zheng, Z. et al., 2015). Collagen meniscus implants can contain Collagen I, Collagen II, or a mixture of both. A scaffold of Collagen I and GAG provides more strength while Collagen II and GAGs increase the rate of cell proliferation and lubrication. Even with natural materials, there are difficulties with cells interacting to form a meniscus matrix and forming biometric meniscus shape. The degradation of the scaffold and suturing to the native tissue are also drawbacks.

One example of a collagen meniscus replacement implant was RenGen’s Menaflex (Fox et al., 2015). This was a scaffold of purified Collagen I from a bovine Achilles tendon. Similar to a surgical mesh, Menaflex was approved by the FDA to repair and reinforce the medial meniscus. The scaffold could be trimmed to fit the damaged site and then be sutured to the remaining healthy tissue. Over time, the patient’s cells would grow into the scaffold. This procedure was considered an alternative to a partial meniscectomy. The Menaflex was successful.
in the amount of cellular integration into the implanted matrix and that the material composition allowed it to be resorbed into the body.

Along with advantages, there were disadvantages with the Menaflex design and validation (Fox et al., 2015). Two design drawbacks were that the scaffold was not made from living tissue and that it shrank in size over time. After implantation, patients with Menaflex were required to restrict their activity level for up to six months. A partial meniscectomy, a procedure the FDA considered of the same caliber, restricted the activity level of patients for three weeks. Menaflex was unsuccessful for complete meniscus replacement and could not be used for lateral meniscus repair. The FDA retracted its approval of RenGen’s Menaflex due to the company’s inability to prove the benefit of the product for patients through proper clinical trials and validation.

**Actifit**

Another approach is the Actifit scaffold (Zheng, Z. et al., 2015). The design is a highly interconnected porous matrix of synthetic polyurethane. It has been tested for chronic meniscal tears but not full replacements. Actifit has shown success for smaller repairs with minimal need for activity restrictions. The architectural structure allows the tissue to grow into the large pores at the edges of the scaffold after three months. The synthetic make up allows for flexibility of material composition, and consequently the material properties such as degradation time. The Actifit scaffold is 80% biodegradable polymer and 20% polyurethane.

Currently, Actifit is suitable for lateral meniscal repairs in Europe (Zheng, Z. et al., 2015). It has not been approved in the United States for implantation. Actifit is not considered to be a long-term solution for repair due to a lack of data. Long-term studies need to be conducted since the short term relief of the scaffold may not outweigh unseen risks of prolonged exposure.
**Mimetix**

The Mimetix scaffold is one company’s version of a cartilage tissue scaffold (Amsbio 2013). The scaffold is constructed out of electrospun polymer poly (L-lactide) microfibers. The medical grade PLLA has a porosity of 80% with consistent pore size and fiber diameters. The fibers can be customized with different coatings and can be purchased in 12, 96, or 364 well plates. The scaffold can grow successfully grow cells in a 3D environment. The electrospun fibers are extruded out of a single needle system and utilize a rotating drum for a collection system.

Using PLLA as the scaffold material, Mimetix is not biodegradable (Amsbio, 2013). The electrospun fibers have a random orientation that is not cohesive to mimicking the anatomy of the meniscus. The scaffold needs to be preconditioned before cell seeding and has only been used in conjunction with human primary, cancer, and stem cells.

**Electrospinning**

One method of fiber scaffold deposition is using an electrospinning machine (Yousefzadeh M. *et al.*, 2012). The current models create nanofibers, which is an appropriate size to overcome the natural perfusion limit. Fiber alignment can be random or parallel, depending on the machine. There is no established design for electrospinning machines and there are many possible modifications to create fibers with alignment. The main two categories for modifications include externally applied forces and collection plate design.

Electrospinning requires charging the fiber material that is being extruded (Li *et al.*, 2005). Some designs take advantage of this and create charged electrodes or plates to guide fiber deposition. One popular design is to have a pair of parallel plates surrounding the extruder. Both plates have an equal charge, the same of the fiber material. When the fiber is extruded, both
plates repel it. The fiber travels in a straight line and is deposited on the collection plate. The fibers can also be guided by charged electrode that repel the fibers to keep them straight or of the same charge to attract them to align parallel on the plate. The electrodes can be as simple as a charged copper wire running along the backside of the collection plate (Yousefzadeh M. et al., 2012).

Modifications can be made to the collection plate, another part of the electrospinning machine (Li et al., 2005). There are numerous approaches to collecting the fibers. The original design is a flat collection plate than randomly aligned fibers were ejected onto. A rotating drum can be placed at the back of the collection plate to pull the fibers tight and wrap them around the drum like thread around a spool to create aligned fibers (Yousefzadeh M. et al., 2012). The collection plate can move to create alignments but the plate must be moving as fast as the injection speed to avoid fiber breakage.

One specific electrospinning collection design brought collection plate modification and electric field manipulation together (Zhang, K. et al., 2009). Electrospun PLLA was deposited on a collection plate of charged needles. The needles extruded from the plate in a square formation, as seen below, while the remaining portions of the plate were insulated (Figure 6).
Figure 6: Collection plate design from literature study

(Zhang, K. et al., 2009)

During collection, the PLLA self-assembled into high-density fiber areas at the top of the charged needles while the bottoms of the needles and plate yielded low fiber density. The advantage of this collection plate system is the ability to create shapes from electrospun fibers. The main drawbacks of this system were the fibers were spun at a micro-scale rather than the nanoscale and were deposited at the tops of the needles. This collection plate could not create a thick layer of threads, as would be possible if the threads deposited at the needle bases and extended to the tips.

Overall, the current electrospinning technology lacks the ability to create a curved fiber scaffold to create cell alignment as seen in natural meniscuses.
3. PROJECT STRATEGY

This chapter provides a description of how our projects client statement, objectives, and constraints were developed.

3.1. Initial Client Statement/Revised Client Statement

The knee meniscus is a two-part three-dimensional structure that provides shock absorption, stability and lubrication to the knee. Meniscal tears and ruptures of the knee are very common injuries. The team will engineer a three-dimensional, biomorphic tissue scaffold to research the ability to grow, ex vivo, replacement menisci. This scaffold will be printed with a commodity 3-D printer modified and re-engineered as necessary by the team to provide higher resolution.

Once our advisor, Professor Ambady supplied us with his initial client statement above, the team set to work conducting background research on the meniscus and learned the capabilities of our available methods for growing 3-D tissue models.

3.2. Technical Design Requirements

The team developed a list of objectives, secondary objectives, functions and means, project constraints, and device constraints to guide in the maturation of the project.

Objectives

The team compiled a list of objectives that were most critical for this design to achieve: safe, customizable, user friendly, compatible, reproducible. Each objective is discussed in detail below, with an explanation of its ranking and the corresponding secondary objectives.
This product must be safe for all stakeholders, including the designers, patients, medical professionals, and manufacturers. Safety is an objective that encompasses all of the stakeholders involved. Without this product being safe for each of the stakeholders, it will not be possible to patent this device for use. If a product puts a stakeholder at any risk of physical harm, then it will not be considered a beneficial product. Therefore, safety is an important objective that this product must satisfy. This product being safe can be broken down further into secondary objectives:

1. **Biocompatible**: This product must be biocompatible to avoid a foreign body response when it is implanted into a patient. The material considerations must all be considered biocompatible. The interaction between the product and the body/surrounding tissue must not cause inflammation or a toxic response. If the material is rejected from the body or broken down within the body, it will not be able to perform its primary function. This is the most significant secondary objective beneath safety because the main function of this product will be to exist in the body and function as normal knee meniscus would. If this product is not biocompatible, the main function of the device will no longer exist.

2. **Sterile**: The product must be able to be sterilized prior to implantation to avoid the contamination. The product will not be biocompatible if it cannot be sterilized before being implanted into the body. Therefore, this secondary step is essential in achieving safety for this product, which is why it is ranked second behind biocompatibility.

3. **Implantable**: This device must also be implantable and safe to handle by medical professionals. The procedure of implanting the product must not jeopardize the safety of the patient or surgeon/medical professional in any way. This secondary objective is
important in the consideration of both the patient and medical professional. It is vital that there is a reliable procedure for implanting a biocompatible meniscus replacement without risking the sterility of the product. This procedure must also be safe for the medical professional. For these reasons, this is why implantable ranks third among the secondary objectives for safety.

4. **Manufacturability**: The product must also be safe to manufacture. The procedure to create this product must not be unsafe to the designers and manufacturers. Also, the materials that are being handled must be safe for human handling. The major function of this device is to provide a patient with a functional meniscus replacement, however, it is still important for the product to be developed without posing health risks to the designers and manufacturers. Therefore, manufacturability safety is an important objective to be considered after meeting the objectives listed above.

5. **Meet FDA Regulations**: When the product is ready to be used clinically, it must meet FDA Regulations in order to allow secondary sources to use the product in practice. If the FDA does not approve the product it will not be able to be used for any medical applications. If the secondary objectives for safety are satisfied then the product should pass FDA approval, which is why the FDA approval is the lowest priority secondary objective for the safety of the product.

*Customizable*

It is essential that the device is designed in a way that can be customized in the future. This is important for implantation, so that the scaffold can be patient specific. Each patient has a meniscus with specific morphology that helps provide all the proper functionality. Therefore, it is important that this product have the ability to be customized to help provide the full
functionality of a meniscal implant to each patient. Below are the secondary objectives for customizable:

1. **Cell alignment control**: The control of cell and fiber alignment is an important and novel aspect of this project. All people have the same general fiber orientation in their meniscus. It is important that the specific size, length, and orientation of the fibers is achieved to help allow for these scaffolds to be customizable for each patient. Cell and fiber alignment is a major focus for this project, which is why it is the most important secondary objective beneath customizable. This can be achieved through using an electrospinning technology to customize the fibers that are developed for the scaffold.

2. **Design any shape**: The control over scaffold shape and morphology is also very important to the customizability of a replacement meniscus. The ability to control the shape of the scaffold is a key aspect of making each scaffold patient specific. This can be done with a combination of techniques. The first technique would be to use computer aided design to mimic the meniscus of a patient from MRI images. Creating that specific shape could then be complete through 3D printing or electrospinning. This is ranked second behind cell alignment because it is not the main aspect of this project but is still very important for making each scaffold patient specific.

3. **Cell differentiation control**: The use of IPCs allows for the differentiation into the cells of the patient. This is important for the use of this product in the long run. For this project, it is not as vital that patient specific cells are developed but it is recognized that this is important for the customizability of the product in the future.
User Friendly

This product must be easy to use for the stakeholders who are directly working with it. If the device is challenging to work with for stakeholders, it will inhibit the ability for the product to be used properly and function properly. The secondary objectives for ease of use are as follows:

1. **Researchers**: The product must also be easy for researchers to use and test to accurately determine the functionality, strengths, and weaknesses of the device. Our main focus is for this project to be used for further research in developing a meniscal implant with proper fiber alignment. Therefore, this secondary objective is most important for the ease of use objective.

2. **Medical Professionals**: This device must be easy for surgeons, doctors, and medical professionals to use when conducting a procedure on a patient to ensure proper implantation and functionality. The end goal is for this product to be used in clinical practice but not before further research is conducted, which is why it ranks behind the researchers.

Compatible

This product needs to be compatible in a couple different ways. If the product cannot cohesively fill the current needs, than it will not work as proof of concept or research tool.

1. **Proper materials**: The product must have the proper materials necessary for function. The materials of the design be manufactured as well as having a product that is biocompatible.

2. **Different lab set-ups**: The main user of this product is thought to be researchers. The design must integrate into current lab equipment and protocols. If the base clientele can
use a product without requiring a lot of additional expenses, the product will be more successful.

**Reproducible**

Reproducibility is important for proof of concept since it is the objective meaning that all results from the project need to be able to be repeated by others. All aspects of the project need to be able to be reproduced, from the manufacturing process to experimental procedures.

1. **Manufacturability**: The design must be able to be manufactured, for the project and in the future. Relating to the project, the design must be made out of resources readily available to the team or available for purchase. The design should incorporate standard measurements for easier machining. Manufacturing standards should be taken into account.

2. **Design Validation**: The design and product should be validated to truly be a proof of concept. All validation should be able to be repeated. The design needs to create similar results to be a dependable research tool.

![Objective tree diagram with objectives and sub objectives](image-url)

*Figure 7: Objective tree diagram with objectives and sub objectives*
The team ranked the objectives and each were then compared to each other and priorities were determined.

**Pairwise Comparison Chart**

<table>
<thead>
<tr>
<th></th>
<th>Safe</th>
<th>User Friendly</th>
<th>Easily Manufacturable</th>
<th>Customizable</th>
<th>Compatible</th>
<th>Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Safe</td>
<td>X</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>User Friendly</td>
<td>0</td>
<td>X</td>
<td>0.5</td>
<td>0</td>
<td>0</td>
<td>0.5</td>
</tr>
<tr>
<td>Reproducible</td>
<td>0</td>
<td>0.5</td>
<td>X</td>
<td>0</td>
<td>0</td>
<td>0.5</td>
</tr>
<tr>
<td>Customizable</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>X</td>
<td>0.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Compatible</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.5</td>
<td>X</td>
<td>3.5</td>
</tr>
</tbody>
</table>

*Table 2: Pairwise Comparison Chart*

From these rankings, customizable and compatibility were determined to be the highest priority. This is because for compatible, the device must be biocompatible and support cell life. Equally important, the scaffold needed to be customizable to mimic varying tissue structures in the body. The next most important objective is that the device must be safe. The next two objectives are that the device must be user friendly and reproducible. Reproducibility is important for proof of concept. Lastly, the device must be easy to use so that researchers won’t have to invest too much.

**Functions and Means**

The team compiled a list of functions that were most critical for this design to achieve: safe, user friendly, manufacturable, customizable, compatible. Each function and corresponding means is discussed in detail below, with an explanation of which means were chosen as a design solution. Table 3 illustrates the design functions and means.
<table>
<thead>
<tr>
<th>Function</th>
<th>Means</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scaffold Creation</td>
<td>3D Printing</td>
</tr>
<tr>
<td>Fiber Collection</td>
<td>Pin Matrix Plate</td>
</tr>
<tr>
<td>Customizable Orientations</td>
<td>Soldered Rows</td>
</tr>
<tr>
<td>Fiber Removal</td>
<td>Forceps</td>
</tr>
</tbody>
</table>

Table 3: Function and means chart with chosen mean in bold

*Scaffold Creation*

The team was tasked with creating a three-dimensional tissue scaffold. This was the first function of the design that needed to be addressed and arguably the most important since the chosen mean influenced the rest of the functions for the design. There were two means available to the team.

1. **3D printing:** The 3D printer available could print SolidWorks designs to a resolution of \( +0.10\text{mm} \) with a precision of 11 microns. These were limiting factors for how precise a scaffold could be printed. In addition, the printer could only print two materials; polyvinyl alcohol (PVA) and polylactic acid (PLA). PVA is a biocompatible material that cells can adhere to. However, it degrades quickly and swells when exposed to cell media.

2. **Electrospinning:** The electrospinning machine in the Jain lab was a means available to the team. The three main components of the machine, the voltage source, syringe pump, and syringe tip, could be interchanged or adjusted. The voltage source could supply over 30kV of charge. The syringe pump flow rate was adjustable and syringe tips of different diameters were available. The electrospinning machine could be used with any polymer.
that would dissolve in water or organic solvent. Lastly, the electrospinning process could create random fibers with nanometer diameters.

The team used the means of electrospinning due to the higher number of material choices and the smaller fiber diameter. The problem to overcome with electrospinning was the random fiber generation.

*Fiber Collection*

The next function of the design involved the need of controlling how the nanofibers aligned. Randomly aligned fibers do not mimic native tissue. Our design needed to control how the nanofibers aligned so the 3D scaffold better represented tissues in the body.

1. **Pin Matrix Plate**: A pin matrix plate collects charged nanofibers on the tips of grounded metallic needles. A collection plate with only specific needles or rows of needles grounded may control fiber alignment. This would require a very fine pin matrix to achieve curvature at the nano-level.

2. **Magnets**: Magnets with polar charges may be placed on either side of the syringe tip. When the polymer extruded down from the tip, it would create varying degrees of straight and curved fibers depending on the proximity to the magnets. This method would have less control on fiber orientation.

3. **Rotating Drum**: The fibers can be extruded onto a flat collection plate randomly aligned. The fibers are then pulled onto a rotating drum to become aligned in a curved fashion. This would require very small drums to achieve curvature at the nano-level.

The team decided on using the pin matrix collection plate to fulfill the function of collecting nanofibers.
Customizable Orientations

To further the versatility of the design and scaffold, the team investigated different means to customize fiber alignment.

1. **Soldered rows**: The bottom of the metallic pin matrix could be soldered together to form the alignment desired by the user. This was a permanent solution for grounding the pins. The user would need another collection plate to create an alternative fiber alignment.

2. **Weaved wire**: The backs of the pins could be attached with wire. This would not be a permanent solution but it would be difficult to form a wire to a fine pin matrix without accidentally charging pins outside the desired alignment.

3. **Brass Caps**: Brass caps could connect the backs of the metallic pins to the voltage source through wires. The alignment could be altered by the user and would only charge the desired pins.

The team went with using the brass caps to customize the fiber collection due to the accuracy and ease of use of the design.

Fiber Removal

The nanofibers need to be removed from the collection plate after electrospinning. The fibers are very delicate. Moving or tugging on the fibers can warp or destroy the customized alignment. The team thought of three means to remove fibers and maintain integrity.

1. **Forceps**: One means of removing the fibers could be with forceps. The fibers could collect onto the pin tips and then carefully removing with forceps. The fibers could possibly stick to the needle tips and lose the alignment. The fibers could be taken off the collection plate and then placed into a petri dish.
2. **Parafilm**: Parafilm could be placed on the needle tips of the collection plate and then fibers could collect onto the parafilm. The removal of parafilm from the plate would be easier but would add another step into the fiber removal process. The fibers would need to come off the parafilm before being placed into the petri dish due to the toxicity of the parafilm to cell culture.

3. **Fine Comb**: The fibers could collect directly onto the needles of the collection plate. A fine comb could be slipped into the pin matrix and drawn up to collect the fibers. This would require an extra tool to be manufactured to the fine dimensions of the needles and would add an extra step to the fiber removal process. The fibers would need to be removed from the comb before being placed on a petri dish.

The team chose to remove the fibers with parafilm as the means of fiber removal. The parafilm was readily available and maintained most of the alignment of the scaffold.

**Specifications**

The team needed to make a collection plate that could withstand the operational ranges for electrospinning which can be found in Table 4.
<table>
<thead>
<tr>
<th>Electrospinning Operation</th>
<th>Range</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Voltage</td>
<td>10-30 kV</td>
<td>higher voltage results in finer fibers and increased material strength</td>
</tr>
<tr>
<td>Flow rate</td>
<td>&lt;0.5 mL/h</td>
<td>Slow to allow organic solvent to evaporate- beading occurs at higher flow rates</td>
</tr>
<tr>
<td>Needle to plate distance</td>
<td>10-20 cm</td>
<td>less mechanical strength at a further distance, too close then solvent can't dissolve in time</td>
</tr>
<tr>
<td>Polymer density</td>
<td>2-20 wt. %</td>
<td>dissolved in water or organic solvent</td>
</tr>
<tr>
<td>Fiber diameter</td>
<td>100-200 μm</td>
<td>need fibers to be a smaller diameter than limit to allow tissue perfusion</td>
</tr>
</tbody>
</table>

Table 4: Operational ranges for electrospinning

The material of the collection plate needs to be able to withstand high voltages. The base must be a non-conductive material as to not attract charged fibers. The pin matrix must be conductive and able to pass voltages up to 30kV. The connective backing for the pins must be conductive with a high voltage capacity as well.

Constraints

The constraints were divided into two categories; project and device constraints. Project constraints are limitations due to the nature of the MQP and academic year. Device constraints address limitations of the product design that must be considered for a successful outcome.

Project

Cell proliferation and differentiation: The ATDC5 cell line requires proliferation and a 3-week differentiation period in the scaffold. Time must be well managed since the academic year is 28 weeks long. The limited budget is also a project constraint.
Device

The product needs to be designed within the WPI machine shop's capabilities. The machine shop was the team's sole source for manufacturing. An example would be that the diameter of the pins must match a drill size within the machine shop. The device must also eliminate electrical hazards for operators.

3.3. Standard Design Requirements

One standard applicable to the team's project was manufacturing standards for designs created in SolidWorks. All drawings needed to be made with the SolidWorks feature for drafting standards for ISO. The collection plate design, if mass manufactured, would need to comply with ISO's standards for dimensional specificity.

Another industry standard applicable to the project involved cell seeding of the scaffold. The group needed to follow an industry approved methodology for counting cells in a high-density cell suspension. This was required to ensure the accuracy of number of cells seeded on the scaffold met industry standards. The team used a C-chip disposable hemocytometer. The dimensions of the hemocytometer grid were 0.3mm x 0.3mm. The cell count was done by the user, counting cells in four of the nine counting squares. This process is the least accurate during low cell counts or high cell counts with clumping.

The team also conducted a risk assessment for safety for the project. The findings of the assessment can be seen in Table 5.
<table>
<thead>
<tr>
<th>Hazard</th>
<th>Who can be harmed</th>
<th>Potential injury or damage</th>
<th>Risk Controls in place</th>
<th>Risk Assessment</th>
<th>Action to control risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrical Electrospinning Machine</td>
<td>Operators</td>
<td>Electrical shock</td>
<td>Plastic barrier</td>
<td>Moderate</td>
<td>Voltage off until barrier is closed</td>
</tr>
<tr>
<td>Pin Matrix collection plate</td>
<td>Operators</td>
<td>Puncture</td>
<td>Carrying case</td>
<td>Low</td>
<td>Contain plate exposure time. Transfer from carrying case to machine and back.</td>
</tr>
<tr>
<td>Chemicals</td>
<td>Operators</td>
<td>Inhalation of organic solvent, eye irritation</td>
<td>Handle chemicals in hood, gloves, safety glasses</td>
<td>Low</td>
<td>Follow procedures closely</td>
</tr>
<tr>
<td>Biohazard</td>
<td>Operators</td>
<td>Exposure to cells, chemicals</td>
<td>Gloves, handle in culture hood</td>
<td>Low</td>
<td>Follow procedures closely</td>
</tr>
</tbody>
</table>

Table 5: Risk Assessment for project

The team identified and assessed various hazards in the project, determining all could be well managed by following procedures.

3.4. Pathway to Revise Client Statement

From the initial client statement, we consistently modified it to what is now our current client statement:

*Meniscal tears and ruptures of the knee are very common injuries. The team will engineer a custom aligned 3D cartilage tissue scaffold to grow, ex vivo, replacement menisci.*

This client statement has been cut down to the basic client need and will likely be carried out with a combination of different construction techniques (electrospinning, 3D printing, etc.).
As it stands right now this client statement shows the ultimate deliverable within the scope of our project.

In the process of conducting background research, our team began making gradual modifications to our client statement based on our findings. Our first revision was redefining our final deliverable. Originally our statement was geared towards a “three-dimensional, biomorphic tissue scaffold”, which would closely model the physical properties of the meniscus. The team believed that creating a universal tissue scaffold would be equally as effective in proving the effectiveness of seeding and growing cells in ex vivo conditions. Once this condition can be proven on a broad scale, the model can be specialized to form a family of products for specific tissue constructs. Through further research in the current “gold standard” technology, we determined that there were already viable ongoing scaffold experiments that focused on the seeding, proliferation and differentiation of cells on biocompatible materials. After a reevaluation of our project goals we determined that a new, novel component was necessary to make our project stand out. Initially we believed this novel idea was to focus on a bi-zonal scaffold, which would closely model a natural meniscus. However after sitting down with both Professor Ambady, we determined the novel aspect of our project is now focused on the ability to create multiple cell alignments within a single scaffold. This is what led us to develop the revised client statement that our group presented above.

3.5. Project Approach

The team expected to hit deadlines for each term involving research, electrospinning, prototype creation, and scaffold creation. Cell culture was an aspect of the project that needed to be carried out throughout the duration of the project. The team needed to follow and achieve
these milestones for a successful project. The success of scaffold seeding directly depended on creating a final design that could achieve fiber collection and alignment.

A term was reserved for research to better the team's understanding of the project and how to find a solution. After conducting research, the team was able to create a revised client statement as well as set up objectives for the project. Overall, A term set up the team up to start prototyping and electrospinning the next term.

The technical aspect of the project began in B term. The team needed to manufacture the first prototype so electrospinning with the team's design could begin by the end of the term. This would confirm that the pin matrix was a design that could lead into customized scaffold development in the next term from a final design.

The final design needed to be determined by the beginning of C term. This would allow ample time for manufacturing of the collection plate as well as scaffold creation. Aligned scaffolds were created in this term to allow for cell seeding. The team wanted to observe cells on the scaffold for as long as possible to ensure design validation. To ensure the success of the yearlong project, the team created a Gantt chart that can be seen in the Appendix B.
4. DESIGN PROCESS

The team followed the engineering design process as highlighted in this chapter.

4.1. Needs Analysis

The functions of the collection plate could be divided into two categories; needs and wants. Needs are requirements or results that the final design must meet or create to be a success. Wants are attributes that can further the success of the final design but are not necessary. Table 6 illustrates the functional wants and needs of the project.

<table>
<thead>
<tr>
<th>Function</th>
<th>Want</th>
<th>Need</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attract and Collect Fibers</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Create Scaffold</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Ability to create fibers in customized alignments</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Accept high amounts of voltage safely</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Compatible with different electrospinning machines</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Fiber removal with perfect alignment</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Quick customization</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Fiber diameters small enough for nutrient perfusion</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Very fine pin matrix for increased alignment control</td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>

Table 6: The functional needs and wants for the project

The needs analysis was done with the client in mind; researchers. The collection plate must be compatible with different electrospinning setups. Some machines use gravity or syringe pumps. The design needed to be compatible with as many setups to be more versatile and appeal to as many research labs as possible. The collection plate must not exceed the dimensions of plastic barriers surrounding the electrospinning machine or extend too close to the syringe tip. The collection plate needed to safely be charged up to 30kV to be used during electrospinning.
The plate needed to attract and collect fibers long enough to create a scaffold with layers of fibers, around 1mm thick after running the electrospinning machine for the average test time of 20 minutes. To improve upon the electrospinning process, the fibers needed to be controlled so the fiber visually followed the alignment under 20X magnification. This alignment must be changed as the user desires. The wants for the project are desired but are not critical for a proof of concept design. The function needs can be found in Table 7.

<table>
<thead>
<tr>
<th>Function Needs</th>
<th>Mean</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attract and Collect Fibers</td>
<td>Pin Matrix</td>
<td>Metallic pins with diameter of drill bit in machine shop</td>
</tr>
<tr>
<td>Create Scaffold</td>
<td>Electrospinning</td>
<td>Fiber diameter of 500-600 μm</td>
</tr>
<tr>
<td>Ability to create fibers in customized alignments</td>
<td>Brass Caps</td>
<td>Diameter slightly larger than pins</td>
</tr>
<tr>
<td>Accept high amounts of voltage safely</td>
<td>Metallic pins</td>
<td>&lt;30kV</td>
</tr>
<tr>
<td>Compatible with different electrospinning machines</td>
<td>Collection plate design</td>
<td>&lt;5x5x5 in</td>
</tr>
<tr>
<td>Fiber removal</td>
<td>Parafilm</td>
<td>Slightly larger than dimensions of pin matrix unstretched</td>
</tr>
</tbody>
</table>

Table 7: The function needs and the corresponding means and specifications

4.2. Conceptual Designs

One initial conceptual design was to use magnetic fields to control and change fiber alignments. Previously, magnetic fields have been used to create straight aligned fibers. This was done by a process shown in Figure 8, with the collecting fibers being spun into the "b" region of the magnetic field. The team’s conceptual design was to use the "c" region of the magnetic field
to create curved fibers. However, the team determined that this would allow for limited control, and very limited customization. Because of this, the team moved on to further design ideas.

Figure 8: Magnetic Electrospinning (Yang et al., 2007)

4.3. Preliminary (Alternative) Designs

The team considered many alternative designs which are highlighted in this subchapter.

Initial Soldered Pins

Benefits

The goal of this initial design was to act as a proof-of-concept, which we demonstrated by producing aligned fibers on a small-scale with successful reproducibility. In performing this soldering the team was able to ensure that all necessary pins were connected to eliminate a potential confounding variable if breakages in the alignment were to occur.

Limitations

As the associated soldering requires permanent fixation of charged orientations, meaning that new plates would have to be produced for each desired fiber alignment. We next experienced restrictions in sizing and pin number as the copper-plated pins used were 0.75 inch, 14 gauge, leaving us with too few pins to create more complex orientations with the given 5x5
matrix. As the initial focus was on collection along each row, these pins were 5 mm apart whereas each row was separated by 6 mm, which could cause skewed results once we began developing angled shapes (Figure 9).

![Figure 9: Isometric and bottom views of Prototype 1](image)

**Insulating Cover**

*Benefits*

This design would allow only the exposed pins to have fibers collected upon, meaning that the team would be able to charge the entire plate instead of individual pins. Additionally, having multiple insulating covers would make transitioning between different fiber collection methods during the testing process fairly simple. This means that layers could be created within the same experiment, hopefully with mixed orientations.

*Limitations*

This would require developing additional shields for each desired fiber orientation, which our team determined to be the most inhibiting drawback. Also as fibers tend to collect on the
closest possible surface, which in this case could result in the collection along the cover, as the needles would not necessarily poke through the insulated layer (Figure 10).

**Figure 10**: Isometric view showing the collection plate with a shielded cover

**Conductive Metal Backing**

The conductive metal backing can be seen in Figure 11.

**Benefits**

Creating this conductive backing would allow for the charging of only the desired rows, causing fibers to be pulled towards these specific pins.

**Limitations**

This would require developing additional conductive backings for each desired charging orientation.
4.4. Feasibility Study/ Experiments

A series of feasibility studies and experiments were performed to ensure the engineering design project was progressing in the correct direction. These studies included learning and optimizing electrospinning, determining feasibility of the pin matrix collection plate method, testing the effects of parafilm on cells, and scaffold seeding differences on different cell culture plates.

Electrospinning is a very precise process that requires a lot of testing to optimize. A small-scale electrospinning setup, shown in Figure 12, was used to perform feasibility studies and experiments.

In order to set operation parameters of the electrospinning setup, the team used the most simple collection plate form that is commonly used, an aluminum foil flat collection plate. This provided a cheap, easily changeable, sure collection plate that would not change while other parameters were altered. While using this collection plate, the flow rate, voltage, polymer weight
percent by volume, and distance were altered. These values were initially set based on the literature review, then optimized for this teams setup. A summary of some of these tests can be seen in Table 8.

Figure 12: Electrospinning feasibility study setup showing the syringe pump, voltage supply, and collection plate.
<table>
<thead>
<tr>
<th>PVA wt. %</th>
<th>Distance needle to plate (mm)</th>
<th>Diameter (mm)</th>
<th>Extrusion rate (mL/hr)</th>
<th>Collection plate</th>
<th>Results/Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>30-80</td>
<td></td>
<td>1.1</td>
<td>foil</td>
<td>PVA stirred, no heat</td>
</tr>
<tr>
<td>20</td>
<td>50</td>
<td></td>
<td>0.1</td>
<td>foil</td>
<td>PVA stirred, heat. Fibers made for first time</td>
</tr>
<tr>
<td>30</td>
<td>50</td>
<td></td>
<td>0.1</td>
<td>foil</td>
<td>fibers but solution difficult to get in syringe</td>
</tr>
<tr>
<td>25</td>
<td>50</td>
<td></td>
<td>0.1</td>
<td>foil</td>
<td>created fibers, getting beads</td>
</tr>
<tr>
<td>25</td>
<td>50</td>
<td></td>
<td>0.05</td>
<td>foil</td>
<td>fibers</td>
</tr>
<tr>
<td>20</td>
<td>36.8</td>
<td>0.08</td>
<td>0.05</td>
<td>pins</td>
<td>fibers gathered on needle points</td>
</tr>
<tr>
<td>20</td>
<td>36.8</td>
<td>0.08</td>
<td>0.02</td>
<td>pins</td>
<td>fibers, collected on parafilm</td>
</tr>
<tr>
<td>20</td>
<td>36.8</td>
<td>0.08</td>
<td>0.01</td>
<td>pins</td>
<td>least beading, lots of fibers</td>
</tr>
</tbody>
</table>

Table 8: Electrospinning feasibility experiment parameters and observations.
In the end of the preliminary experiments, the team was able to create nanofibers on the collection plate as shown in Figure 13.

With the electrospinning parameters refined, the next step taken was to test the feasibility of the pin matrix system for collection of nanofibers. This system is not readily used, so a feasibility study was necessary. To do this, similar electrospinning parameters were used as determined in the preliminary electrospinning feasibility study, but the collection plate used was changed from flat aluminum foil to the prototype 1 pin matrix collection plate. Figure 14 shows the polymer fibers collected to the pin matrix collection plate of prototype 1.
Once the feasibility of the pin matrix system as a collection plate was determined, the ability of the collection plate to collect along a charged row was studied. When one row of pins was charged, the polymer fibers would collect and organize along those charged pins. Figure 15 shows the collection plate with polymer fibers aligned across two rows. This multi-row scaffold was achieved by collecting along one charged row, then switching the charged row and continuing to electrospin. This alignment shows the feasibility of the pin matrix system to collect fibers along a defined charged row.
4.5. Final Design Selections

The final design for the electrospinning collection plate can be seen in the Figure 16 below:

<table>
<thead>
<tr>
<th>Top View</th>
<th>Bottom View</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Top View Image" /></td>
<td><img src="image2.png" alt="Bottom View Image" /></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Isentropic View</th>
<th>Side View</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image3.png" alt="Isentropic View" /></td>
<td><img src="image4.png" alt="Side View Image" /></td>
</tr>
</tbody>
</table>

Figure 16: Images of our final prototype with leg extensions and brass cap fittings
This electrospinning collection plate pin matrix is made with a base insulating Delrin plastic with Stainless Steel minutien pins press fit into the plastic to make 10x15 pin matrix. These pins had a diameter of 0.4mm and a diameter at the pin tip of 0.03mm. This diameter allowed for a much finer matrix to be created to help foster more precise fiber orientations. The fine diameter at the tip of the pins was also advantageous as it concentrates the charge being applied from the voltage source at the very ends of the pins for precise fiber collection. The pins extended 25mm above the collection plate for fiber collection and 5mm on the back of the collection plate. The exposure of the pins on the backside of the collection plate allowed for customized charging of individual pins. The original pins were 38mm in length with a ball shaped head at the base of each pin. These pins were modified to a length of 33mm by removing the head of each pin. This was done to allow for the pins to be placed in the matrix 2mm apart without the heads on the pins colliding with one another. Removal of the head on the pins also allowed for a more effective method of charging individual pins. The dimensions of the collection plate in inches can be seen below (Figure 17):
The method of charging the pins on the backside of the collection plate was achieved through a brass cap method. Brass caps were drilled with holes on each end (Figure 18):

Figure 18: Shows the hole cut into each brass cap fitting
Each hole was equivalent to the diameter of the pins in the collection plate. One end of the cap was used for the charging of individual pins by being press fit onto any pin of choice. The other hole allowed for an electrical wire to be inserted and soldered into the hole to help achieve an electrical current to the caps (Figure 19):

![Figure 19: Brass cap fitting attached to soldered wire](image)

These caps allowed for any pin to be charged, which allowed for unique shapes and patterns of fiber collection to be achieved. The wires extending from each brass cap were weaved together at the ends. The weaving of the wires allowed for the alligator clip of the electrospinning unit to charge all of the brass caps at once (Figure 20):
For this research project, the brass caps were placed on the pins in a curved orientation to charge pins in this orientation and produce a curved PCL fiber scaffold.

Because the brass caps were longer than the legs of the collection plate, the collection plate was modified. The height of the collection plate needed to be increased to allow for clearance of the brass pins when the collection plate was taped to the rubber backing. 10 mm of Delrin plastic was added on two opposing sides of the collection plate to increase the height. Therefore, extending the length of the sides of the device allowed for the brass caps to be placed on the pins and the collection plate to be tapped to the rubber backing at the same time.
## Collection Plate Material Selection

<table>
<thead>
<tr>
<th>Material</th>
<th>Function</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Chosen</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVC</td>
<td>Collection plate base</td>
<td>~Insulating plastic</td>
<td>~Not easily manufactured ~Shrinking and swelling of holes during manufacturing</td>
<td>No</td>
</tr>
<tr>
<td>Delrin</td>
<td>Collection plate base</td>
<td>~Insulating plastic ~Easily manufactured</td>
<td>N/A</td>
<td>Yes</td>
</tr>
<tr>
<td>Stainless Steel Minutien Pins</td>
<td>Collection plate pins</td>
<td>~Decent electrical conductivity ~Small diameter ~Fine pin tip</td>
<td>~Not optimal electrical conductivity</td>
<td>Yes</td>
</tr>
<tr>
<td>Brass Cap</td>
<td>Pin charging</td>
<td>~Strong electrical conductivity ~Easily manufactured</td>
<td>N/A</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Table 9: Collection plate material selection analysis
The materials that were chosen to manufacture the collection plate were Delrin plastic for the base, the stainless steel minutien pins, and the brass caps. Although the stainless steel minutien pins did not provide the optimal ability to conduct electricity compared to other metals, the small diameter provided for stronger advantages. The diameter of the pins allowed for a fine matrix to be created, thus, improving the precision of fiber alignments. The fine diameter at the tip of the pins helped concentrate the voltage at a very specific point on the pins, which helped for organized fiber collection. The Delrin plastic was chosen over the PVC plastic because the Delrin plastic was easily manufactured in comparison to the PVC. When drilling the holes into the plastic to create the pin matrix, the PVC plastic heats due to friction with the drill and the holes expand to larger holes than desired and later shrink when the plastic cools. This behavior of the PVC does not allow for accurate manufacturing.

**Design Benefits vs. Drawbacks**

<table>
<thead>
<tr>
<th>Benefits</th>
<th>Drawbacks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Customized pin charging/fiber alignment</td>
<td>Difficult for user to manually apply brass caps for pin charging</td>
</tr>
<tr>
<td>Fine pin matrix for precise control of fiber alignment</td>
<td>Difficult fiber removal process</td>
</tr>
<tr>
<td>Reproducible scaffold creation</td>
<td></td>
</tr>
<tr>
<td>Compatible</td>
<td></td>
</tr>
<tr>
<td>Portable</td>
<td></td>
</tr>
</tbody>
</table>

*Table 10: Benefits and drawbacks table for our final prototype*
This collection plate design met the main requirements that were desired for the goal of electrospinning customizable polymer fibers. This collection plate and brass cap method allows for any pin orientation to be charged, thus, allowing for any fiber alignment to be achieved. This collection plate also allowed for a reproducible procedure when creating polymer scaffolds. It also is a compatible design that can be used with any electrospinning unit and is easily transportable. Areas of improvement include the ability of the fibers to be removed from the collection plate in a more effective manner. Lastly, because the brass caps were a press fit to the pins and the pins were also a press fit into the collection plate base, it was difficult to manually apply the brass caps to the pins for charging as some pins would become dislodged at times. Overall, the advantages this collection plate provided outweighed the drawbacks.

**Preliminary Experimentation**

Based on preliminary experimental results, modifications to the project were made. The original electrospinning unit that was being constructed did not allow for voltages above 10kV to be reached (Figure 21):

![Initial electrospinning setup from A Term](image)

*Figure 21: Initial electrospinning setup from A Term*
From research, this voltage was not high enough for effective electrospinning testing. The team researched the availability of a sufficient voltage source but could not find one within a range that could be supported by the budget. Therefore, the team was granted access to use the electrospinning unit in Professor Anjana Jain’s Lab. The voltage source in the Jain Lab had the capability to reach voltages of 30kV (Figure 22):

![Electrospinning setup from Jain Lab utilized for all testing B-D Term](image)

Initial electrospinning testing was being completed with polyvinyl alcohol (PVA) solution. The PVA solution was dissolved in distilled H$_2$O and experimented at 10, 15, 20, and 30 weight percent. PVA was primarily used as an electrospinning material to help with the proof of principle that straight and curved aligned fibers could be achieved. Advantages of using this material for initial testing was that it was readily available, did not cost money, and was simple to dissolve into solution. These advantages made PVA a great initial polymer to test the proof of electrospinning varying fiber orientations with. However, the group moved from PVA to PCL
once it became time to start seeding the polymer scaffolds with cells. This is because PVA is a rapidly degrading polymer when in contact with cell media. Therefore, PCL became the primary polymer used when electrospinning scaffolds to seed cells with. The two major advantages it provided over PVA was its structural integrity and its ability to promote cell adhesion to the scaffold.

The early stages of electrospinning were also completed with Prototype 1. This prototype was a proof of principle model as well. The goal with this electrospinning collection plate was to prove that polymer fibers could be collected in a straight aligned fashion. Once straight fiber alignment had been achieved, the final collection plate pin matrix was designed to allow for customizable fiber orientations. The initial prototype proved the concept that fiber alignment could be controlled with the pin matrix method. This allowed the group to move forward with a more advanced collection plate as a final design that promoted customizable fiber alignment using the pin matrix method in a more refined manner.
5. DESIGN VERIFICATION

This project used electrospinning technology to create customizable polymer fiber alignment. A curved fiber orientation was desirable to mimic the fibers that exist within the meniscus. The curved fibers were then taken and seeded with ATDC5 cells and a GFP expressing cell line to prove that cells could attach to the scaffold.

5.1. Electrospinning

For the final collection plate design, the main goal was to use electrospinning technology to create custom aligned fiber scaffolds to be used for cell seeding to prove that native tissues orientations within the body can be mimicked. Through preliminary testing with Prototype 1, the group was able to prove that straight aligned fibers could be achieved with a pin matrix method. A final collection plate was designed and tested with a 10% PCL polymer solution dissolved in HFIP. This formulation was proven to be successful in previous research as the team would make batches of PCL solution that could be used for up to three weeks before needing a fresh PCL solution. The PCL formulation for electrospinning can be seen in the table below:

<table>
<thead>
<tr>
<th>PCL Mass</th>
<th>HFIP Volume</th>
<th>Dissolving time on magnetic stir plate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5g</td>
<td>5.0ml</td>
<td>24 hours</td>
</tr>
</tbody>
</table>

Table 11: 10 % weight PCL Electrospinning Solution

With the final collection plate design, the initial tests were conducted to produce straight aligned fibers to validate that the pin matrix design and pin charging method could support
controlled fiber alignment. An image of the straight fiber alignment can be seen below (Figure 23):

![Figure 23: Straight fiber collection on parafilm using final prototype](image)

Once straight aligned fibers were achieved, curved PCL fibers were then created. After extensive experimentation, the optimal parameters for creating curved PCL fiber scaffolds are featured in Table 12 below:

<table>
<thead>
<tr>
<th>Test duration</th>
<th>Voltage range</th>
<th>Flowrate</th>
<th>Syringe diameter</th>
<th>Distance from syringe tip to pin matrix tips</th>
<th>10% PCL solution volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-20 minutes</td>
<td>8-12kV</td>
<td>0.15ml/hr</td>
<td>12.06mm</td>
<td>10cm</td>
<td>0.5-1.0ml</td>
</tr>
</tbody>
</table>

Table 12: Electrospinning Test Parameters

Curved fibers were created to mimic the native fiber alignment of meniscal circumferential fibers. The electrospinning procedure was able to produce these curved scaffolds
consistently. This test procedure is repeatable as the results were mimicked on numerous occasions. Images of the scaffolds can be seen below (Figure 24):

![Figure 24: Successful curved alignment along charged brass caps with final prototype](image)

The electrospinning process for creating curved fibers worked very effectively at times but due to certain factors, the quality of electrospinning could be inhibited. Weather conditions had an impact on the success of electrospinning curved scaffolds. When the weather was abnormally dry, internal static was created and often caused fiber collection to be much more scattered and random or for the fibers to collect in a non-optimal location. On the contrary, if the weather was abnormally humid, the fibers tended to be clumpier and cause dripping at the syringe tip. Weather conditions caused fluctuations in the results depending on the humidity levels in the air. Improper cleaning of the syringe tip also inhibited the electrospinning of PCL scaffolds. The PCL solution could solidify in the syringe tip if the tip was not cleaned between each test, causing difficulty for the solution to be extruded. The images below indicate a proper Taylor cone, a clumpy solution, and random fiber collection due to internal static (Figure 25):
5.2. Cell Seeding

Cell seeding of the scaffold could only begin once electrospinning produced results with the collection plate. The team conducted a population doubling study with a cell line of mouse chondrocytes, ATDC5. The cell culture procedure followed can be found in Appendix A. The numerical results of the study can be seen in Table 13 and visually in Figure 26:
<table>
<thead>
<tr>
<th>Days</th>
<th>Starting Cell #</th>
<th>Ending Cell #</th>
<th>PDL</th>
<th>Cumulative PDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.00E+05</td>
<td></td>
<td>1.00E+05</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1.00E+05</td>
<td>3.85E+06</td>
<td>5.26E+00</td>
<td>5.26</td>
</tr>
<tr>
<td>12</td>
<td>1.00E+05</td>
<td>4.60E+06</td>
<td>5.52E+00</td>
<td>10.78</td>
</tr>
<tr>
<td>18</td>
<td>1.00E+05</td>
<td>2.45E+06</td>
<td>4.61E+00</td>
<td>15.40</td>
</tr>
</tbody>
</table>

Table 13: Population doubling study results

The population doubling study allowed the team to plan out sub culturing and timing for future experiments. The team also practiced cell freezing and thawing. These procedures can be found in Appendix A.

The team met with members of the Gaudette lab as well as with Alex Beliveau. Alex worked with seeding electrospun PCL and the team created a scaffold seeding protocol based on his experiences.
Once scaffolds of PCL were being created with prototype 1, cell seeding began. ATDC5 were seeded with the protocols found in Chapter 6. The cells were difficult to visualize on the scaffold. To increase visibility, a GFP expressing variant of breast cancer cell line MDA-MB-231 were used. The scaffolds were seeded at a density of 250,000 cells per mL and 750,000 cells per scaffold. After seeding, the cells were imaged for three weeks. Below are the GFP expressing cells seeded onto PCL scaffolds (Figure 27).

<table>
<thead>
<tr>
<th>Days</th>
<th>Brightfield, 5X</th>
<th>FITC, 5X</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Brightfield, 5X</td>
<td>FITC, 5X</td>
</tr>
<tr>
<td>2</td>
<td>Brightfield, 10X</td>
<td>FITC, 10X, 18ms exposure</td>
</tr>
<tr>
<td></td>
<td>Brightfield, 20X</td>
<td>FITC, 20X, 18ms exposure</td>
</tr>
<tr>
<td>6</td>
<td>Brightfield, 10X</td>
<td>FITC, 10X</td>
</tr>
<tr>
<td>---</td>
<td>----------------</td>
<td>------------</td>
</tr>
<tr>
<td>7</td>
<td>Brightfield, 10X</td>
<td>FITC, 10X, 60ms exposure</td>
</tr>
<tr>
<td>8</td>
<td>FITC/Hoechst, 20X</td>
<td>FITC, 20X</td>
</tr>
</tbody>
</table>

Figure 27: Images taken daily of PCL scaffolds seeded with GFP expressing cells

To determine PCL degradation time, one scaffold remained seeded in media for a month. The degradation of the PCL could be seen with the naked eye after two and a half weeks. At this time point, the cells were no longer clearly visible, and appeared to be dying.
6. FINAL DESIGN AND VERIFICATION

Based on the battery of tests that the team performed using the electrospinning setup we determined that our device was successful as we proved the ability to control the fiber alignment along multiple orientations with the brass cap grounding method as well as meeting each of our objectives as described below.

6.1. Objective Verification

Verification of each of our previously described objectives are outlined in this subchapter.

Safe

This objective focused on the ensuring that the designed collection plate would not remain active if the operator touches said plate after the completion of an electrospinning cycle. The safety of the electrospinning operation was established with a two-tiered approach: (1) establishing that the voltage supply was shut off before attempting to touch the collection plate and (2) the plastic enclosure, which prevented the user from accidently touching any charged portion of the electrospinning setup during the fiber collection process.

User Friendly

Regarding this objective, the team achieved the desired function for the proof-of-concept and has subsequently set in place more refined guidelines to be used in future prototypes given more time and department funding to produce. Specifically, this entails a simplification of the conduction step that currently consists of installing each individual pin with a conductive brass cap to ensure connection with the other pins for a desired orientation. Refinement of this process will allow for easier transition between various fiber alignment shapes to better model the conditions present in cartilage/tissue scaffolds. In relation to portability, our proof-of-concept
represents a fairly mobile device with the small restriction that the brass caps may be subject to shifting depending on the nature in which they are handled as each is installed in a double press-fit fashion. While there are a fair amount of parts due to the fine pin matrix, these will be attached during the manufacturing process meaning that the only additional part to be attached is the cluster of conductive brass caps.

**Manufacturability**

During the development and manufacturing process, the team modified segments of the final collection plate design to best fit the standard drill bit sizes available within the Goddard Hall machine shop. Given this restriction, we narrowed our search down to pins of 0.4 mm diameter, equivalent to an available drill bit size of 0.016 inches. While this modification slightly limited our possible pin sizes, the varying accessible drill bit sizes offer a wide range of available pin diameters that hardly limited our desired sizing requirements. This prototype was produced with the help of Tom Partington, the Goddard Hall machine shop Lab Manager, who developed them by hand, cut from a block of polyoxymethylene (Delrin). In the future if predetermined design specifications are established for mass production, the development process could be streamlined and automated to enhance the efficiency and precision of each plate design.

**Customizable**

Within the scope of our project, the customizability of our design was focused on the ability to adjust to different charged pin orientations. In this respect, our final prototype has succeeded in producing all possible orientations when including between 1-21 pins as the team prepared, wired and soldered a total of 21 brass cap fittings for the purpose of our testing. This customizability comes with the stipulation that there is a small margin between connected pins as
the fibers tend to seek out the closest possible collection areas in regards to the charged collection plate sections, thus inhibiting large jumps from one charged pin to another.

**Electrospinning Compatible**

Referencing the electrospinning syringe pump setup, our testing has proven that our collection plate method is viable with this electrospinning approach, whereas the gravity-induced pump has yet to be determined but based on our research, the gravity-induced pump collection can be controlled based on the collection plate angle, which is comparable to modifications in the flow rate of the polymer solution in syringe pump induced electrospinning (Thoppey 2010). Additionally, our team protected against unnecessary confounding variables by confirming that the materials used to create the collection plate, not including the conductive pins, were insulating and would not misdirect the conduction to non-metallic portions of the collection plate.

**6.2. Final Prototype Experimental Methods**

Once the team prepared their final collection plate for electrospinning tests, the initial tests were performed using polyvinyl alcohol (PVA) to eliminate possible confounding variables. Once these tests were deemed successful, the team shifted their focus to polycaprolactone (PCL) due to its higher biocompatibility to promote cell adhesion and survival.

Once this polymer change was made, the first three rounds of testing were run under the following conditions:

*Solution of 10% PCL (w/v), Syringe to plate distance of 15 cm, with a fluctuating voltage between 10-15 kV and tests were run for between 10 – 20 minutes depending on fiber control.*

After meeting with Alex Beliveau who had previous experience spinning with PCL, he recommended moving the plate closer to the syringe tip to inhibit random collection and testing
at lower voltages to allow for more controlled fiber collection. After this meeting the testing conditions were adjusted to our final specifications of:

*Solution of 10% wt. PCL (w/v), Syringe to plate distance of 10 cm, with a lower voltage range of 8-10 kV and tests were run between 10 – 15 minutes.*

6.3. Electrospinning Protocol

The following procedure describes the process to electrospin customized Polycaprolactone (PCL) fibers, specifically in a curved orientation.

**PCL Solution Preparation**

(10% weight PCL in 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) (Figure 28))

![PCL and HFIP Solution used for polymer dissolution](Image)

1. Obtain 0.5g of PCL by weighing the PCL in a weigh boat on a scale. The scale should be zeroed with the weigh boat on the scale before the PCL mass is measured.

2. Place the 0.5g of PCL and a magnetic stir bar into a plastic capped vial (Figure 29):
3. Bring the HFIP bottle, a stir plate, tape, pipetter, 5ml pipette tip, and the vial with 0.5g of PCL to a sterile lab hood.

4. In the hood, remove the cap from the HFIP bottle and extrude 5ml of the solvent. Place this 5ml of HFIP into the vial with 0.5g PCL and the magnetic stir bar.

5. The plastic capped vial should now have a 10% PCL weight solution in HFIP.

6. Cap the vial loosely (in order to ensure pressure buildup does not occur) and place the vial onto the magnetic stir plate. Tape the vial onto the stir plate to secure it to the plate as seen in the image below (Figure 30):
7. Turn on the magnetic stir plate to allow for the solution to be mixed. If the stir bar is not stirring properly, remove the cap from the vial and stir the solution manually with a sterile rod to dislodge the stir bar. Once the stir bar is unstuck, place the vial back onto the stir plate with the cap on lightly and tapped to the stir plate.

8. Label the vial with the project name, date, and weight percent solution that has been made.

9. Allow for the solution to mix for 24 hours before using to electrospin.

10. Each PCL solution that is created has a shelf life of 2-3 weeks.

11. When the PCL solution has been mixing for 24 hours, remove the vial from the stir plate and cap the solution tightly. Use parafilm to cover the cap and seal the vial from allowing the solvent in the solution to evaporate (Figure 31).
Figure 31: Mixed PCL solution covered with parafilm to prevent evaporation

12. Store the solution at room temperature.

**Controlled Fiber Alignment Electrospinning Procedure**

1. Obtain a 5ml syringe, 22-gauge stainless steel syringe tip, and 10% PCL solution.

2. In a sterile lab hood, extract 0.5 to 1.0ml of solution into the syringe through the stainless steel syringe tip (if the solution cannot be extracted through the syringe tip any longer, it has become too thick and is no longer suitable for electrospinning). Cap the PCL solution tightly and seal the cap with parafilm to minimize evaporation of the HFIP. Store the vial at room temperature.

3. Remove all air bubbles from the syringe and syringe tip.

4. Obtain a syringe pump and place it into the electrospinning unit. Turn on the syringe pump and set the syringe diameter to 12.06mm and the flowrate to 0.15ml/hr. The flow rate can be adjusted to a faster flowrate if the polymer is not being drawn from the syringe tip or adjusted to a slower flowrate if the polymer is dripping from the syringe tip.
5. Place the syringe with the PCL solution on the syringe pump and secure it into place with the syringe placed as forward as possible, the top clamp tightened over the syringe, and the moving plate in contact with the back of the syringe (Figure 32):

![Figure 32: Syringe with PCL secured in the injection pump holder](image)

6. Obtain the pin matrix collection plate and on the back of the collection plate, place the brass caps onto the pins that desired for charging. For a curved fiber collection, the brass cap orientation should be similar to Figure 33 below:

![Figure 33: Final collection plate set up with curved charging](image)
7. Using electrical tape, cover the other brass caps that are not being used to charge the collection plate.

8. Obtain a pair of scissors and cut a 2x2 inch piece of parafilm. Place the parafilm onto the collection plate pin matrix so that the tips of the pins are exposed. A flat-head stainless steel rod can be used to assist in applying the parafilm over the collection plate to expose the heads of the pins through the parafilm (Figure 34):

![Figure 34: SS rod used to lay parafilm onto pin tips](image)

9. Using electrical tape, cover the collection plate onto the rubber backing so that the pin matrix is at the same height as the syringe tip.

10. Place the collection plate 10cm from the syringe tip measuring the distance from the end of the syringe tip to the tips of the pins on the collection plate.
11. Attach the positive lead from the voltage source (the green alligator clip) to the syringe tip and attach the negative lead (the yellow alligator clip) to wire endings of the brass caps (Figure 35):

![Figure 35: Shows the alligator clips grounding the plate and charging the syringe needle](image)

12. Start the syringe pump.

13. Close the door to the electrospinning unit. Do not ever have the door open or any body parts close to the electrospinning unit while the voltage source is on.

14. Turn on the voltage source and adjust the voltage to 10kV (Figure 36). The voltage can be adjusted between 8-12kV during the test if needed. If the polymer is dripping from the syringe tip, increase the voltage that is being applied. If the polymer is not visible from the syringe tip, decrease the voltage until a proper Taylor Cone is achieved.
15. Run the test for 15-20 minutes or until the desired scaffold thickness has been created. The test/voltage may need to be stopped during the duration of the test to remove excess fibers that are inhibiting proper fiber collection. Use chem-wipes to remove any of these excess fibers.

16. When the test is complete, turn off the voltage source. Remove the alligator clips from the syringe tip and the collection plate. If another test is going to be run, clean the syringe tip out with acetone.

17. Carefully remove the parafilm from the collection plate pin matrix so that the fiber orientation is preserved. Remove the parafilm manually with two hands.

18. Label the parafilm with the test number, the date, and the polymer solution that the fibers were created from.

19. Place the scaffold on the parafilm in a container and store it at room temperature for 24 hours.
20. Put all materials back where they were stored. Clean any excess fibers off of the collection plate pin matrix and using 70% isopropanol clean the rubber backing used to hold the collection plate.

6.4. Scaffold Seeding Procedures

This subchapter highlights the scaffold removal and scaffold seeding procedures used.

Scaffold Removal Procedure

1. Obtain forceps and non-tissue culture dish.
2. Remove scaffold and parafilm from container after a minimum of 24 hours.
3. On lab bench, carefully remove scaffold from parafilm with forceps starting at aligned row and lifting towards edge of the parafilm.
4. Place scaffold into open petri dish.
5. Place lid on petri dish. Avoid dragging or rubbing dish as to not create static electricity.

Scaffold Seeding Procedure

1. Place petri dish with scaffold into UV sterilizer. Leave lid on petri dish.
2. Turn on sterilizer by flipping bottom button to on position.
3. Expose scaffold and dish to UV light for 30 minutes.
4. During sterilization time, follow cell culture procedure steps 2-15 with cells in incubator.
5. Re-suspend cells in sufficient amount of media to create a cell suspension with a density of 250,000 cells/mL.
6. Remove petri dish from sterilizer and bring to culture hood.
7. Clean dish with 70% isopropanol and place in hood.
8. Fill 10mL pipette with cell suspension.

9. Lift petri dish lid up just enough to pipette cell suspension on to the scaffold. Do not remove lid entirely or blowing air will interfere with scaffold alignment.

10. Pipette until scaffold is entirely covered with suspension. Do not cover entire bottom of dish and pipette only where scaffold is present.

11. Count number of drops to be able to calculate total cell count.

12. Place lid on petri dish.

13. Gently move dish to incubator. Avoid moving scaffold in dish or displacing cell suspension from scaffold.

14. After six hours, pipette media into sides of dish. Entire bottom of dish should now be covered.

6.5. Validation process methods

The regulations are very stringent for devices used for medical purposes. However, the designed collection plate will currently only be used for research purposes, so the regulations are not as strict. However, there are still engineering standards that would need to be followed for manufacturing and marketing the designed collection plate.

One resource used to find standards to follow is www.nssn.org/search/IntelSearch.aspx which is a search engine provided by American National Standards Institute (ANSI). Below is a list of some standards that would need to be considered to manufacture and market the developed collection plate design. One standard to highlight would be the ISO 9000 series, which define, establish, and maintain a quality assurance system for manufacturing. Another highlight would
be the family of standards ISO 14000, which works to help organizations decrease their negative effect on the environment and increase compliance with laws and regulations.

- ISO 9000 – Quality standard
- ISO 14000 – International Environmental Management Guideline (standard) - product life cycle and sustainability standard for business, industry, and engineering design
- ANSI/ASME Y14.1 and Y14.5 – International drafting standards for engineering drawings
- ANSI B11.20-2004 (R2015) -- Safety Requirements for Integrated Manufacturing Systems
- ISO 1101 – Geometric Dimension and Tolerancing Standard
- IEC 60060 Ed. 3.0 b:2010 – High-voltage test techniques

6.6. Effects and Considerations

The results of this project can have many effects, and there are many factors for the team to consider. The following list was compiled to show the thoughts behind some considerations of the potential effects of this project, including economics, environment, societal, political, ethical, health/safety, manufacturability, and sustainability.

1. Economics: This project has the ability to greatly affect the economics of the nation. One opportunity for this device to affect economics is if it progresses to the point where a tissue-engineered meniscus can be implanted into patients. This would have a great
impact on economics, as meniscus injury is a four billion dollar market. In the short term, this device has the ability to affect the economics on a smaller scale. Research labs around the world are using scaffolds for a wide variety of purposes. The desire to make tissue engineering more biomimetic persists, and this projects device provides just that. If research labs want to use or make customized scaffolds, this device can be used to make the custom scaffolds then sold to the lab or the lab could buy the collection plate directly and use it in the lab to create their own custom scaffolds.

2. **Environmental impact:** The project impacts the environment by using synthetic polymers and organic solvents. The new collection plate design required less solvent and solute due to the higher level of control over the polymer extrusion. The level of waste reduced and so did the polymer and organic solvent needed initially. Use of organic solvents impacts the environment as well. Organic solvents release volatile compounds into the atmosphere. On a large industrial scale, the compounds can create smog. The organic solvent HFIP had a half-life of one to seven days in the atmosphere and one to five days in water.

3. **Societal influence:** This technology and this product specifically have the ability to influence many lives in a positive way. Meniscus injuries are very common in athletes and ordinary people. These injuries often can be detrimental to the quality of life of the individual who has sustained the injury. There is currently a gap in treating significant meniscal tears to help patients reach full health again due to the avascular nature of the meniscus. Customizable fiber orientations in a curved alignment that mimics the fiber alignment within a meniscus could be used to support the growth of patient specific cells. This technology could significantly advance how meniscal tears are treated. Mimicking
the fiber alignment of a meniscus and seeding the artificial scaffold with patient specific cells could potentially allow for the most advanced meniscal replacements to this day. The functionality of the replacement meniscus could most effectively be achieved through mimicking the orientation native tissue itself. Not only does this product have the ability to change the way meniscus injuries are treated, but also how other tissues in the body can be mimicked. This technology could be used to develop fiber orientations that mimic any native tissue in the body. This versatility can then be used to create treatment options for any injury to any type of tissue by most accurately mimicking the structure and form of any tissue of interest.

4. **Political ramifications:** Based on the team’s foreseeable scope of this project, the potential political impacts are very minimal as the intended use of our design is to allow for research and teaching opportunities for university laboratories. The only concern our team could determine from this design is if the produced fibers were then used for controversial purposes, which could lead to political friction on the utilization of the technology. For example, these could include the use of stem cells for seeding onto the align fibers but given the team’s level of operation, this was not deemed to be a serious concern.

5. **Ethical concerns:** This project is extremely ethical as it aims to solve a gap in the medical field to help treat patients who need replacement tissues after sustaining an injury or losing function. Specifically, this project focused on the challenge with treating meniscal tears. Therefore, the target population is individuals who have meniscal injuries. However, this technology has the potential to mimic many different types of tissues in the body, thus, allowing this technology to cover a wide range of patient needs. The
fundamental goal that this product is trying to achieve is to improve the quality of living of a patient in need. Whether that need be an engineered meniscus implant or a cardiac muscle implant, this technology can potentially be used to help these types of individuals improve their quality of living.

6. **Health and safety issue**: The collection plate as designed is a research tool for three dimensional tissue scaffolds. The ideal future use would involve implanting ex vivo tissue within the body for tissue replacement or repair. One example would be with replacement meniscus. It would increase the standard of care for the health industry.

7. **Manufacturability**: Manufacturability in this case can be broken down into two categories, the collection plate pin matrix and the customized scaffolds. The collection plate pin matrix can be easily manufactured in large quantities, with minimal materials, and efficiently. The procedure for producing the collection plates could be improved to an automated production line as the plastic base could be developed through simple machining and the pins secured into the plastic base. With an automated manufacturing procedure, the collection plates could be created in mass quantities without sacrificing time or cost. On the other hand, manufacturing the customized scaffolds is also achievable. Electrospinning experimentation would not allow for the scaffold creation to be as time efficient as the collection plate manufacturing. However, with multiple electrospinning units, the ability to efficiently manufacture scaffolds significantly improves. Scaffold creation requires very minimal material and would be relatively cheap to produce.

8. **Sustainability**: The base of the collection plate is made out of insulating plastic. If mass-produced, the base can be made out of a recycled thermoplastic and reformed to be used
as the base material. This would decrease the need for synthesizing more plastic for production.
7. DISCUSSION

Electrospinning is very attractive due to the extremely high surface area to volume ratio it produces. A high surface area to volume ratio means that more cells can be seeded more densely on the scaffold. Electrospinning also allows for high control of the fibers diameter. However, current electrospinning methods provide limited control of fiber arrangement.

The team improved the most advanced pin matrix collection plate. The team’s collection plate had a finer pin matrix as well as conductive backings to allow for different fiber collection shapes. The finer a pin matrix is, the more flexibility there is when attempting to mimic tissues within the body. Moving from Prototype 1 to the final collection plate design indicates that a finer matrix allows for micro-geometries in the body to be mimicked with improved accuracy. The collection plate was compatible with varying test parameters including, changing voltage, materials, and polymer flow rate.

One of the main benefits of the plate was the design’s ability to be integrated into different electrospinning machines. The plate design can replace current system’s flat collection plates. Most strategies for alignment seen in literature require applying an additional external force to the fibers. For example, containing fibers in chambers of flowing gas can induce straight fiber collection. This adds another piece of expensive equipment into the process as well as needing to stock gaseous material.

The largest advancement the collection plate added to the electrospinning process was fiber alignment customization. The user may choose the orientation the fibers align in by changing the orientation of the brass caps on the back of the plate. As a proof of concept, curved scaffold alignment was created but this was one of the many possible shapes the team could have created. This a function that is not available on the electrospinning market, especially within one
device. Customized fiber alignment is an area of electrospinning that can be used to mimic various tissues within the body. This technological advancement provides tremendous potentially to treating tissue-engineering needs, such as replacement menisci for patients with significant injury to this cartilage tissue.

The collection plate design also was able to produce scaffolds consistently. Various tests were conducted with the collection plate and the design and procedure were able to consistently produce scaffolds of curved fibers. There were times where complications arose and certain scaffolds were not as high of quality compared to other scaffolds. However, the results were reproducible for creating scaffolds.

The safety level and user friendly level of the collection plate design was achieved but could be improved. The collection plate pin matrix is hazardous for the user and can easily puncture the skin of someone using it for testing. If the collection plate is handled properly and transported properly then the safety is not as much of an issue. It is very small and compact, which makes it easy to handle and easy to bring between different labs. The proper transportation method to improve safety for the user is to transport the device in a container. Along with that, when handling the collection plate, the user must just be aware and cautious that the pins are exposed. It is a lightweight device and easy to handle in the lab but should be used with caution. As mentioned before, it is user friendly in the sense that this device can be used for any electrospinning setup and is easily used during electrospinning. The one major downfall for the user is that the pin-charging method with the brass caps is manual and tedious procedure.

7.1. Cell Seeding and Alignment

Through the project experiments many results were obtained regarding cell seeding and alignment. The team was able to show that cells could grow on and throughout the PCL scaffold.
Additionally, the cells aligned along the created fiber alignment. This means that the designed collection plate can control fiber alignment, therefore controlling final cell alignment. This is a very useful ability to have. A major limitation to tissue engineering and regenerative medicine is the lack of functionality the engineered tissues have. Better mimicking the microenvironment and macro-environment of native tissues may help the engineered tissues function closer to native tissues. For example, the circumferentially aligned fibers and cells allow for a high compressive strength with all the GAG’s present in the tissue. The compression pushes down on the tissue, which then pushes out on the circumferential fibers. If engineered scaffolds mimic this circumferential fiber alignment, the resulting tissue may be able to support similar compressive forces.

The PCL nanofiber scaffold remained in geometry for 2-3 weeks in cell media. This is enough time to observe cell attachment and alignment on the scaffold fibers. However, the desirable degradation time should be explored more moving on in this project. The idea of this scaffold is to allow for cells to be seeded to the scaffold, align in the custom fiber orientation, lay down their own extracellular matrix mimicking the custom fabricated matrix, then have the polymer degrade away. The exact time frame for when the ECM would be produced needs to be balanced with the polymer degradation time.

7.2. Assumptions

One assumption is that mimicking native tissues through custom alignment is better and will lead to an increase in functionality of the final engineered tissue. The need for improving tissue engineering is to improve the functionality of the engineered tissue. The idea that mimicking native tissues more closely will lead to better functionality is one that is believed throughout the tissue engineering and regenerative medicine field.
Another assumption that was integral to the proof of concept of this project is that because cells attached to the straight aligned fibers they would also attach to curved fibers. Cell seeding of scaffolds primarily focused on promoting growth along the straight aligned fibers. Minimal cell seeding was done on the curved scaffolds. The fiber handling process was also not effective enough, therefore, removing fibers from the collection plate and to the culture dish often resulted in compromising of the fiber orientation. Because it was difficult to maintain the curved nature of the scaffolds, it became difficult to test whether or not the cells would attach and grow along the curved fibers that were created. Cells were proved to be able to grow along straight aligned fibers, from which the group made the assumption that the cells would also grow along curved fibers.

A GFP expressing cell line was used to seed the scaffolds with in order to improve imaging capabilities of the cell behavior on the fibers. The GFP cell line that was used was not a chondrogenic cell line that is seen in the meniscus. Therefore, the group made the assumption that the chondrocytes or patient specific cells would behave like the GFP expressing cell line in the sense that the cells would attach and grow on the fiber scaffolds. ATDC5 cells were opted against when seeding the scaffolds because the ability to collect useful images of these cells on the scaffold was extremely challenging. As a proof of principle, the GFP expressing cell line was chosen to improve imaging capabilities, thus, rendering the assumption that patient specific cells would provide similar results when being seeded to a scaffold.

The current gold standard for electrospun scaffolds is the Mimetix scaffold. Table 14 compares Mimetix to this project's final design.
<table>
<thead>
<tr>
<th>Attribute</th>
<th>Mimetix</th>
<th>Final Design</th>
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<td>Syringe pump</td>
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<td>Collection plate</td>
<td>Rotating drum</td>
<td>Pin matrix</td>
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<td>Scaffold material</td>
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<td>PCL</td>
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<td>Custom Alignment</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

**Table 14: Comparison between the Mimetix and custom scaffold**

There are similarities and differences between the attributes. Both electrospinning machines used voltage and a syringe pump with a single extrusion needle to pull polymer solutions into fibers.

The two collection plates varied, resulting in major differences in fiber alignment. The rotating drum pulls randomly aligned fibers along its circumference to form straight alignment. The team's collection plate grounds pins to align fibers along the pin tips. This collection plate allowed the team to create customized alignments while the Mimetix scaffold is available in straight or random alignments.

There were different material choices for the two scaffolds. The Mimetix used PLLA that degrades very slowly in culture while the team chose PCL for degradation time of four weeks. Both materials have the potential to be coated prior to cell culture, such as with collagen. Mimetix offered this as a customizable option but is not necessary for cell culture. For scaffold
seeding, the Mimetix scaffold requires preconditioning 12 hours before use and the custom scaffold needs twenty-four hours after electrospinning to allow the organic solvent to fully evaporate. Overall, both scaffolds are successful in 3D cell culture.

One drawback of the team's scaffold design is increased handling by the user. The scaffold needs to be removed from the collection plate and placed in a petri dish for seeding. The Mimetix scaffold comes in a petri dish. Increased user handling time increases the likelihood for human error, especially when maintaining alignment in delicate fibers is important.

7.3. Limitations

The data in this project have some limitations to consider, as with any proof of concept design project.

One major question that might be asked when looking at this project is the use of MDA-MB-231 GFP expressing cells to validate the cell seeding and viability on the customized scaffold. These GFP expressing cells were chosen to seed due to their capability to fluoresce green under specific wavelength exposure. The limitation in using these cells is that they are breast cancer cells. This means that they grow much more rapidly, and in harsher environments than non-cancerous cells. The translation between these breast cancer cells to chondrocytes, which are the cells in a native meniscus, is difficult. However, the team feels that the use of the breast cancer cells was necessary to monitor cell attachment and alignment over an extended period of time. The attachment and alignment of breast cancer cells does not mean chondrocytes will act in the same manner, but it is a step that supports the viability of chondrocyte use. In the end, cells were able to attach to the created scaffold and had access to nutrients, which are two key factors in supporting cell life.
Related to the use of breast cancer cells to seed the scaffold, the study only involved immortalized cell lines that are designed to continue to replicate outside of the body. Because of this, the cells continue to proliferate, even when the scaffold reaches its capacity. This prevents cells to live on the scaffold, as they overgrow and cannot be passages when seeded on the scaffold.

Another major limitation to the data is the sample size used in the research. A very small number of scaffolds were actually seeded with cells and observed throughout two weeks. The small sample size is useful for observing cell behavior, but it is difficult to relate the samples to the population of all cells. It is unclear if cells will always act the way the observed cells acted.

The degradation time of PCL in cell media is another limitation to the data. The PCL nanofiber scaffold degraded in 2-3 weeks. Because of this, cell monitoring is not possible past three weeks.
8. CONCLUSIONS AND RECOMMENDATIONS

The completion of this project has led to many conclusions, accomplishments, and recommendations.

8.1. Conclusions

Many conclusions can be made from the research completed in this study. Firstly, custom alignment of a nanofiber scaffold was created using a pin matrix collection plate design. This creation shows that the pin matrix technique is a viable technology for custom fiber alignment.

Another conclusion is that electrospun PCL allows for cell attachment and alignment along fibers. MDA cells were seeded to the scaffold and were monitored by a fluorescent microscope. This is an important conclusion, as cell viability is a key aspect to most created scaffold designed for tissue engineering and regenerative medicine purposes.

The PCL nanofiber scaffold remains in shape for 2-3 weeks when seeded with cells in cell media. This means that chondrocyte progenitor cells would have enough time to differentiate into chondrocytes once seeded to the scaffold, as this differentiation time is 1-2 weeks long. The timeframe of 2-3 weeks will need to be researched further, to determine if that is an ideal time frame for degradation for seeded cells to create their own extracellular matrix, or if this degradation time needs to be altered by modifying or exchanging the material.

The optimal weight percent by volume of PCL in HFIP was determined to be 10% for electrospinning. This was determined after much research and experimentation. The optimal flow rate and voltage range were found to be 0.15ml/hr and 8-12kV. The optimal distance for electrospinning was determined to be 15cm.
8.2. Accomplishments

Over the course of the year, the team was able to achieve marked progress and make positive strides within each of the three main areas of concentration of collection plate development, 3D scaffold production and successful cell adherence to the nanofibrous PCL matrix. Each of these areas and the associated sub-accomplishments are evaluated below:

Collection Plate

Customizable Grounding

After initially proving the successful, albeit permanent, grounding abilities of soldering each row, the team developed a cluster of brass conductive caps that were custom-fitted to the pin diameters, which were then soldering to electrical wiring and merged on the opposite side allows for a single alligator clip to be used for the entire set. Using this method the team was able to ground various different pin orientations, thus proving the present and future viability of this technique.

Multiple Setup Compatibility

Even in the short term the team was testing we were able to prove that portions of our setup (i.e. collection plate, injection pump, voltage supply) could be switched out and still operate effectively in producing fibers. Based on the conducted research, it was also determined that this design would be able to work with a gravity-induced electrospinning setup.

Portable and Straightforward

As the final collection plate measured a very reasonable 22 x 38 mm, it was highly mobile for transportation between labs when brass cap installation or testing warranted these location changes. Once the plate was fully manufactured, the plate setup actually only required two parts: the main collection plate and the cluster of brass caps soldered, each soldered to an
electrical wire. With this minimal setup, the device is very user-friendly and the setup is easy to learn for any new individuals going through training.

3D Scaffold

*Increased Level of Fiber Control*

While the research paper that sparked the interest in pin collection methods proved alignment was possible along straight lines, the team took this concept one step further and on multiple occasions was able to produce fibers in both straight and curved orientations. This proved the advancement of fiber control with our device and the reproducibility by testing successfully throughout final prototype testing days.

*Curved Alignment*

As briefly discussed above, the team was able to orient the charged pins along diagonals and straight lines within the same orientation and managed to produce a fully-connected alignment through the entire charged shape, thus deeming the success of curved fiber shapes.

Cell Culture

*Cell Adherence*

With 6-8 scaffolds produced with curved fiber alignments tested with cell seeding protocols and confirmed by closer inspection with the Salisbury Labs microscopes, the cells showed strong adherence to the electrospun fibers.

*Growth Along Fibers*

Expanding upon the above achievement and outlined in our results section, the team recognized cells aligning more consistently along the aligned fibers than in the randomly oriented, less dense fiber networks.
8.3. Recommendations

There are many areas that this study could be improved through further research. The major areas that future research could help improve this project are the following: refining fiber removal process, electrospinning with other polymers or with a cell/polymer solution, fiber surface coating for cell viability and growth along fibers, improving the process for charging collection plate pins, and developing a layered scaffold to achieve different alignments in 3-D.

The current method of fiber removal from the collection plate is achieved by using parafilm that is placed on the pin matrix so that the ends of the pins are exposed. The fibers collect on the ends of the pins and the parafilm is then slowly removed from the pin matrix taking the fibers with it. The fibers are then removed from the parafilm and placed into a culture dish for cell seeding using forceps. The parafilm and forceps fiber removal methods disrupt fiber orientation and can damage the fibers very easily. A method of effective fiber removal is an area of research for this project and this technology that could help validate customizable fiber orientations.

This study focused on electrospinning PCL for scaffolds that were used for cell seeding. With cost and availability being large factors as to why PCL was chosen as a polymer for electrospinning, no further research was conducted on electrospinning other biomaterials. It would be advantageous to conduct further research into other biomaterials that could be used for electrospinning and cell seeding. For example, materials such as silk fibroin, collagen, and PLGA along with others could be tested to determine how effective they are for electrospinning and seeding the scaffolds with cells. Another area of research that could be furthered is creating a cell/polymer solution to be electrospun. Therefore, the cells would already be intact with the
scaffold and cell-seeding methods could be bypassed because the cells are already a part of the scaffold.

The PCL scaffolds were seeding with cells without coating or modifying the surface of the fibers. Cell viability and adhesion were achieved, however, a study could be conducted on the advantages of coating the fibers with collagen or modifying the structure or surface of the fibers to promote better cell growth along the fibers, attachment, and proliferation.

Another area of this study that could be improved is the method of charging the pins of interest for customized fiber collection. The current method of the brass caps allows for customized fiber alignments to be possible, however, it is a manual application of the caps to the pins that is time consuming and tedious. The pins often come out of place because the pins are press fit into the collection plate base and are also press fit to the brass caps. Therefore, an improved method for charging the pins would be highly advantageous. An automated method of pin charging or a much more user friendly manual pin charging method is an area of interest for further advancement of this technology.

Lastly, improvement to the three-dimensionality and layering of the electrospun scaffolds could be an area of future work for this study. The current study has developed some three-dimensionality with the scaffold creation; however, this area can be improved upon. Specifically, the scaffold could be layered in three-dimensions so that the fiber alignment could differentiate as the scaffold is built upward. For example, a layer of the scaffold could be made with one fiber orientation and the next layer of the scaffold could be made with a different fiber alignment. This improvement could further help mimic tissues within the body more accurately.
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Yan, Le-Ping, Joaquim M. Oliveira, Ana L. Oliveira, Sofia G. Caridade, João F. Mano, and Rui L. Reis. "Macro/Microporous Silk Fibroin Scaffolds with Potential for Articular Cartilage


doi:10.1039/c5ra13859k
Appendix A. Protocols

Cell Culture Protocol

Note: This protocol was developed by the Worcester Polytechnic Institute Biomedical Engineering Department.

1. Dilute 0.25% trypsin-EDTA stock 1:5 in 1X Ca++, Mg++ free DPBS to make a 0.05% working stock solution. This is done by adding 1 part of trypsin to 4 parts sterile DPBS. Warm the working stock to 37o C in the incubator or in a water bath. Return the 0.25% trypsin stock to the freezer.

2. Wipe the outside of the trypsin bottle with 70% ethanol and place it in the culture hood.

3. Take out the culture dish to be split and place inside the hood.

4. Aspirate the culture media from the dish using a Pasteur pipette attached to the vacuum pump.

5. Carefully add 5 ml of Ca++, Mg++ free DPBS to the dish and rinse the cells by gently swirling the dish. Do not squirt DPBS directly onto the cells. This might detach cells from the dish and be lost while aspirating the DPBS after rinse.

6. Aspirate DPBS using a Pasteur pipette. Repeat the DPBS rinse once again.

7. Add 5 ml of warm 0.05% trypsin-EDTA to the dish and incubate at 37 °C in the incubator for about 3-5 minutes.

8. Observe the dish under a light microscope to see the degree of cell detachment. If the detachment is not complete, incubate an additional 2 minutes. If complete, go to the next step.

9. Using a serological pipette, add 5 ml of complete medium to the dish.

10. Mix the contents by repeated pipetting to break up cell clumps to individual cells.

11. Take out a small sample for cell counting if necessary.

12. Place the tube in the centrifuge along with a balancing tube containing an equal volume of sterile water.

13. Centrifuge the tubes at 200g (RCF) or corresponding RPM for 5 minutes.

14. Take out the tube and watch for a cell pellet at the bottom of the tube.

15. Using a sterile Pasteur pipette, carefully remove the supernatant leaving the cell pellet intact.

16. Re-suspend cells in sufficient amount of complete medium to conveniently split to desired number of plates. For e.g., if you are going to split the cell pellet from one T25 flask to two T25 flasks, re-suspend the cell pellet in 10 ml complete medium. Add 5 ml of cell suspension to the two T25 flasks.

17. Make sure that the media is spread uniformly in the dish by gently rocking the dishes gently.
18. Observe cells under an inverted light microscope and transfer the dishes to the cell culture incubator for overnight incubation.

**Cell Freezing Protocol**

Note: This protocol was developed by the Worcester Polytechnic Institute Biomedical Engineering Department.

*Preparation for freezing cells*

A. Prepare enough cryovials for freezing cells. This is done by marking the following details on the cryovials using a permanent marker. More specific details that cannot be noted on the cryovial should be entered in the freezer log.

1. Name of cell line or cell type
2. Passage #
3. Cell count per vial
4. Date of freezing

B. Prepare freezing solution A. This consists of basal media (DMEM) containing 10% FBS (e.g. 9 ml DMEM + 1 ml FBS). Mix thoroughly.

C. Prepare freezing solution B. This consists of basal media (DMEM) containing 10% FBS and 20% DMSO (e.g. 7 ml DMEM + 1 ml FBS + 2 ml DMSO). Mix thoroughly.

*Trypsinization and freezing*

• Trypsinize and re-suspend cells as described earlier.
• Note the total volume of cell suspension.
• Load a small amount (~50 ul) into the hemocytometer to determine cell count. Dilute cells if necessary.
• Centrifuge cells at 200xg for 5 to 10 minutes.
• While the cells are spinning, determine the total cell count.
• Determine the number of vials to be frozen based on total cell count. For e.g. if you have a total of 4 million cells and if you want to freeze @ 500,000 cells per vial you have a total of 8 vials to freeze.
• After centrifugation, aspirate the supernatant carefully without disturbing the pellet.
• Using a serological pipette, re-suspend the pellet thoroughly by gentle pipetting in half the final freezing volume of freezing solution A (4 ml in this example).
• Using a fresh serological pipette, add equal amount of freezing solution B slowly (4 ml in our example), a few drops at a time while at the same time mixing the contents by gentle rotation or inverting the tube. Finally, invert the tube several times to mix the contents thoroughly.

• Aliquot 1 ml of this mixture into pre-marked cryovials. Make sure to mix the cells intermittently lest you end up with vastly different cell numbers in the cryovials as the cells tend to settle to the bottom.

• Cap the vials tightly.

• Transfer the vials to a freezing container and place in a -80 °C freezer overnight.

• The following day, transfer the vials to a liquid nitrogen container for long-term storage.

**Cell Thawing Protocol**

Note: This protocol was developed by the Worcester Polytechnic Institute Biomedical Engineering Department.

1. Take out the required number of vials from liquid nitrogen and quickly transfer to a small container with lukewarm (not hot) water. The cells will thaw out in about 2 to 3 minutes.

2. In the meantime, prepare sufficient number of cell culture plates or flasks. Mark the flasks with all relevant details that pertain to the cells that you are culturing (cell type, cell #, passage #, date etc).

3. Inside the culture hood, transfer 9.0 ml complete media into a fresh 15 ml conical centrifuge tube.

4. Once the contents of the cryovial have thawed, wipe the outside of the cryovial with 70% ethanol and bring it inside the hood.

5. Carefully open the cryovial. Using a P-1000 pipette or 2 ml pipette, carefully transfer all the contents into the centrifuge tube containing 9.0 ml complete media. Close the cap tightly and mix the contents by gentle inversion.

6. Place the tube in the clinical centrifuge. Balance it with a control tube containing an equal volume of sterile water.

7. Centrifuge the tubes at 200g (RCF) or corresponding RPM for 5 minutes. Take out the tube and watch for a cell pellet at the bottom of the tube.

8. Using a sterile Pasteur pipette, carefully remove the supernatant leaving the cell pellet intact.

9. Using a 10 ml serological pipette, add 10 ml of warm complete media to cell pellet and mix gently to disperse the cells uniformly. This can be best accomplished by pipetting multiple times and gently forcing the media on to the sides of the centrifuge tube.

10. After the cells are completely dispersed in the medium, transfer the entire content into a sterile culture flask(s). Plate 10 ml per T75 flasks or 100 mm plate. If using this step is important to ensure smooth functioning of the centrifuge. Make note of the cell type, passage number, cell number per vial, date of freezing and any other relevant details described on the vial, cell freezer log or details provided by your instructor. Wear protective eyeglasses and
gloves while handling frozen vials in liquid nitrogen. Vials are known to explode as the contents in the vial expand rapidly upon thawing due to the dramatic temperature differential between liquid nitrogen and ambient room temperature. 25 T25 flasks or 60 mm plates use 5 ml culture per dish.

11. Make sure that the media is spread uniformly at the bottom of the dish by gently rocking the dishes back and forth and sideways (Do not mix by rotation as this will result in cells accumulating in the center of the dish)

12. Observe cells under an inverted light microscope
Appendix B. Gantt Chart
Appendix C. Product Codes

Polycaprolactone
Average molecular weight of 80,000
Sigma Aldrich product code 440744

1,1,1,3,3,3-Hexafluoro-2-propanol
Molecular weight 168.04
Sigma Aldrich product code 105228