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Design of an Automated Microthread Processing System

A Major Qualifying Project Report

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By

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Alexandra Birch

_____

Spencer Coffin

_____

James Perez-Rogers

_____

Heather Peruffo

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Approved by:

_____

Professor George Pins, Advisor

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1 Introduction

Musculoskeletal injuries, which include damage to tendons, ligaments, and skeletal muscles, are widespread in the United States and accounted for approximately $127.4 billion in healthcare costs in 2004 (Andersson, 2008). Small-scale injuries are often resolved through natural regeneration in the body, but large-scale injuries, which more than 20% of native tissue is lost, require additional resources to prevent scar formation and promote regrowth of functional tissue (Page, 2011). There are an estimated 33 million musculoskeletal injuries annually in the United States, with over 50% caused by tendon and ligament damage (Calve, 2004).

Tendon and ligament tears, as well as significant muscle loss due to trauma, are some types of large-scale musculoskeletal injuries that require assisted healing to restore full function. Current therapeutic options for tissue repair are allografts, autografts, and xenografts. Although there are other options on the market currently, use of an autograft is currently the gold standard for anterior cruciate ligament (ACL) reconstruction (Spencer, 2003). Although autografts are used extensively and considered the best solution because they use patient-specific tissue, they are limited in terms of their availability and the risk of donor site morbidity because of the creation of a second wound site on the patient (Cleland, 2007). Allograft and xenograft surgeries are complicated by immune rejection as a response to foreign tissue, which is a deterrent to patients (Cleland, 2007). These limitations drive the need for the development of an alternative solution.

A promising alternative to allografts, autografts and xenografts are biomimetic scaffolds, which facilitate wound healing by mimicking the structural and biological properties of native tissue (Cornwell K.G., 2007). Fibrin microthreads, a scaffold material that is morphologically similar to skeletal muscle, ligaments, and tendons, are comprised of natural materials and represent a promising scaffold. Fibrin microthreads were originally created through an extrusion and stretching process (Cornwell, 2007). Since their development, fibrin microthreads have been used for the restoration of skeletal muscle injuries in mouse models (Page, 2011) and the delivery of human mesenchymal stem cells (hMSC) on culture plates and other applications (Potapova IA, 2007). Although fibrin microthreads have been used in many applications, the way in which the threads are produced is limiting their large-scale use in research laboratories.

Fibrin microthreads are currently fabricated in batches through a hand-drawn extrusion process, coupled with a manual stretching process, and various optional post-production

modifications (Cornwell K.G., 2007). Manual extrusion and stretching of the threads induces high batch-to-batch variability in terms of the mechanical and structural properties of the threads. In order to develop more uniform threads with consistent properties, the need for this project was to develop a stretching system to interface with the current extrusion process and minimize human handling of microthreads during their production.

Although previous attempts have been made to automate the production of microthreads, development of a system that encompasses automated extrusion and stretching of the threads does not exist. A previous Major Qualifying Project (MQP) team successfully developed a semi-automated extrusion system for collagen microthread production in 2010. Work conducted by that MQP team showed decreased variation in automatically extruded microthreads, showing that decreased human handling of threads increased the uniformity of the threads between batches. Because of the similarities in the production of collagen and fibrin microthreads, it was possible to incorporate the collagen extrusion head in the automated fibrin microthread processing system.

A key component of microthread production that had not been addressed previously was the automation of the stretching process. According to unpublished data in Pins' lab, stretching fibrin microthreads is a critical step during production because it hypothesized that it aligns the proteins present in the amorphous threads. Additionally, increases in the stretch percentage of threads led to increases in the elastic modulus (or stiffness) and ultimate tensile strength, as well as decreases in the strain at failure of the threads. The increase in the mechanical properties of threads with respect to stretching is why stretching is a critical and essential part of the production process. However, threads are currently stretched in a manual stretching process, and this increases variability within the threads during stretching. The scope of this project involved the creation of an automated post-production modification system for the threads, and integration with the previous automated extrusion system.

This project included the design, development, and testing of a prototype device that performed stretching of the threads automatically. It aimed to maximize automation and minimize manual contribution to the microthread fabrication process. Functionally, the device was customizable and produced threads to desired parameters in terms of extrusion and stretching. The design team used the design process to determine design goals, develop alternative solutions, and determine the appropriate steps to create a functional system.

The team performed proof-of-concept tests, including clamp-mechanism tests and non-adhesive surface tests to determine the feasibilities of alternative designs. This allowed the team to make accurate assessments to determine the final design for the project. The team was able to choose squeegee clamps that would anchor the threads to the stretcher plates that, with a motorized stretcher in an angled bath, would remove the threads from the extrusion pan by stretching them.

As soon as the final design was chosen, a prototype was developed to validate the design, and the design team completed initial validation tests to ensure that each component of the system would function properly. During this testing, modifications were made to the stretch-to-remove system, the motorized stretcher, and the previous extrusion system in order to fix production problems with the system that would prevent it from making consistent threads or performing within ± 10% of each setting. Once these modifications were made, the design team evaluated the prototype based on the initial objectives, constraints, and systemic needs and wants for the system. This evaluation of the prototype confirmed that the design team had created a working prototype that performed up to initial standards.

After the final prototype was completed, the design team ran validation tests to ensure that the system parameters and thread mechanical and structural properties were within the tolerances that the design team identified. Results of the system studies showed that the settings for the parameters of extruder head rate, stretch percentage, and stretch speed accurately performed to within ± 10% for each setting.

The purpose of this project was to design, construct and test an automated fabrication system for fibrin microthreads. Although the designed device performed within desired parameters, time was a limiting factor and it was necessary to modify the process parameters in order to complete thread validation. The biopolymer extrusion system was designed to extrude collagen microthreads, but the design team ran into problems customizing it to fabricate fibrin microthreads and interfacing it with the stretching system within the given time frame. To try and fix this problem, the team moved to validate the system through hand-drawn extrusion and automated stretching, and it was this semi-automated process that was used to validate the stretching system.

Although full automation of the fibrin microthread processing system was not achieved, the design team developed a process to create fibrin microthreads at an acceptable failure

percentage of 25% with decreased variability in their mechanical properties. This work represents significant progress in the automation of fibrin microthread production to include automated stretching. In combination with a fully automated extrusion and removal system, this stretching system has the potential to eliminate human handling of fibrin microthreads during the entire fabrication process.

2 Background

One of the goals of tissue engineering is to develop a provisional scaffold, with a morphological structure similar to native tissue that aids in tissue healing and regeneration. These scaffolds can be applied in the healing of musculoskeletal injuries, as seen in car accidents, severe burns, and combat injuries, and these injuries account for approximately 61.2 million treated cases annually (Andersson, 2008). Skeletal muscle consists of a hierarchy of long, cylindrical fibers, each of which consists of smaller bundles of thinner fibers. Fibrin microthreads are a scaffold material used in the treatment of musculoskeletal injuries because of their three-dimensional morphologic similarities to skeletal muscle. Additionally, fibrin is a natural biological protein critical in the healing of injuries because of its role in clot formation and its ability to direct the wound healing process.

Collagen and fibrin microthreads have the potential to create artificial ligaments, tendons, skeletal muscle, and promote wound healing by mimicking the provisional matrix and encouraging cell migration and alignment onto their substructure. However, the current production of fibrin microthreads is entirely manual, which induces batch-to-batch variations in the structural and mechanical properties of the threads. In order to test the efficacy of fibrin microthreads as a possible scaffold in tissue regeneration, an automated processing system must be developed to create threads uniformly and consistently. This automation will allow researchers to fully characterize the properties of fibrin microthreads and provide a prototype for future, large-scale production systems.

First, this chapter addresses the clinical importance of tissue engineering and the role of wound healing in its application. First, the clinical need for this project with relation to musculoskeletal injuries is discussed, understanding the need driving scaffold research. One concept that tissue engineering often tries to mimic or augment is the wound healing cascade, therefore it is important to understand this process before using it in engineering applications. After understanding the biological processes, the design team discusses current alternative treatments to tissue engineered products, which include autografts, allografts, and xenografts from a similar tissue source.

The next section of this chapter focuses on scaffolds, which provide structure and mechanical stability to the wound while encouraging cell growth. Because of the fibrous structure of the musculoskeletal system, the types of scaffolds for this application should fibrous

and load bearing. As a final point in this section, the types of fibrous scaffolds the currently exist are discussed.

The final section of this chapter focuses on fibrin microthreads, which are one type of scaffold material with applications in musculoskeletal injuries. The remainder of this project will focus on fibrin microthreads, so it is important to discuss the concept, applications and production process for this material. This information gave the design team a full picture of product, as well as identified flaws within the production process that drove the need for this project. Finally, this section discusses patents and previous work that aim to automate and increase the precision of thread making processes. This research helped the design team in developing a solution for this project.

2.1 Tissue Engineering

The repair of human tissue, which is damaged through organ failure or severe trauma, is a constantly evolving problem in the medical industry. In small scale injuries, the wound healing process is capable of removing damaged tissue, guiding new native tissue growth, and remodeling the injury site. In larger scale injury, where the full structure of native tissue is lost, the body is unable to regenerate full functional tissue by the same mechanism (Page, 2011). When tissue loss is large enough to interrupt normal function, collagen is deposited to limit the amount of tissue loss and protect the body from pathogens and other harmful material. Although limiting the overall damage to the body by blood loss and infection, this process does not reestablish functionality of muscle tissue or necessary vascularization. The current gold standard for large scale tissue repair in musculoskeletal injuries, like muscle loss or tendon and ligament tears, is the placement of autograft or allograft tissues, from a secondary donor site or another patient, which limit scar formation and replace lost tissue (Cleland, 2007). However, this process is limited in terms of available donor tissue. Synthetic replacements such as polyester and polytetrafluoride meshes are another option for tissue replacement, but are limited in terms of their capacity to restore total normal function and vascularization to the wound site (Silver, 1991). Synthetic replacements provide mechanical stability and structure to the effected site, but are limited in their ability to direct the regrowth of cells to replace what was lost.

Tissue engineering aims to replace donor tissue with fully functional, patient-specific tissue grown *in vitro* or an acellular scaffold into which native tissue can grow, and eliminate the need for large amounts of donor tissue in wound repair. This interdisciplinary field aims to regenerate

tissue by creating a microenvironment in which regenerative cells attach, proliferate, and differentiate into functional tissue (Ma, 2008). These microenvironments mimic biological processes within the body such as cell signaling, and encourage the proliferation and alignment of cells either *in vitro* or *in vivo.*

2.1.1 Clinical Need

Musculoskeletal injuries refer to any injuries related to joints, muscles, ligaments, or tendons. In the United States alone, musculoskeletal injuries accounted for approximately $127.4 billion in healthcare costs in 2004 (Andersson, 2008). In 2006, there were more than 61.2 million treated cases of musculoskeletal injuries. Within these 61.2 million cases, open wounds and contusions accounted for $10.2 million and $10 million respectively (Andersson, 2008). Open wounds and contusions consist of structural damage to muscle tissue and when significant damage occurs, the muscle cannot repair itself (Andersson, 2008). An artificial scaffold has the potential to bridge the gap in larger muscular trauma and promote healthy tissue regeneration, but there is a need to further develop this method to better mimic the properties of the native tissue.

One of the uses for these scaffolds is in the repair of joint injuries. Two of the most commonly injured joints are the shoulder and the knee. Four million Americans seek medical care for shoulder injuries each year, with 100,000 of these resulting in surgery to repair shoulder tendons, ligaments, and rotator cuff muscles (Bergfeld, 2012). Approximately 10.8 million patients report knee injuries annually. One of the more common injuries to the knee is an anterior cruciate ligament (ACL) tear, which results in approximately 150,000 surgeries per year. (Bergfeld, 2012)

In 2006, approximately 4 out of every 100 patients that reported musculoskeletal injuries, also reported limited performance in daily activities as a result of their reported injury (Andersson, 2008). This represents a significant population that suffers from limited mobility. To improve mobility and promote full tissue regrowth, fibrin microthreads can be applied as a provisional scaffold in musculoskeletal injuries, and may result in more effective treatments with minimal complications.

2.1.2 Wound Healing and the Extracellular Matrix

One microenvironment that scaffold engineers look to mimic is the wound healing environment, because of its capacity to restore tissue through the recruitment of cells, growth factors, and other products. The wound healing process in the body is complex and involves many proteins and cell signaling processes. Breaking down and studying this process has helped scaffold engineers to design more effective scaffolds using specific proteins and biological functions in order to regrow native tissue instead of replacing it with a synthetic alternative .

The wound healing cascade in the body is activated by both the extrinsic and intrinsic pathways. The extrinsic pathway is activated by blunt tissue trauma and disruption to blood vessels (Monaco, 2003). Injuries which activate the extrinsic pathway generally heal quickly with little scar tissue formation. The intrinsic pathway is activated by exposure of blood to foreign material, and results from a cut to the skin or from the implantation of a synthetic implant (Monaco, 2003). Because of this, the intrinsic pathway is the focus of many tissue engineering applications. After severe trauma to tissue and the exposure of blood to foreign material, blood flow rushes platelets and erythrocytes to the site of injury, getting caught and coagulating in a matrix of fibrin to form a blood clot at the injury site. This process establishes the provisional matrix, which fills the damaged area and protects the body (Clark, 2006). The provisional matrix also directs cell growth and allows for tissue repair by releasing proteins and other factors that recruit cells to the site of injury. This process contributes to restoring homeostasis and exhibits the dynamic reciprocity of cells within their microenvironment.

The first step in the regeneration of tissue is the formation of the provisional extracellular matrix, which is comprised of fibrin, fibronectin, and vitronectin. After serving to initially restore homeostasis, the function of the provisional matrix is to attract monocytes, fibroblasts, endothelial cells, and other cells to the wound site (Clark, 2006). Fibrin and the other matrix proteins are essential in controlling the differentiation of endothelial cells and initiating the process of angiogenesis to restore blood vessels in the new tissue (Clark, 2006). The provisional matrix also serves as a temporary scaffold that provides mechanical and morphological support at the wound site until it can be replaced by regrown tissue.

Degradation of the provisional matrix after cells have begun to regenerate is equally as important as its formation. Within days of the initial injury, proteolytic enzymes, plasminogen activators, and plasmin work in conjunction to degrade the provisional matrix and provide space

for healthy proliferating cells (Monaco, 2003). Plasminogen inhibitors increase degradation time of the provisional matrix around only newly formed cells. However, inadequate removal of the provisional matrix may lead to fibrosis in which scar tissue forms within the wound site and functional tissue is not regrown (Salonen, 1989). It is important for tissue engineers to consider both the formation and degradation processes so that they can have a controlled balance between cell ingrowth and connective tissue degradation at the wound site.

As mentioned previously, in larger scale traumatic injury, the provisional matrix and its directed inflammatory response are not adequate to regenerate fully functional tissue. In these injuries, fibroblast activity increases to create scar tissue, in which cells deposit collagen and other connective tissues at the wound. In order to reduce the formation of scar tissue and re-establish healthy tissue in large scale injuries, tissue engineering aims to create scaffolds for wound healing that mimic the structural and biological properties of the provisional matrix. Engineered for larger traumatic wound sites, artificial biomimetic scaffolds aim to facilitate cell proliferation and differentiation into functional tissue and limit scar tissue formation.

2.1.3 Autografts, Allografts, and Xenografts

Large scale musculoskeletal injuries often require the addition of a graft to replace lost tissue to supplement tissue the bulk of the tissue that cannot regenerate on its own. These grafts are taken from another large area of similar tissue such as the thigh, and are used to provide a healthy portion of functional tissue in areas that there has been a lot of tissue damage. Three different natural types of grafts are available as grafting agents for patients.

Autograft refers to when a graft is taken from a donor site from the patient and placed into the damaged area. Autografts are currently the gold standard of treatments for large scale musculoskeletal injuries, but require a donor site which leaves the patient with multiple wound sites. Some patients are unable to go through this surgery due to weakness and the risk of loss of blood, which for victims with multiple lacerations could potentially be a serious problem. Patients could also have a problem with a lack of available tissue to harvest for the graft (Cleland, 2007).

Allografts and xenografts are not considered the first choice for surgeries, but are an option for patients with large scale tissue damage where a donor site would be difficult to utilize. Allografts are grafts taken from another human donor, and xenografts are grafts taken from another species. These can provide temporary wound coverage, but issues with rejection,

availability, and disease transfer are prevalent (Cleland, 2007). Xenografts are often taken from pigs, cows or horses, and currently there is no good way to screen for common viruses in the graft and even decellularized xenografts may still be contaminated by viruses (Cleland, 2007).

2.2 Scaffolds

Scaffolds are three-dimensional structures onto which cells can be seeded, cultured, and implanted into the body. Scaffolds provide mechanical stability to newly grown cells to allow for tissue development. Scaffolds are a key component of tissue engineering and it is important to discuss both their purpose and the different types of scaffolds

2.2.1 Purpose

Biomimetic scaffolds are engineered to mimic the structural and biological properties of native tissue and facilitate directed tissue growth in large scale injuries (Ma, 2008). The goal of biomimetic scaffolds is to facilitate cell proliferation and differentiation in large injuries by mimicking certain functions and morphologies of the ECM.

There are well-established criteria for the necessary functions and characteristics of an engineering scaffold. Foremost, the scaffold should facilitate cell adhesion, proliferation, and differentiation into the desired tissue type for full restoration of function of different systems (Chen, 2002). In addition, scaffolds must be biocompatible, not elicit an immune response, and biodegradable. Morphologically, the scaffold should be three dimensional, porous, and mechanically stable. These properties allow for nutrients to move within the matrix while still providing mechanical stability for cells as well as to serve as a guide for cell alignment (Chen, 2002). Another critical characteristic of scaffolds is that they are specific to different types of tissue engineering applications. While scaffolds that aim to facilitate the regeneration of bone are generally porous and mechanically stable under tension, scaffolds for connective tissue and skeletal muscle applications are often fibrous and are also mechanically stable under tension.

2.2.2 Types of Fibrous Scaffolds

There have been many different scaffolds developed for tissue engineering, each for a wide variety of applications within the body. Two distinct types of scaffold materials are natural and synthetic materials. The two types each have their own advantages and disadvantages, and some research aims to combine the two types of materials to get some of the more desirable characteristics from each. Natural materials, which include but are not limited to collagen, silk, and fibrin, are advantageous because they have low toxicity, low inflammatory response and can

be naturally degraded by enzymes (Vats, 2003). Some of their disadvantages, however, are that they have relatively low mechanical strength and are sometimes in short supply because they need to be sourced from available organisms. The advantages of synthetic materials are that they are easy to produce and manipulate to varying mechanical strengths, but they are disadvantageous in that they can have toxic byproducts that render them not biocompatible (Gunatillake, 2003). Both synthetic and natural scaffold materials have been used in the design of fibrous scaffolds for tissue engineering applications.

Collagen was one of the early biomaterials that was considered for load-bearing, fibrous scaffolds for use in applications such as musculoskeletal injuries. Collagen was investigated because it had long been used as a suture material and clotting accelerator (Petrigliano, 2006), as well as being the principle protein found in ligament and tendon (Liu, 1995). Although collagen scaffolds enhanced cell proliferation and attachment on the scaffold, it was found to have decreased mechanical strength and stability over time from degradation and fatigue failure from continued tensile loading (Calve, 2004). It is still explored, however, for many tissue engineering applications because of its role in the wound healing process.

Silk is another natural biomaterial that is used in load bearing, fibrous scaffold applications for tendon and ligament repair. Like collagen, it has been used in sutures as an inexpensive and biocompatible scaffold material (Petrigliano, 2006). For a natural material, silk is characterized by having high mechanical strength and can be braided and twisted to resemble the architecture of ligaments and tendons. Silks have also been modified with short synthetic peptide sequences such as arginine-glycine-aspartic acid to increase cell attachment and proliferation (Petrigliano, 2006). However, silk needs to be isolated from small organisms so is not normally available in large quantities, which make it difficult to work with (Petrigliano, 2006).

In order to combat some of the problems with availability and mechanical strength faced by natural scaffold materials, one direction that researchers have moved in is into the development of synthetic polymer scaffolds to combat some of the problems caused by natural models. Synthetic polymer scaffolds are designed to provide mechanical stability to the scaffold site and then gradually degrade to allow for native tissue growth into the wound site (Petrigliano, 2006). This greatly decreases the need for long term mechanical strength of the scaffold, as it does not permanently remain in the body. Poly glycolic acid (PGA), poly lactic acid (PLA), polycaprolactone (PCL), and poly(L-lactic) acid (PLLA) are synthetic materials that are often

used in fibrous scaffold applications. They have also shown potential to increase cell attachment and proliferation, especially when using tailored pore sizes (Petrigliano, 2006), but still have many of the disadvantages of synthetic biomaterials. These disadvantages include loss of mechanical properties early in degradation, as well as acidic byproducts that are released during degradation (Gunatillake, 2003).

Although there are many different types of scaffolds for musculoskeletal applications, each has its advantages and disadvantages. While synthetic scaffolds are easy to manipulate and tailor to relevant mechanical properties, natural scaffolds are advantageous in that they are more biocompatible and naturally exist within the body. Another natural scaffold material which has been explored for use in musculoskeletal applications is fibrin, and this material will be the focus of the remainder of the project.

2.3 Fibrin Microthreads

Fibrin has been explored as a suitable material for biomimetic scaffolds because of its involvement in the provisional matrix during the wound healing process. Additionally, fibrin can limit the foreign body response in patients because it can be derived from the patient's blood (Jockenhoevel, 2001). Fibrin products have been produced for applications such as blood clotting, sealing of wounds, and low strength mechanical scaffolds in medicine since the early twentieth century (Jockenhoevel, 2001). Fibrin has been commercially available as a topical sealant, skin adhesive, and hemostat since 1998 (Spotnitz, 2010). Historically, fibrin has been combined with other strengthening components to increase its mechanical stability, such as poly-lactic acid (PLA) (Ahmed, 2008).

One application of fibrin in tissue engineered scaffolds was the development of fibrin gels for cell seeding (Matsumoto, 2007). Fibrin gels have been used in vascular and heart valve prosthesis (Matsumoto, 2007). They were effective because they could be manipulated into three dimensional structures with complex morphologies. It was shown that scaffolds made from fibrin gels were capable of supporting the attachment of collagen and other components at an increased level when compared to porous scaffolds made from other materials (Jockenhoevel, 2001). These gels were also capable of releasing growth factors into the surrounding tissue space (Jockenhoevel, 2001). Although fibrin gels had many ideal scaffold properties, their mechanical strength and stability were not adequate for all scaffold applications. This research led to further manipulation of fibrin into a form which would support mechanical loading.

2.3.1 Fibrin Microthread Concept

During wound healing in the body, fibrinogen and thrombin react with one another in the wound healing cascade to form, in conjunction with the aggregation of platelets, the provisional extracellular matrix. During this process, fibrin forms long fibers that aggregate and create branched networks to form a fibrin clot. When this process is repeated *in vitro*, the produced clots typically possess poor mechanical properties because natural fibrin is amorphous. These properties are not ideal for load bearing applications, including ligament and muscle repair (Cornwell, 2007). In order to develop fibrin-based scaffolds for use in these applications, the *in vitro* process needed to be modified to produce a stronger, more aligned form of fibrin.

The thread making process for fibrin threads involves coextruding fibrinogen and thrombin into a solution of HEPES, which mimics the biological environment while the fibrin polymerizes. During initial production and development of fibrin microthreads, different variables were tested to determine the optimal parameters for thread production (Cornwell, 2007). Variables tested included: syringe pump extrusion rates, or flow velocity (0.125, 0.250, 0.500 ml/min), speed of the tubing through the bath, or the plotter velocity (550, 1,100, 2,200 mm/min), the temperature (20°C and 37°C), and pH (6.0, 7.4, and 8.0) of the HEPES bath. The rate ratio was determined by dividing the flow velocity by the plotter velocity. As demostrated in the research, a rate ratio of less than 1 was insufficient form functional threads (Cornwell, 2007). These tests determined the properties and relevant tolerances necessary for the production process, which were importance parameters to consider in the development of an automated process.

The rate ratios were varied to determine the effect on microthread properties, based on the ultimate tensile strengths of the threads. When looking at the thread diameters, there was a positive linear increase with respect to the rate ratio; meaning that as the rate ratio increased an increase was also observed in the wet diameter. A positive linear relationship was also observed in the strain at failure plotted against rate ratio (Cornwell, 2007). The varied temperature and pH of the HEPES bath also changed the microthread mechanical properties. Threads produced in a pH of 6.0 had a significantly lower ultimate tensile strength than threads produced in 8.5 and 7.4 pHs. Threads produced in a bath with a pH of 7.4 were shown to have the highest tensile strengths. As for the temperature of the bath, the room temperature (25^{o}C) bath produced threads with significantly higher tensile strengths than those produced in the 37^{o}C bath (Cornwell, 2007).

During the design of an automated system, it was important to take these parameters and all attempted values into consideration. This research determined the importance of having accurate parameters for bath temperature and pH, as well as extrusion rate in a new system, in order to ensure threads with the highest ultimate tensile strength.

One of the primary advantages of using fibrin microthreads in tissue engineering applications is the potential interactions with cells and growth factors (Cornwell, 2007). Fibroblasts were seeded to bundles of 10 threads at a concentration of 300,000 cells/ml. The two groups that were tested were control threads and fibroblast growth factor – 2 (FGF2) loaded threads. Cell proliferation was examined at days 2, 5, and 7, using a 17mM Hoechst nuclear reagent. Images were taken at each of these days and cell counts were determined. The results of this study showed that fibrin microthreads supported cell adhesion and proliferation (Cornwell, 2007). This research showed that fibrin microthreads were capable of facilitating cell and tissue growth similar to its natural function in the body, by showing fibroblast proliferation and alignment along the thread's longitudinal axis (Cornwell, 2007).

Another function of fibrin microthreads that was evaluated was cell outgrowth. Culture plates with fibroblast-populated collagen lattices were placed in combination with the microthreads. Every 24 hours the threads were imaged and the measurements of the furthest cell from the thread to the edge of the platform were recorded (Cornwell, 2007). No significant difference for fibroblast attachment to the fibrin microthread bundles was observed between the FGF2 loaded bundles and the control bundles. Fibroblast proliferation occurred on both FGF2 loaded and control bundles, but as time increased the fibroblast proliferation was much greater in the FGF2 loaded microthread bundles. The addition of FGF2 into the microthread bundles increased the outgrowth rate, but the concentration of FGF2 did not change the velocity of fibroblast outgrowth. In this portion of the study, cell and cytoskeleton alignment was also investigated. Fibroblasts, throughout the course of this study, aligned along the long axis of the microthreads and the microthread bundles. When microthread bundles were used, cells aligned and aggregated in the grooves between individual microthreads, forming a thin elongated morphology. The cytoskeleton of the fibroblasts showed preferential direction oriented with the direction of the microthreads. Figure 1 and Figure 2 show images of some cell alignment and attachment to fibrin microthreads.

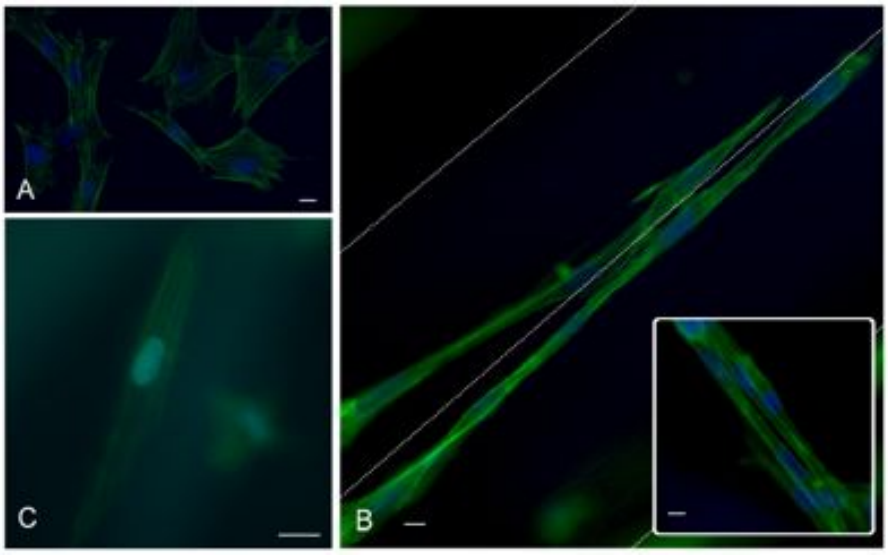


Figure 1: Cell and cytoskeleton alignment on fibrin microthreads. Fibroblasts were seeded on bundles of fibrin microthreads and stained for actin using phalloidin (green) and nucleic acid using Hoechst (blue) after 4 hours of attachment. (A) Control fibroblasts showed preferential alignment or orientation, cultured in plastic culture dishes. (B) Fibroblasts on fibrin threads showed alignment along the long axis of the fibers (white dashed line) and in the grooves between fibers (inset shown without drawn thread boundary line). (C) Fibroblast actin is oriented in parallel arrays along the long axis of fibrin microthread. **(Cornwell, 2007)** [Scale bars = 5 μm].

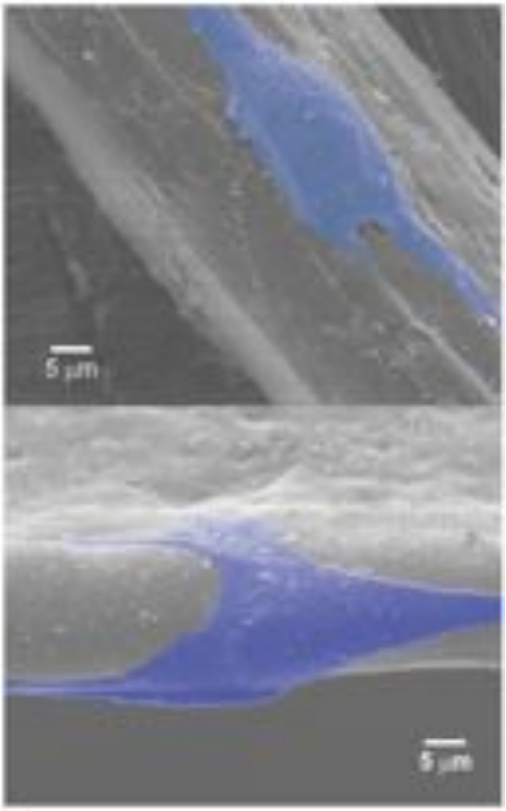


Figure 2: Cell alignment on individual fibrin microthreads. Samples were taken during the late stages of the cell outgrowth assay (Day 7), fixed, and dehydrated for imaging with SEM. Individual cells can be seen aligned along the long axis of the microthread (blue highlighting added for emphasis during analysis, post-acquisition) **(Cornwell, 2007)**.

One way to further increase the mechanical strength of the threads was through crosslinking. A study investigated the effect of UV crosslinking on the mechanical properties of fibrin microthreads. Normal human fibroblasts were cultured and attachment and proliferation was also investigated. The mechanical properties of the threads are summarized in Table 1 (Cornwell K.G., 2007). Results show a UV exposure time of 40 minutes produced threads with the highest ultimate tensile strength and modulus.

Table 1: Mechanical properties of fibrin microthreads with increased UV crosslinking,

UV Exposure Time (min)	Power (J/cm^2)	Sample Size (N)	Strength UTS (MPa)	Failure Strain, SAF	Modulus, E (MPa)
0	0.00	22	4.48 ± 1.79	0.31 ± 0.15	60.70 ± 25.71
20	8.55	19	5.29 ± 2.78	0.26 ± 0.13	88.54 ± 27.53
40	17.10	19	7.82 ± 3.10	0.27 ± 0.08	111.39 ± 67.48
60	25.66	19	6.58 ± 3.03	0.25 ± 0.11	103.89 ± 53.47
120	51.31	11	5.88 ± 3.45	0.19 ± 0.12	81.41 ± 66.90

Human fibroblast migration, attachment, and proliferation on fibrin microthread bundles were assessed to determine the biocompatibility of the microthread bundles. For the migration, proliferation, and attachment study, polypropylene was used at the control because it is generally considered biocompatible and would not greatly encourage cell proliferation or outgrowth. After one day of being seeded on the microthread bundles, fibroblasts attached to both the crosslinked and uncrosslinked threads and both showed more attachment than on polypropylene threads. On all thread types, the fibroblasts aligned with the long axis of the thread and in the spaces between individual threads in a bundle. By day 7, viable cells were visualized on all threads, polypropylene and fibrin. Fibroblasts on uncrosslinked threads exhibited robust proliferation and were completely confluent, taking up the whole area of the dish and threads (Cornwell K.G., 2007).

2.3.2 Fibrin Microthread Applications

A scaffold that is morphologically similar to tendon, ligament, and muscle that can be produced *in vitro* and seeded with cells for delivery is necessary to help heal large scale wounds. In large defects where the body cannot completely regenerate tissue, fibrin microthreads can provide a scaffold that is morphologically and mechanically similar to native tissue, while promoting regeneration of native tissue and revascularization to restore normal function. Unlike fibrin gels, which can support cell growth and proliferation, fibrin microthreads have greater

structural similarity to many native tissue including skeletal muscle, tendons, and ligaments (Page, 2011).

2.3.2.1 Skeletal Muscle

Designed as a scaffold and delivery vehicle for cells in tissue regeneration, a previous study has used fibrin microthreads to deliver cells to skeletal muscle defect sites and promoted tissue regrowth (Page, 2011). When compared to a control group of wounds allowed to heal without microthreads, fibrin microthreads seeded with mature muscle cells showed increased muscular regeneration at 1 and 2 weeks (Page, 2011). According to these results, Page *et al* concluded that fibrin microthreads were a suitable scaffold and vehicle for cell delivery in large skeletal muscle defects. It was also determined that microthreads aided in promoting regeneration of native muscle tissue, reduced collagen formation, and restored muscle strength to near 100% after 90 days when compared to original tissue strength (Page, 2011).

2.3.2.2 Ligament and Tendon

Tendons and ligaments are made up of many small collagen fibers. These individual fibers are used together to form bundles, which form tendons and ligaments, as seen in Figure 3:. Fibrin microthreads can be bundled together and have shown the potential to exhibit similar morphology to collagen thread bundles and patient tendon/ligament (Cornwell, 2007). Fibrin microthreads show morphological similarities to collagen (Figure 4). This shows similar diameters in the fibrin and collagen threads, but fibrin threads seem to exhibit a smoother surface than that of collagen (Cornwell, 2007).

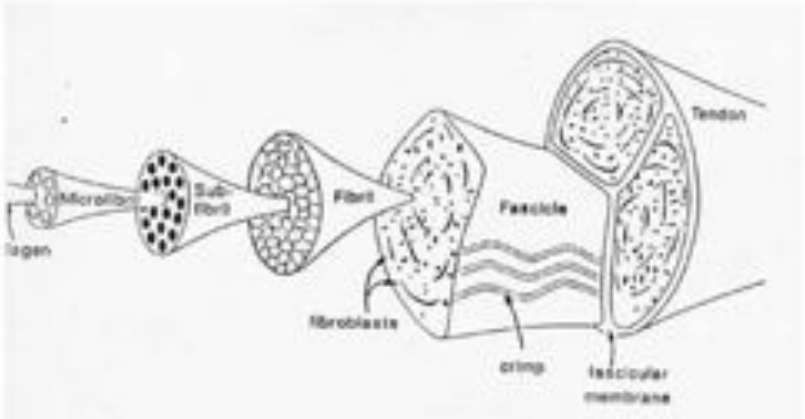


Figure 3: Structure of tendon. The tendon is a bundle of fascicles, comprised of fibril, sub-fibril, and microfibrils bundles, which are made up of individual fibers of lagens. Image from **(Tissue Mechanics II - Soft Mechanics., 2013)**.

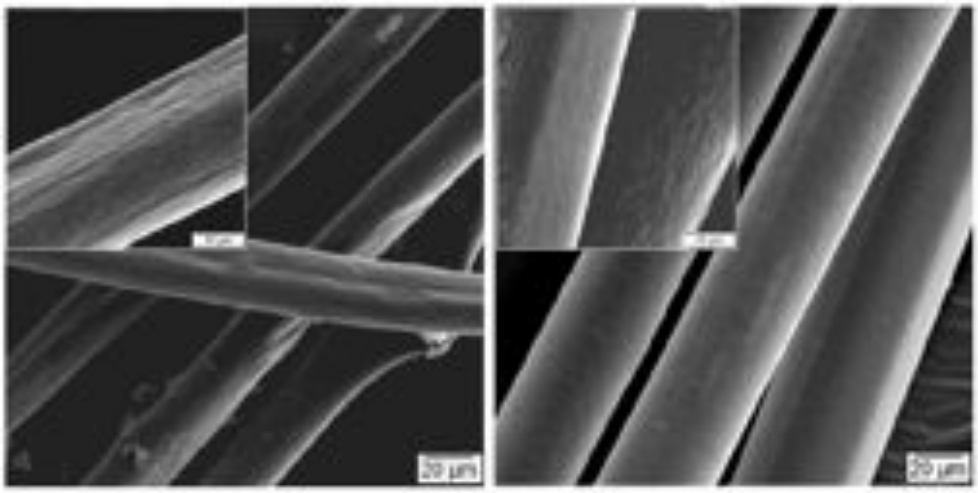


Figure 4: SEM comparison of collagen and fibrin microthreads. Collagen is shown on the left and Fibrin on the right. Note, both threads have consistent diameters and exhibit cylindrical morphology. Image from **(Cornwell, 2007)**.

2.3.2.3 Cell delivery

Fibrin microthreads have the potential to fill a niche in biomaterial scaffolds for tissue engineering and site specific cell delivery. Fibrin microthread bundles have been used experimentally to structurally support attached human mesenchymal stem cells (hMSCs) *in vitro* (Proulx *et al*, 2010). This study showed that hMSCs can be secured and cultured to fibrin microthreads and remain multipotent with the ability to diffrentiate. The fibrin microthreads were bundled and sutured to collagen gels via surgical needles while maintaining cell abilities including vacuole formation, differentiation into adipocytes, and osteocyte differentiation. The maintaining of hMSC differentiation indicated that the microthread bundles could be a viable method to deliver stem cells for tissue regeneration (Proulx, 2011).

2.3.3 Production of Fibrin Microthreads

Fibrin microthreads are currently fabricated through a manual production process. This manual process has several aspects that cause variations in the properties of the threads, all of which depend on the lab technician. Inconsistencies in fibrin microthread production have slowed down progress in terms of fully characterizing uniform microthreads for tissue engineering applications as well as limited the reproducibility of threads with uniform properties between labs. This section explores the current manual production process, the variability within specific steps, as well as the effects of post-production modifications on fibrin microthread properties such as including stretching.

2.3.3.1 Manual Process

Originally designed to coextrude solutions of collagen and fiber formation buffer for production of collagen microthreads (Cornwell K.G., 2010), a dual extrusion system has been modified for production of fibrin microthreads (Cornwell K.G., 2007). The process includes co-extrusion of fibrinogen and thrombin using a blending connector (Figure 5) into a bath containing a solution of distilled water and 10 mM HEPES. According to previous methods, thread materials were coextruded and hand-drawn across the bottom of the bath and allowed to polymerize. The system used an extrusion machine with a stabilizing crosshead on a threaded rod to extrude two 1 cc syringes filled with thawed solutions of thrombin and fibrinogen. The thread materials were coextruded through polyethylene tubing onto a Teflon-coated pan to increase polymerization and reduce adhesion to the pan. The threads were hand-drawn across the Teflon pan at a rate dependent on the movement of the lab technician.

After approximately 12 minutes of polymerization, the threads were removed from the bath of HEPES using two forceps at the ends of each thread. Each thread was removed from the pan using a lifting, turning motion until the thread was completely removed from the pan but remained in the bath. The lab technician stretched each thread at a constant rate to approximately 225% of its initial length and placed it to dry overnight (Grasman, 2012). After drying, the threads were stored in a desiccator until use.

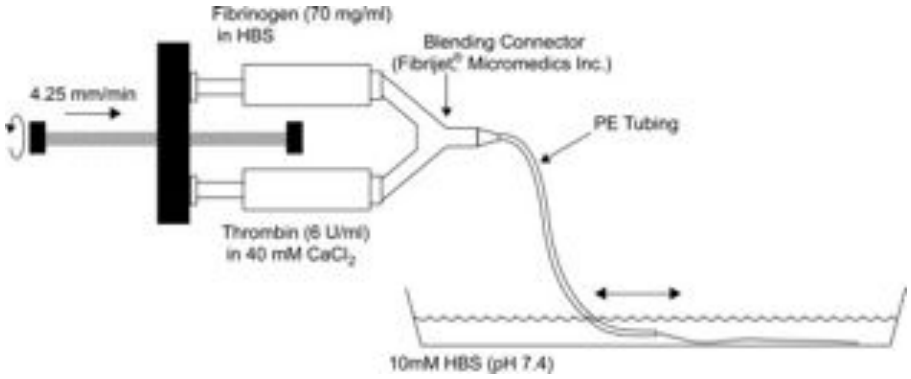


Figure 5: Schematic drawing of fibrin extrusion system – Illustrates the coextrusion system used to manually produce fibrin microthreads into a bath. (Cornwell, 2007)

The manual production process does not necessarily stop after stretching and drying but can include other post-production modifications such as crosslinking and sterilization. Crosslinking of threads increases the bonding between thread molecules and stiffens the threads (Grasman, 2012) and (Cornwell, 2007). A previous study examined threads crosslinked using UV light to produce varying mechanical properties as well as varying proliferation properties

(Cornwell, 2007). Sterilization can be done as another post-production modification using either isopropyl alcohol or ethanol to produce threads free of pathogens. These post-production processes can manipulate thread properties but are subject to the variability within thread production.

Ideally, threads would be extruded and handled uniformly throughout the production process to ensure consistent properties. However, the current process is inconsistent, and leads to variable threads. Variability is the most pertinent obstacle that needed to be overcome for this project. Areas of variability in the current manual process stem primarily from the hand-drawing of threads into the HEPES bath. The extrusion head rate is held constant, but the lab technician controls the speed of drawing threads into the bath. Other variables that produce inconsistent threads include bubbles in the extrusion system, and user error in terms of consistent drawing rate. Bubbles within the system hinder production process because the thread may tear when removed from the bath or not form at all. An inconsistent drawing rate greatly influences the threads because it produces threads with beaded diameters. Threads produced with minor pauses in drawing rate have bulges in diameters that lead to changes in mechanical properties.

2.3.3.2 Stretching of Fibrin Microthreads

After extrusion, drawing, and removal of fibrin microthreads from the Teflon pan, each thread is stretched to increase production yield and enhance mechanical properties. This manual process is another source of variability created in the production process. Stretching procedures align the polymer fibers within the microthreads and enhance mechanical and structural properties. While unpublished data indicates that stretching threads to differing percentages produced threads with increased mechanical and structural properties, manual stretching is limited in terms of inconsistent stretch speed and stretch percentage.

Unpublished data suggests stretch percentage is a factor that determines mechanical properties of fibrin microthreads during production. During the study, fibrin microthreads were stretched to different stretch percentages and mechanically loaded to failure. Mechanical properties affected by stretch percentage include ultimate tensile strength (UTS), strain at failure (SAF), and stiffness (which is referred to as maximum tangent modulus (MTM)) depicted in Figure 6. Ultimate tensile strength, which was defined as the maximum stress a thread experienced before failure, was significantly affected by stretch percentage. Other mechanical

parameters affected by stretch percentage included strain at failure, which was defined as the maximum strain a thread experienced before failure, and maximum tangent modulus, which was calculated as the maximum stiffness of a thread before failure (Figure 7C). As illustrated in Figure 7, threads stretched to greater stretch percentages showed significant decreases in SAF values (Figure 7A), significant increases in UTS values (Figure 7B), and significant increases in MTM values (Figure 7C). However, the standard deviations in mechanical property averages are large due to the manual production and stretching processes.

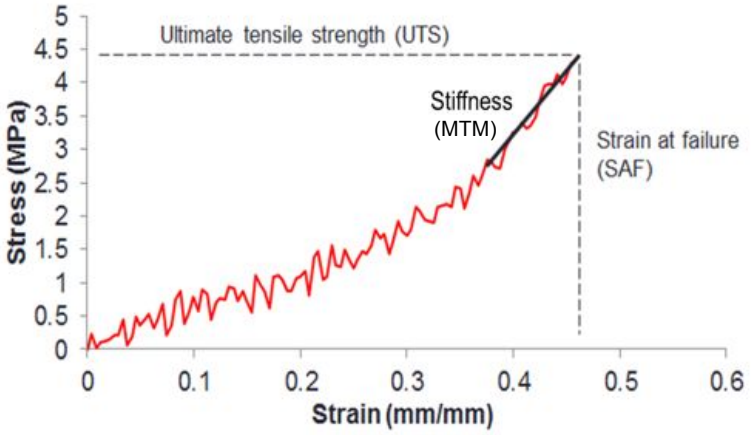


Figure 6: Stress strain curve of fibrin microthreads under mechanical loading

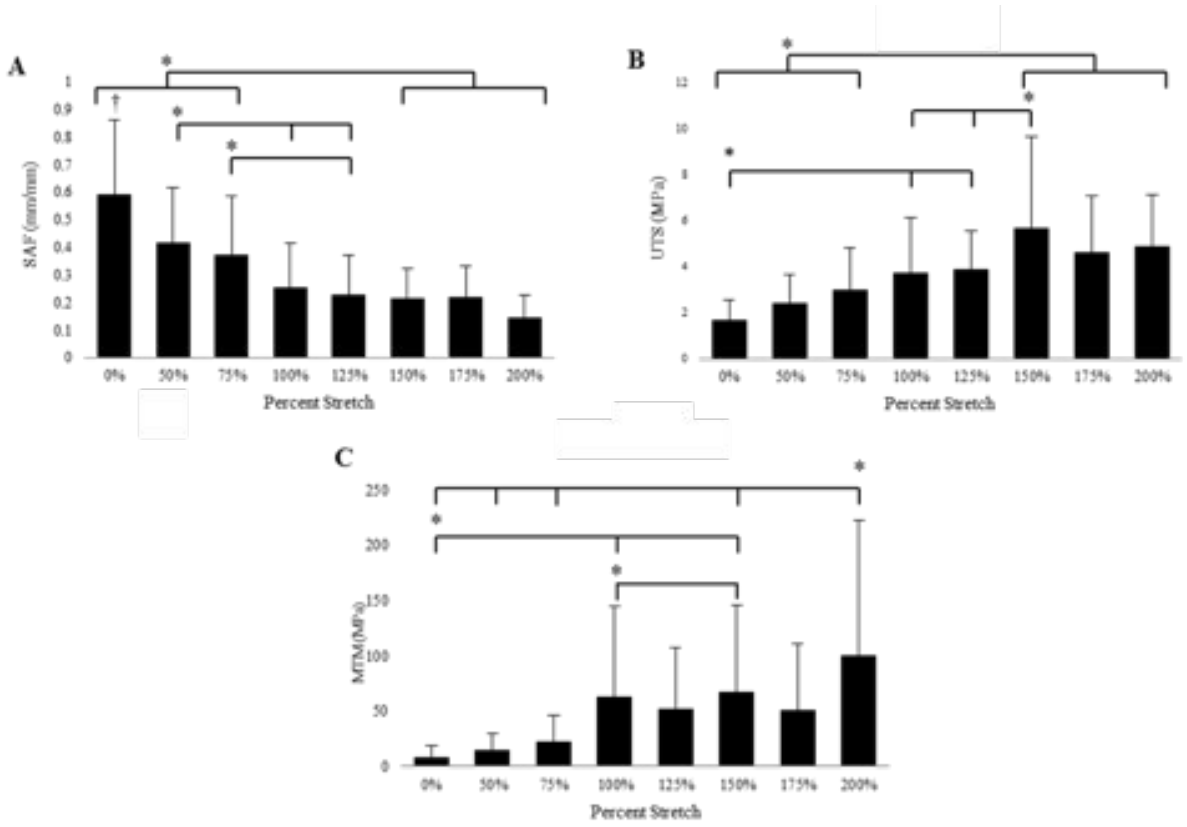


Figure 7: Mechanical data from unpublished research highlighting differences in mechanical properties in relation to stretch percentage. (A) Significant decreases in SAF between higher and lower stretch percentage groups. (B) Significant increases in UTS as stretch percentage increases. (C) Significant increases in stiffness (MTM) values as stretch percentage increases. Note: Deviation bars depict standard deviations († indicates statistical significance from all other groups. * indicates statistical significance from indicated groups using one-way ANOVA with Holm- Sidak post hoc analysis ($p \leq 0.05$), $n \geq 29$).

Stretching fibrin microthreads also plays a significant role in terms of structural stability of the polymer molecules. Stretching microthreads to different stretch percentages significantly decreases dry and wet diameter measurements when comparing low stretched threads to high stretched threads (Figure 8). The degree of alignment may be correlated to stretch percentage and can be measured in terms of thread diameters and scanning electron microscope (SEM) images (Matsumoto, 2007) (Figure 9). Large standard deviations in thread diameters indicate a high level of variability, which can be attributed to the manual production process. The frequent incidence of high variability between batches of stretched microthreads confirms the need for an automated stretching system to decrease the variability.

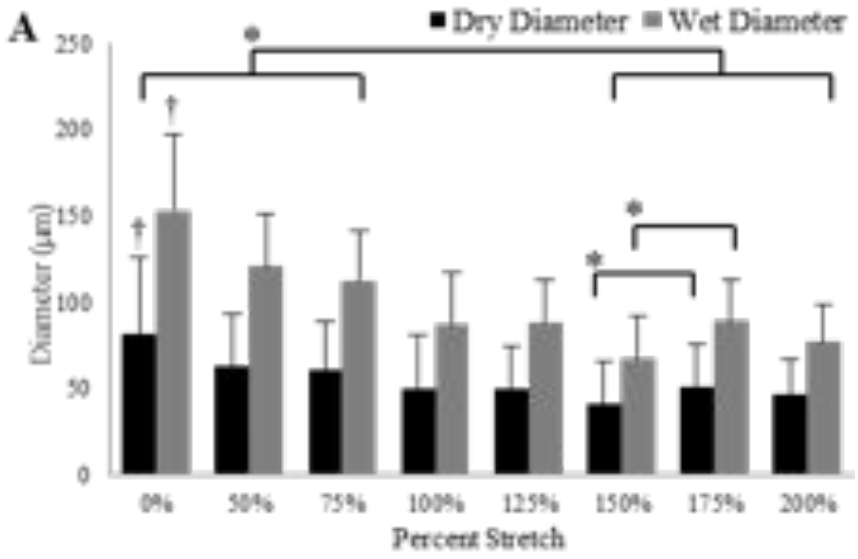


Figure 8: Structural data from unpublished research indicating changes in diameter in correlation to stretch percentage († indicates statistical significance from all corresponding groups. * indicates statistical significance from other groups using one-way ANOVA with Holm- Sidak post hoc analysis ($p \leq 0.05$), $n \geq 29$).

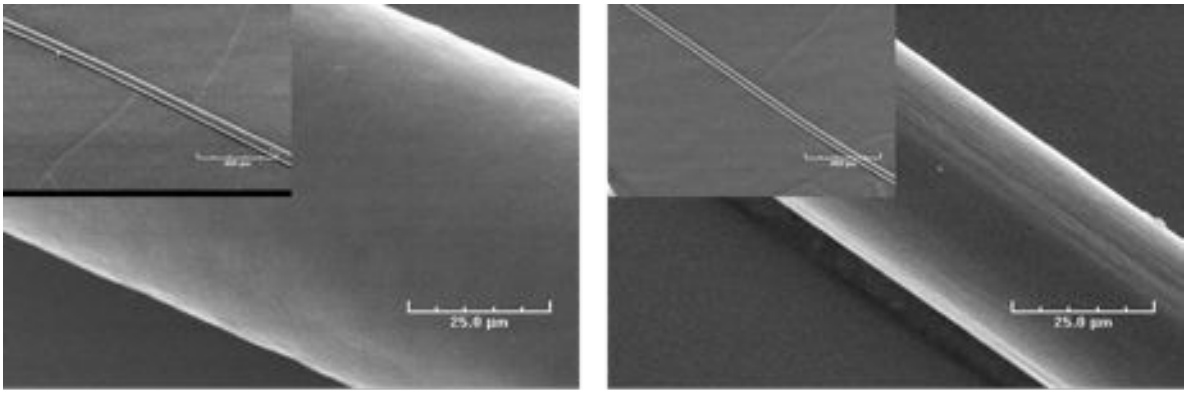


Figure 9: SEM Images highlighting polymer alignment – show diameter change between 75% stretch (left) and 175% stretch (right) for microthread highlighting alignment of polymer molecules.

Based on preliminary data presented on controlled stretching of fibrin microthreads, researchers could be able to tailor the properties of fibrin microthreads based on how much they are stretched. Mechanical properties, including UTS and stiffness were altered to significantly different values when compared to unstretched threads. Significant changes to mechanical and structural properties can also be achieved through crosslinking but it may decrease cellular adhesive and alignment properties (Cornwell, 2007). Stretching fibrin microthreads is advantageous to crosslinking procedures because it can produce similar mechanical properties while maintaining cellular properties.

Stretching fibrin microthreads has a significant effect on their mechanical and structural properties and currently, as mentioned previously, is a manual process. The manual process produces threads with variable properties due to the manual inaccuracy of the lab technician. To ensure stretching is accurate and properly characterized, it must be incorporated into an automated process to control stretch percentage and stretch speed according to the user's needs.

2.3.4 Patents and Previous Work

As part of the design process, it is important to consider what has been previously designed and built to ensure innovation in terms of design ideas and solutions. This process creates a wealth of knowledge regarding successes and failures, allowing new designs to evolve. In terms of microthread production, devices have been built for different types of threads including collagen and fibrin. Collagen microthreads and fibrin microthreads have different processing techniques, but collagen processing systems provided a base from which the design team created their design.

2.3.4.1 Current Laboratory Method

The current laboratory method of producing fibrin microthreads begins with the co-extrusion of fibrinogen and thrombin, which when combined, create the provisional matrix in blood clot formation (Cornwell, 2007). Currently, fibrin microthreads are produced using a syringe pump that coextrudes controlled volumes of fibrinogen and thrombin. The combined solution is guided through polyethylene tubing by hand into a 10 mM HEPES bath. To create threads, the solution is drawn in lines into the HEPES solution on a Teflon pan. The threads must polymerize for ten to fifteen minutes in the salt solution before they are removed, stretched by hand, and suspended on boxes to dry. This process can be done at room temperature, indicating it does not require the use of a heated water bath (Cornwell K.G., 2007). Currently, threads are removed from the pan using forceps and guided through the HEPES solution as they are stretched to approximately 225% of their initial lengths (Grasman, 2012). However, during hand-drawing of threads, threads can be non-uniform in morphology and vary in mechanical strength, including ultimate tensile strength, stiffness, and modulus.

2.3.4.2 Current Automation Capabilities

Various projects have focused on designing an automated production process for microthreads, such as collagen. Previous work has been done to automate the extrusion process

of collagen thread. A system built in 2010 focused on the incorporation of an automated extrusion head for collagen threads. This device, as shown in Figure 10, ensured threads were drawn at a constant rate and drawn in straight, consistent lines to minimize variability of collagen threads (Ellis, 2010).

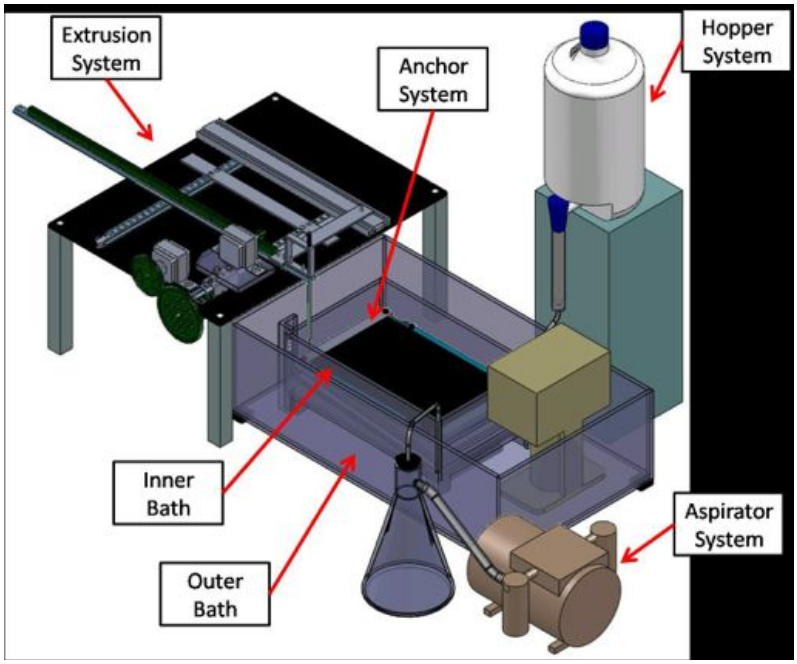


Figure 10: Model of 2010 MQP extrusion and bath system (Ellis et al, 2010).

The collagen extrusion system was able to successfully reduce the variability between threads within a batch, and batch-to-batch. The system was programmed using EasyC Pro to draw 15 threads, 1 cm apart, across a 16 cm pan. The system automated the extrusion process while minimizing variability. The uniformity of the threads was verified using dry and wet diameter testing and mechanical tests to determine the ultimate tensile strength (Ellis, 2010). Without full automation, the process was still very much hands on, and the device did not assist with buffer or bath changes. The system was able to accomplish its goal of automated extrusion and can be applied for different threads including fibrin; however, it does not incorporate any post-production modifications such as stretching. The system is designed to manufacture collagen threads with uniform properties and can serve as a model for fibrin production systems. Even still, because of a lack of automated stretching in this system, there exists a need for a newly designed system that produces fibrin threads and automatically controls stretching.

2.3.4.3 Patents

It is difficult for a manual production process to extrude uniform threads because human error becomes a factor. Different extrusion systems have been created to automate the production of an amorphous polymer and produce uniform threads. For this reason, the design team performed patent searches to evaluate the systems used before and understand current production designs. The following patents are related to this project in that they pertain to the creation of stretched threads for different applications.

Organogenesis Inc. designed a system (Kemp, 1995) that could produce un-crosslinked collagen fibers with ultimate tensile strengths greater than 1 MPa and crosslinked collagen fibers with ultimate tensile strengths of 45 MPa. Collagen threads could then be modified post-production to form a scaffold for tissue constructs. Figure 11 shows the device as threads are stretched and pulled through a multiple bath system (as shown by points 10 thru 20). The device extrudes collagen from a syringe pump (3), through a tube (4) and a blunt needle (5), into a dehydrating salt bath (12), with a recirculation pump (13). As the threads are pulled through the bath system, they are rinsed with a rinsing agent (22). As they are pulled up on the pulleys at the end of the system (43 thru 47), they are brought into a drying cabinet (30) and dried with a heated blower (32) as they are slowly pulled and stretched along the pulley system (Kemp, 1995).

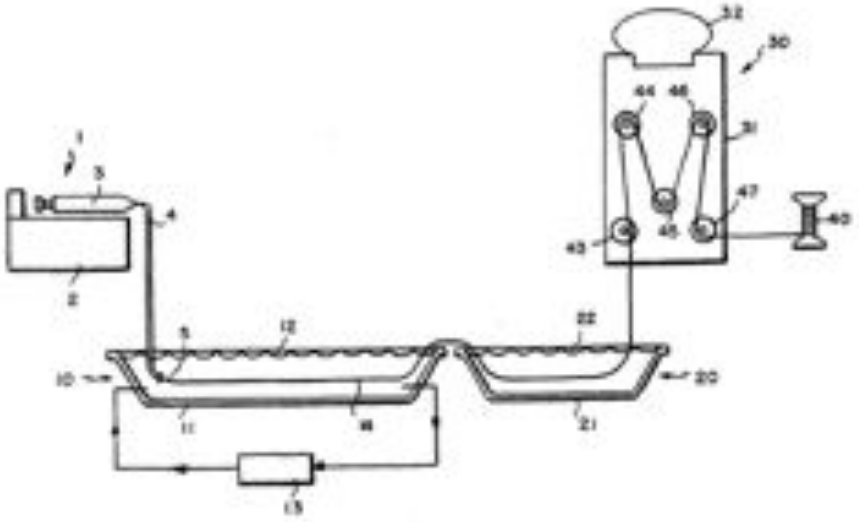


Figure 11: Organogenesis Inc. collagen thread extrusion device (Kemp 1995).

Salo *et al*. patented a device in 1952 to extrude collagen fibers. The patent claimed the orientation of the thread structure affected the strength and enzymatic resistance. The Salo *et al.* design, as shown in Figure 12, consists of a single collagen thread extruded by a pump, and pulled into a nozzle over a dehydrating bath, where the thread is stretched by gravity while traveling into an acetone bath. The fiber is then further stretched by a pulley. There is another distilled water bath which washed away the acetone, and the fibers were finally dried in tension and rolled onto a spool (Salo Torsti P., May 27 1952).

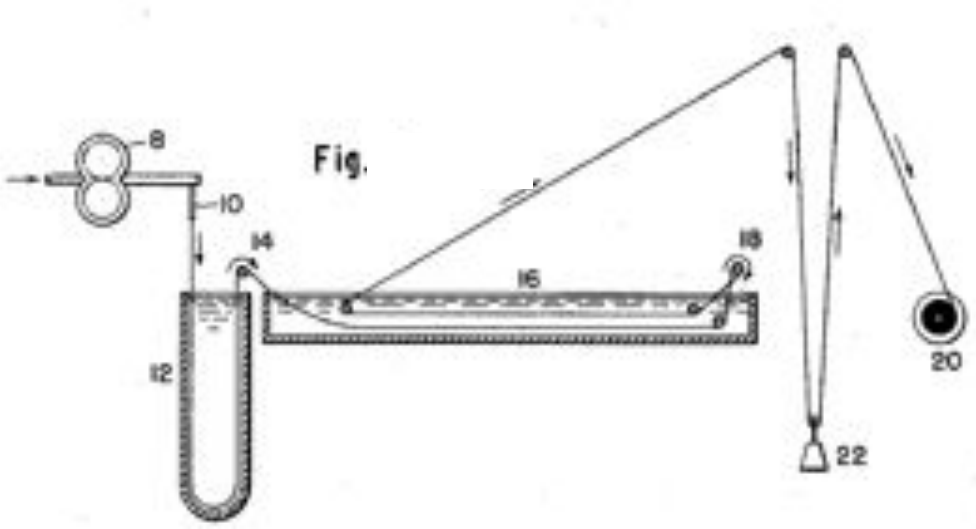


Figure 12: Collagen extrusion device (Salo *et al*., 1952).

Review of these patents gave the design team ideas as to how biopolymer microthreads are produced and spooled. This process is very similar to the process needed to produce fibrin microthreads, and the information from these patents allowed the team to better understand different methods of bath processing and stretching as well as extruding. This patent review has refined the design team's understanding of the design space and the design requirements in terms of what has worked for collagen, and narrowing what might work for fibrin. These design requirements include low stress on the threads, performed an adequate hydrating bath and drying system, and were able to include a co-extrusion system to create the threads.

3 Methodology

In order to create a complete project strategy for an automated processing system for fibrin microthreads, the team needed to interpret the initial client statement, develop objectives from which to establish functions of the device, and revise the client statement based on a qualitative assessment of the important goals of the project. With this information the team gained a full understanding of the problem statement and established preliminary design ideas.

3.1 Initial Client Statement

During the design team's first meeting with the advisor and client, the team was given the challenge of developing an automated system for the production and modification of fibrin microthreads. The current manual production of fibrin microthreads includes extrusion, stretching, removal, and drying, which produces inconsistent threads. The following is the initial client statement the team received:

"Design and develop a system to facilitate fully automated fabrication and post-production modification of fibrin microthreads." – Professor G. Pins and Jon Grasman

Although there have been previous attempts to create processing systems for biopolymer microthreads, current fibrin-specific production systems fall short in terms of modifications and reproducibility. From our initial client statement and the team's knowledge of the problem at hand, the team was able to develop a project strategy and work toward a list of objectives.

3.2 Objectives and Constraints

In order to create a plan for the project, it was necessary to develop a working set of objectives and constraints which could be discussed and manipulated between the client and design team. Initial project objectives helped the team create a design space for the project. Through meetings with the team's advisor, the objectives and constraints were appended and clarified. Ultimately, the design team was able to separate objectives from constraints and build an objectives tree to base the project on. The design team created a series of pair-wise comparison charts which helped to determine the relative importance of each objective. From there, the team moved forward with a clear definition of the project strategy.

3.2.1 Initial Objectives

To gain a better understanding of the project, the team consulted literature in the fields of tissue engineering, applications of fibrin microthreads, and current production and post-production modifications of the current systems. Additionally, the team reviewed the work of a previous MQP team to understand the fibrin production process and limitations. Finally, the design team created fibrin microthreads with the current manual fibrin production process. Based on the current understanding of the fibrin production process, the team formed a list of preliminary objectives that served as the framework to start the project, as listed in Table 2.

Table 2: Initial objectives and definitions

Initial objectives	Definition
Automated	No human handling of threads from production to use
Extrude threads	Must be able to extrude usable complete threads
Stretch threads	Must be able to stretch threads to between 0 and 400% accurately and uniformly
User friendly	Must be easy to use, and easy to clean
Reliable and durable	Produce threads once a day for 5 years

The design team presented these initial objectives to the advisor and client. After discussion and further evaluation of the project, the list of initial objectives and constraints was updated and clarified in order to better characterize the goals of the project. Evaluation of the initial objects led to a revised list of objects and constraints, further refining the team's design space and project goals, as discussed later.

3.2.2 Constraints

The team understood that for the project to be practical in a lab setting, certain criteria had to be met. Some of the initial objectives were considered essential but after subsequent comparison proved to be constraints due to their significance. Table 3 shows our initial constraints and a definition of each.

Table 3: Initial constraints and specifics

Initial constraints	Definition
Money	Budget of $524
Size	Maximum dimensions of 4x6x4 feet
Time	Must be finished by April 2013
Safety	Must not harm user or bystanders
Materials	Non-reactive or leeching materials
Function	Must stretch threads to accurate percentages
Interface	Must interface with existing extrusion machine

Of the initial objectives, the team concluded that safety and basic system functions were constraints, and thus were taken out of the initial objectives table. If the system was unsafe for use or did not perform desired tasks, the system would go unused. The system also had to be made of non-reactive, safe materials to ensure that the integrity of the threads was not compromised. If the threads reacted to the material it was made on, the threads could be deemed toxic or unusable. The system must also stretch the threads, if it does not accomplish this, then would be an unsuccessful system. The system was required to interface with the previous extrusion machine in the lab to automate the extrusion of threads. Other constraints included time, space, and a limited budget. The project must be completed within the confines of the academic year while constructing within the constraints of our lab work space. The maximum design space for the device as decided by the client was 4x6x4ft. Of the $624 team budget, $100 will be used on basic lab materials; leaving the team with $524 to build a functional device.

3.2.3 Revised Objectives

After evaluation of initial objectives and subsequent discussion of redefined constraints, the team finalized an objectives tree with four high-level objectives. From these high-level objectives, the team was able to break down the objectives further into a branched objective tree (Figure 13). This tree allowed the team to rank sub-objectives under corresponding high-level objectives and understand the relative importance of all objectives.

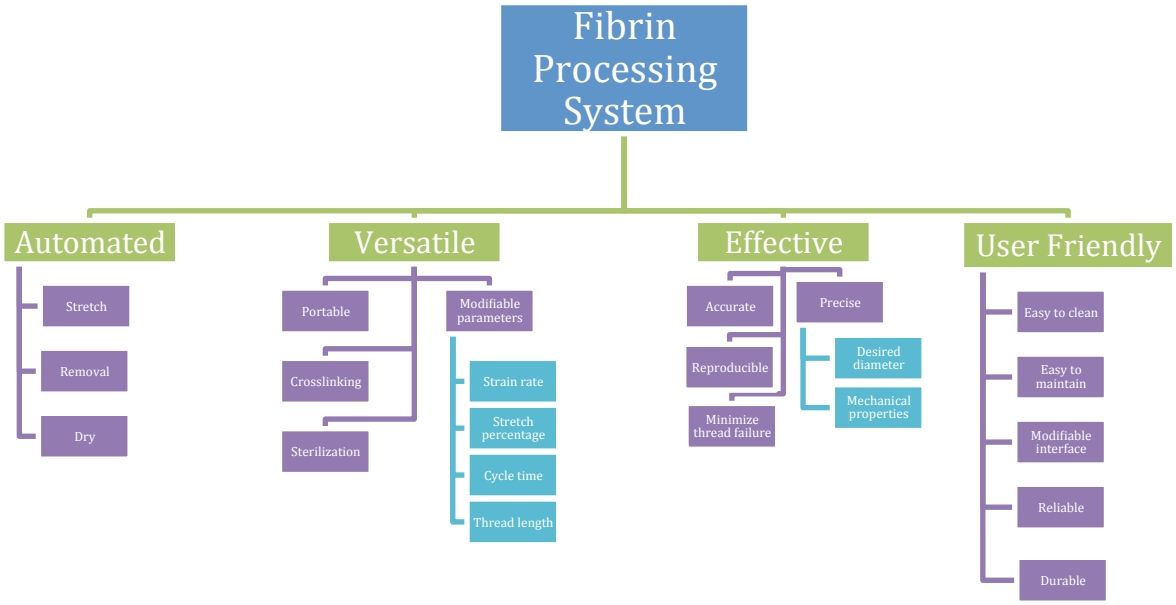


Figure 13: Objectives tree - Hierarchical breakdown of project objectives.

The four high-level objectives of the project were automation, versatility, user friendliness, and effectiveness. Each of these objectives was broken down further into sub-objectives, which helped further describe the project goals and gave the team the ability to prioritize its goals in a qualitative assessment.

One of the major project objectives was automation of the processing system. This was understood as the minimization of human interaction of the fibrin microthreads during production and post-production modifications. Limiting the human interaction in the system would reduce the manual labor and variability. Previous efforts to automate production of fibrin microthreads were not able to completely eliminate manual interaction during production. Automating the entire process from extrusion to stretching, and then to possibly a post-production modification, would minimize variability while optimizing controlled production of threads. The team decided that creating a fully automated device with added production modifications was an essential objective.

The versatility of the system was an important objective to outline because a versatile system would allow for individualized, controlled modification of threads. Although post-production modifications including sterilization and crosslinking were important, the design

team defined a versatile system as one with modifiable parameters such as stretch speed and stretch percentage because sterilization and crosslinking were steps outside the scope of this project.

The team determined that effectiveness of the system was an essential objective because the system must create reproducible threads with uniform properties. For the system to be effective, the team specified that the system needed to be accurate, precise, create reproducible batches of threads, and minimize the failure of threads.

Lastly, the team considered user-friendliness to be an important objective. To create a usable system that could be practical for laboratory thread production it must be easy to use. A practical system must be durable and reliable to minimize manual production and ensure proper production with minimal error. Similarly, the team decided that a user-friendly interface would promote consistent and proper use of the system. A user-friendly device would be easy to clean, easy to maintain, and have a modifiable interface. A modifiable interface would allow the user to input parameters and produce consistent threads to exact specifications.

3.2.4 Qualitative Assessment of Objectives

Once the objectives had been fully defined and grouped, it was necessary to qualitatively rank them to determine the most important objectives and to revise the team's client statement. Rankings were done by the client, the user and the design team via pair-wise comparison charts to determine the relative importance of each objective and respective sub-objectives. These pair-wise comparison charts can be found in **[Appendix A]**. Each objective was compared side by side with the other objectives and ranked based on importance. The objective in the horizontal row scored a 1 over the objective in the vertical column if was more important, a 0 if it was less important, and a score of ½ if both objectives of were of equal importance.

In order to determine the relative importance of each objective, the design team compared the results from each of the pair wise comparison charts. The total was derived from developing an average from the input from the design team, client and user, with the user and client weighted each 40% and the design team only 20%, using the following equation:

$$Total = (0.4 \text{ x User}) + (0.4 \text{ x Client}) + (0.2 \text{ x Design team})$$

The first pairwise comparison chart compared the high-level objectives and determined their relative importance. Table 4 shows the resulting totals from the client, user and design team's pair-wise comparison exercise for high-level objectives.

Table 4: Level one idea comparison chart

Objective	Design team	Client	User	Totals
Automated	2	3	2	2.4
Versatility	0.5	1	1	0.9
User friendly	0.5	0	0.5	0.3
Effectiveness	3	2	2.5	2.4

The two most important main objectives, as identified by the pair-wise comparison chart, were automation and effectiveness, followed by versatility. Originally, the team thought effectiveness of the system would be the most important main objective because of the current reproducibility problems. After discussion, it became clear that human handling of the threads created batch-to-batch variability, not necessarily just the effectiveness of the production process as a whole. Because of this, a system that was automated to minimize any human interaction in the process was ranked the most important main objective. Tables 5 thru 9 show the results of the pair-wise comparison charts for the sub-objectives for each of the high-level objectives.

Table 5: Pair-wise comparison chart for the sub-objectives of the main objective user-friendliness

Objectives	Design Team	Client	User	Totals
Easy to clean	0.5	0.5	1	0.7
Easy to maintain	1.5	0.5	1.5	1.1
Modifiable interface	3	2	2.5	2.4
Reliable	3.5	3.5	3	3.3
Durable	1.5	3.5	2	2.5

When evaluating the sub-objectives of user-friendliness, the client ranked reliability and durability in the system as the most important objectives (Table 5). The client felt that if the system was not reliable and durable for lab use, then it would not be used, and thus the project as a whole would be a failure. Second to these objectives was the option of having a modifiable interface for the system that would allow for the input of different constraints, which are ranked in subsequent charts.

Table 6: Pair-wise comparison chart for the sub-objectives of the main objective automated

Objective	Design Team	Client	User	Totals
Automated stretch	2	2	1.5	1.8
Automated removal	1	0.5	1.5	1
Automated drying	0	0.5	0	0.2

The design team anticipated the results of the pair-wise comparison chart for the automation sub-objectives (Table 6). Removal and drying of the threads were of equal importance after the main sub-objective of stretching. Because automation was the major objective and stretching was the highest ranked sub-objective, drying and removal were the objectives which the design team decided to add to the initial client statement in order to develop a more accurate, revised client statement.

Table 7: Pair-wise comparison chart for the sub-objectives for the main objective effectiveness

Objective	Design Team	Client	User	Totals
Accuracy	2.5	0	1.5	1.1
Precision	2.5	2.5	1.5	2.1
Reproducibility	1	2.5	1	1.6
Minimize thread failure	0	1	2	1.2

According to the client, the most important sub-objectives categorized under effectiveness were precision and reproducibility (Table 7). It is important to note that the design team defined accuracy as production of threads to published standards. Precision and reproducibility were defined as the system's ability to produce threads of the same properties in a single batch and between batches, within 10% of other threads or batches. At first, the team believed that accuracy would hold a similar weight to precision and reproducibility, but after analysis the team understood that producing consistent threads within a single batch and between batches were more important objectives than reproducing data collected from the hand-drawn method.

Table 8: Pair-wise comparison chart for the sub-objectives of the main objective versatility

Objective	Design Team	Client	User	Totals
Sterilization	2	0.5	0.5	0.8
Portability	0	2	0.5	1
Crosslinking	1	0.5	2.5	1.4
Modifiable parameters	3	3	2.5	2.8

Clearly defining and ranking the relative importance of the sub-objectives of versatility was important to narrow the scope of the project. The client clearly stated the importance of having modifiable parameters (Table 8), which might include stretch percentage and stretch speed. Although initially evaluated as the second most important sub-objective in this group, it was determined that crosslinking was outside the scope of this project. Because portability was ranked higher than sterilization, the design team placed greater importance on making a smaller, portable system over adding more post-production features.

Table 9: Pair-wise comparison chart for the sub-objectives of the sub-objective modifiable parameters

Objective	Design Team	Client	User	Totals
Stretch speed	2.5	1	2.5	1
Stretch percentage	2.5	2.5	2.5	2.5
Cycle time	1	0.5	0	0.5
Thread length	0	2	1	2.5

The results of the pair-wise comparison chart for the modifiable parameters sub-objectives were critical in understanding what aspects of the system needed to be programmed for variability. As seen in Table 9, the stretch percentage for the threads was most important because stretch percentage affects thread mechanical properties. Consequently, cycle time was not considered an important variable parameter in thread production when compared to stretch percentage and thread length.

3.3 Discussion of Desired Functions and Specifications

The pairwise comparison charts of the design team, user, and client illustrated which functions were the most important. The highest-ranked level 1 objectives indicated that the

machine needed to be automated and effective. These objectives were most important because automation should reduce the variability from batch-to-batch and within each batch. As for effective, a successful device would extrude, stretch, and dry the fibrin microthreads.

For specifications, the device should be able to stretch threads to up to 400% of the initial length. The device must be able to make reproducible and precise threads. The device must have components that minimize thread failure; this includes clamps that minimize the stresses on the microthreads. The device must have automated stretching and removal of the microthreads from the extrusion surface. The modifiable parameters and modifiable stretch percentages are important for making customizable threads.

3.4 Revised Client Statement

The team revised the client statement based on pairwise comparison charts filled out by the design team and client. The pairwise comparison charts allowed the team to fully understand the important objectives according to the client and formed the following revised client statement:

"Design and develop a **reliable**, **durable** system to facilitate fully **automated** fabrication of **precise**, **reproducible (±10% UTS, diameter, and stiffness)** fibrin microthreads, with an **automated**, **modifiable** post-production system that **stretches threads (between 0% and 400%) according to the parameters of stretch percentage and stretch speed, set by the user.**"

From the pairwise comparison charts evaluated by both the team and client, the team determined that stretching, automation, and precision were the most important objectives for the system. For the system to be effective in terms of client needs, it must produce precise, reproducible threads which verify the modifiable parameters including stretch percentage and final thread length. It was most important to the client that the system be automated. An automated system that produces threads eliminates manual involvement in fibrin production and thereby reduces variability. The next important objective was the precision and reproducibly of the system. The client needed an automated system that produces consistent threads within batches as well as in between batches. The system must be reliable and durable to avoid malfunctions and constant upkeep. While producing consistent threads, the system must also allow the user to modify thread parameters including stretch percentage, thread length, and

stretch speed. A modifiable system allows the user to produce consistent threads in accordance with desired parameters based on client needs.

4 Alternative Designs

In order to create a complete project strategy for an automated processing system for fibrin microthreads, the team needed to interpret the initial client statement, develop objectives from which to establish functions of the device, and revise the client statement based on a qualitative assessment of the important goals of the project. With this information the team gained a full understanding of the problem statement and established preliminary design ideas.

4.1 Needs Analysis

Once the design team designed and produced a full objective tree and used pairwise-comparison analysis to edit the initial client statement, it was crucial to develop specific needs of the system. A needs analysis was conducted to understand the requirements and specifications of the system.

To understand the requirements of the system, the design team identified objectives and classified them under “needs” and “wants”. A system need was defined as a crucial system function that must be met for the system to be considered a success. A system want was defined as a desired objective by the user and client, but did not have to be met for success. Objectives were classified as wants or needs through pairwise comparison charts and conversing with user and client. Table 10 lists the systemic needs and wants of the system and clearly defines each according to the design team.

Table 10: Systemic needs and wants for the system

Needs	Definition
Reproducible	Ability of the system to produce threads which have consistent batch-to-batch structural and mechanical properties
Precise	Ability of the system to produce threads which have consistent structural and mechanical properties within one batch
Minimize thread failure	Ability of the system to minimize the amount of threads that break during production
Automated stretching	Ability of the system to stretch threads without human handling
Automated removal	Ability to remove the threads from the bath without human handling
Modifiable parameters	Ability of the user to modify the stretch speed, stretch percentage and extrusion rate of thread production
Modifiable stretch percentage	Ability of the user to be able to modify the amount of stretch applied to the threads
Wants	**Definition**
Modifiable interface	Ability of the system to have an interface through which the user can modify parameters
Reliable	Ability of the system to perform desired tasks whenever desired by the user
Durable	Ability of the system to last for over 5 years, being used approximately once a day
Sterilization	Ability of the all parts of the system to be sterilized
Portability	Ability of the system to be easily moved and be placed under a hood to create a completely sterile environment
Crosslinking	Ability of the system to be able to facilitate crosslinking of the threads or interface with a crosslinking system
Modifiable stretch speed	Ability of the user to be able to modify the rate at which the threads are stretched
Modifiable thread length	Ability of the user to be able to modify the initial length of the threads

4.1.1 Systemic Needs

Pairwise comparison charts evaluated by the user, client, and design team were analyzed and quantified based on a procedure discussed later in this section. Briefly, the highest quantitative objectives were identified as needs for the system, including automated stretch and removal of microthreads. These objectives were classified as needs because partially automated collagen system that could be modified to the design team's needs already existed. Therefore, the new fibrin system must automatically produce, stretch, and dry microthreads and integrate with the modified collagen system designed in 2010.

Reproducibility, precision, and minimal thread failure were also classified as needs for the system. One of the main problems with the current manual production system was the batch-

to-batch variability. There is a need for the automatic system to decrease the variability of threads. Without precision, reproducibility, and minimal thread failure, the system would not improve upon the current production and would fail.

Lastly, modifiable parameters and its sub-objective, modifiable stretch percentage were classified as additional needs for the system. After discussion with the client, it was clear that the system needed to stretch threads repeatedly to varying stretch percentages as well as other potential parameters. Modifiable parameters were considered needs for the system because stretch percentage plays a major role in determining microthread mechanical, structural, and cell adhesion properties. The system must be modifiable to allow for a variety of stretch percentages as well as expand stretch research characterization.

4.1.2 Systemic Wants

Understanding the needs for the system was important, but understanding the wants was almost equally as important during the design process. Although needs were essential objectives which should be met for a successful project, wants were objectives which would ideally also be met. Wants were classified during meetings with the client and user, as well as through the analysis of objectives in pairwise comparison charts.

Although the objective user-friendly was not identified as a need, some of its sub-objectives were identified as wants. Reliability, durability, and the existence of a modifiable interface were all identified as wants. These objectives were not essential in terms of device success, but would be included in an ideal system. For the system to be ideal, it would need to be completely reliable and durable, and produce threads precisely, but with limitations in terms of time and budget they were identified as wants.

Sterilization, crosslinking, and portability were also identified as objective wants for the system. The primary goal of the system was to produce and consistently stretch threads while minimizing thread failure. However, in an ideal system, the threads would be produced, stretched, and modified in terms of crosslinking and sterility for other experimental needs, which did not fall within the scope of this project.

Lastly, modifiable stretch speed and modifiable thread length were identified as wants. Even though modifiable parameters were identified as needs for the system, only modification of stretch percentage was a necessity. Modification of stretch speed and thread length were

identified as wants because they were secondary when compared with modification of stretch percentage.

4.1.3 Systemic Needs and Wants Design Matrix

After the objectives were defined and classified as necessary parameters or as wants, they were compared to each other and considered in system design. A design matrix was set up to link design considerations to the identified objectives. The purpose of the design matrix was to understand the quantitative specifications needed to accomplish the needs of the project and determine if client and user wants would conflict with project needs (Table 11).

Table 11: Systemic need vs. want design matrix

Needs	Reproducible	Precise	Minimize thread failure	Automated stretching	Automated removal	Modifiable parameters	Modifiable stretch percentage		**Wants**	Modifiable interface	Reliable	Durable	Sterilization	Portability	Crosslinking	Modifiable stretch speed	Modifiable thread length
Size of system													X	X	X		
Programming of system				X	X	X				X							
Bath material					X						X	X	X				
Frame material			X		X						X	X	X				
Adhesion material	X	X	X														
Thread capacity	X	X															
Maximum stress on threads			X	X	X												
Maximum stretch percentage			X				X										
Maximum stretch speed			X													X	
Total weight of system														X			
Different liquid types				X									X	X	X		
Drainage system					X						X						

As seen in Table 11, the completed design matrix compares needs and wants with corresponding systemic specifications. The columns denote the identified needs and wants for the system, whereas the rows denote specific design criteria. An X denotes affected objectives by

the design criteria. For example, the specifications for the bath material directly affect the automated removal of the threads, as well as the reliability, durability, and sterilization of the system. Because automated removal of threads was identified as a need, any material must meet this objective, even at the cost of sterility and durability. The purpose of this design analysis was to identify the relationships between specific designs and the needs and wants of the system.

4.2 Functions and Specifications

The fibrin microthread production and processing system must perform certain functions to be considered efficient and regularly used. This system must extrude, stretch, remove, and dry the threads automatically and according to user parameters to eliminate manual variability and ensure consistent thread properties.

In terms of extruding threads, the system incorporates an automated extrusion mechanism from a previous MQP, which extrudes threads at a fixed rate onto a surface. The extrusion head can be programmed to move bi-axially and draw threads to specified lengths. In terms of specific functions, the system is programmed to extrude the thread equivalent volume of one thread batch (equivalent combined syringe volume of fibrinogen and thrombin aliquots). The extrusion head is not considered a variable in terms of design parameters because it can be programmed around the extrusion plate and stretching system. A bath system, which both hydrates threads and provides an extrusion surface, must not denature or affect thread properties such as ultimate tensile strength or elastic modulus. The threads are extruded into a 10 mM bath of HEPES solution with a pH of 7.4 at room temperature to ensure proper polymerization.

In terms of stretching threads, the system must secure threads within the extrusion bath and stretch them uniformly for client needs. Functionally, the system must secure threads while minimizing thread failure while accurately stretching threads precisely to desired input parameters including stretch percentage and stretch speed. The threads must be secured to the stretching frame to maximize thread stretch effectiveness. The stretching system must be precise and produce threads within batches that have mechanical properties that at within ± 10% of one another. It must also be accurate; producing threads between batches with consistent properties. In terms of specifications, the extrusion process must stretch threads to between 0 and 400% based on initial length within the bath system. The stretch percentage parameters are important specifications because they will influence the extrusion length of the threads and the overall size of the processing system. The threads must be submerged in the bath system while they are

stretched because of inconsistent drying times. The purpose of creating and stretching threads accurately and precisely to controlled lengths ensures that threads will have consistent structural and mechanical properties.

After the threads have been stretched, the system must automatically remove the threads for the drying phase of the process. This can be accomplished by removing the stretching system completely, or by draining the extrusion bath. The system must remove the threads from the bath system to complete the polymerization process, while minimizing thread failure and minimizing extra stretching.

Other general functions of the processing system include modifiable parameters and full automation. The system must be modifiable in terms of stretch percentage, stretch rate, and thread length to meet the user needs. A user-friendly computer interface would ensure proper system use and allow for input parameters from the user. Full automation of the production process from start to finish would eliminate manual involvement and therefore limit variability.

4.3 Design Alternatives

Once the design team fully quantified the important needs for the system, they brainstormed ways to satisfy these needs. In two main brainstorming sessions, the team generated and shared ideas concerning the entire system and individual steps including frame adhesion, stretching, hydration bath, removal, and drying. Initially, each design team member created design alternatives for various aspects of the production process. After initial brainstorming, secondary ideas and branched ideas surfaced and were debated in terms of general feasibility.

4.3.1 Frame and Adhesion

During fibrin production, threads are extruded and must be anchored to a frame that is later used to facilitate stretching. This frame must minimize thread damage and anchor threads during stretching and drying. Proper thread adhesion is essential in determining the success of the system because it ensures uniform stretching of threads while maximizing production value but can be accomplished in many diverse ways including metal pegs, rollers, flat clamps, rotational clamps, roughened surface, Velcro, a compressed seal, or a slanted gear.

Metal Pegs

The metal pegs design consisted of a frame with pegs at the ends of the frame that serve as anchors around which the threads could be extruded (Figure 14). The fibrin would be extruded as

a single thread, wrapped around the outside of the pegs, and the threads could be cut into multiple threads after drying. This design was seen as easy to maintain and reliable, but increased the risk of thread damage. This design would also be more difficult to clean with the microthread dried and wrapped around the metal pegs (Table 12).

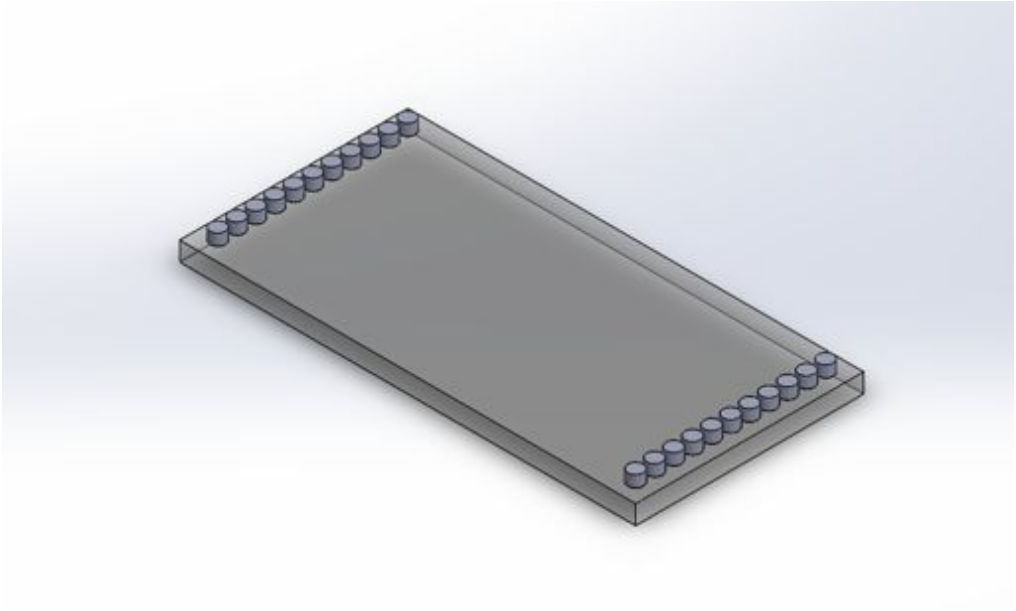


Figure 14: **Metal pegs -** This figure depicts the metal pegs concept of fibrin microthread attachment. In this design the threads would be extruded around the metal pegs that would serve as anchor points. Because threads tend to adhere to metal under production conditions (hydrated threads within HEPES solution), this idea was promising because after thread drawing, the threads would adhere naturally to the metal rods.

Table 12: Pros and cons of metal pegs method

Pros	Cons
Good anchor for threads	Risk damaging threads
Easy to maintain	Difficult to clean
Reliable	

Single Roller

The roller design consisted of a roller on the end of the frame (Figure 15). As the threads were extruded upon the roller, they would spin once or twice to wind the threads around the ends, and slightly stretch them to secure them. This design had the potential to make stretching very easy, but the thread adhesion was questioned in terms of feasibility. As the roller rotated, there was no way for the team to prevent the microthreads from sticking to each other and becoming entangled (Table 13).

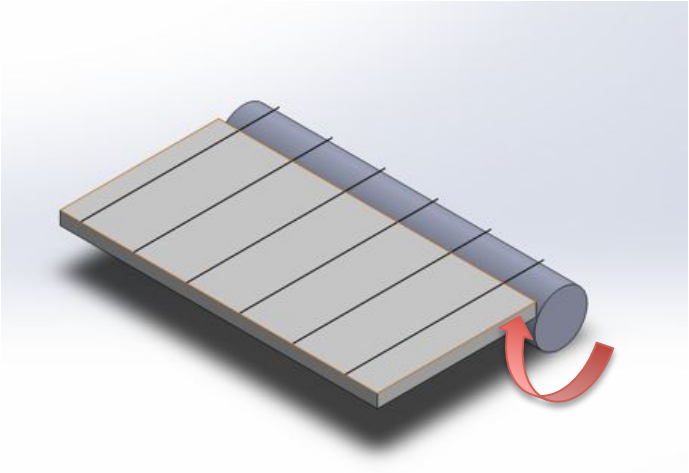


Figure 15: **Single roller -** This figure illustrates the concept of using a roller to attach fibrin microthreads. The concept uses a cylindrical roller that rotates in the opposite direction of the threads. The roller would be metal and adhere the threads, and as it rolled the threads would remove from the pan for secure stretching.

Table 13: Pros and cons of single roller

Pros	Cons
Potentially simple stretching	Microthreads would stick to eachother
Easy to maintain	

Flat Clamps

The flat clamp design consisted of two pieces of Acrylic that formed a flat clamp on each end of the frame (Figure 16). After the threads were extruded onto the frame, a piece of Acrylic would descend to form a flat clamp. This design was simple, easy to clean and maintain, but increased shear stress on the threads during adhesion and could increase thread failure (Table 14).

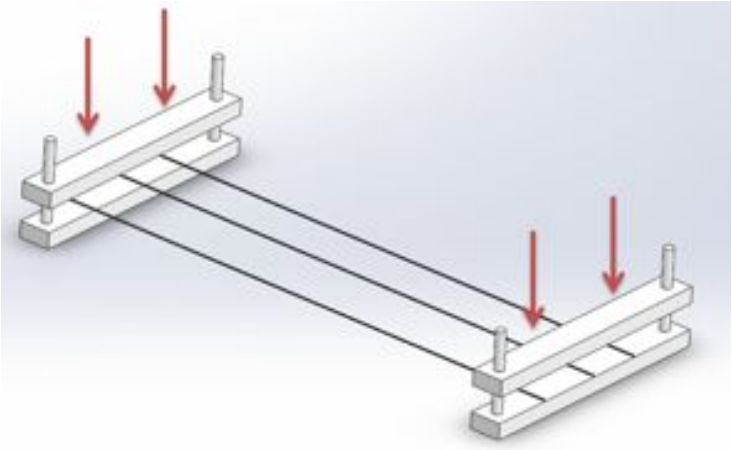


Figure 16: Flat clamps - Illustrates the concept of using a roller to attach fibrin microthreads. The clamps would use an equal, downward force to clamp the threads to the base frame.

Table 14: Pros and cons of flat clamps

Pros	Cons
Simple construction	Potentially increased shear stress on threads
Easy to maintain	Potentially increased thread failure
Easy to clean	

Rotational Clamps

The rotational clamp design consisted of a squeegee that would be attached to the base frame with a hinge. After the threads were extruded onto the frame, the squeegee would come down and clamp the threads to the frame. This design was simple, but involved moving parts. The plastic squeegee should reduce the shear stresses on the microthreads (Table 15). Figure 17 depicts the rotational clamp.

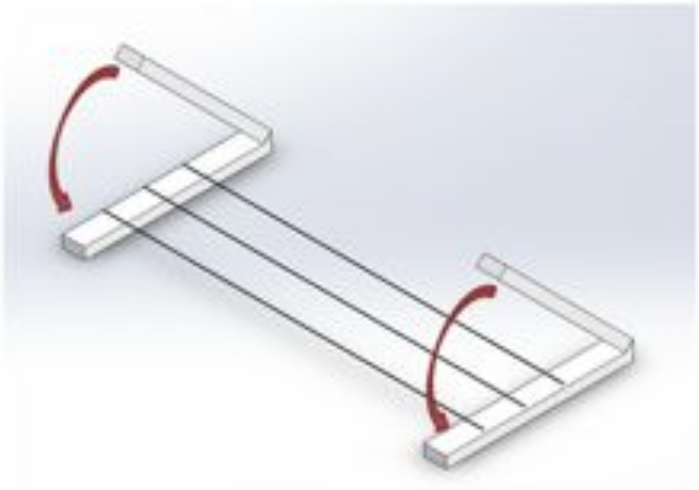


Figure 17: Rotational clamps - This figure illustrates the concept of using a rotational clamp to secure threads to a frame. The rotation of the clamp would be easier to control and produce a uniform compressive stress on the threads.

Table 15: Pros and cons of rotational clamps

Pros	Cons
Simple	More moving parts
Plastic squeegee should reduce shear stress on threads	

Roughened Surface

The concept of the roughened surface was to roughen a piece of Acrylic and allow the threads to attach to the surface without additional manipulation (Figure 18). This simple design would be easy to maintain, but the effectiveness of the roughened surface needed to be tested.

The team also considered the effects of sharpened Acrylic edged on the threads as possible areas for damage of extruded fibers (Table 16).

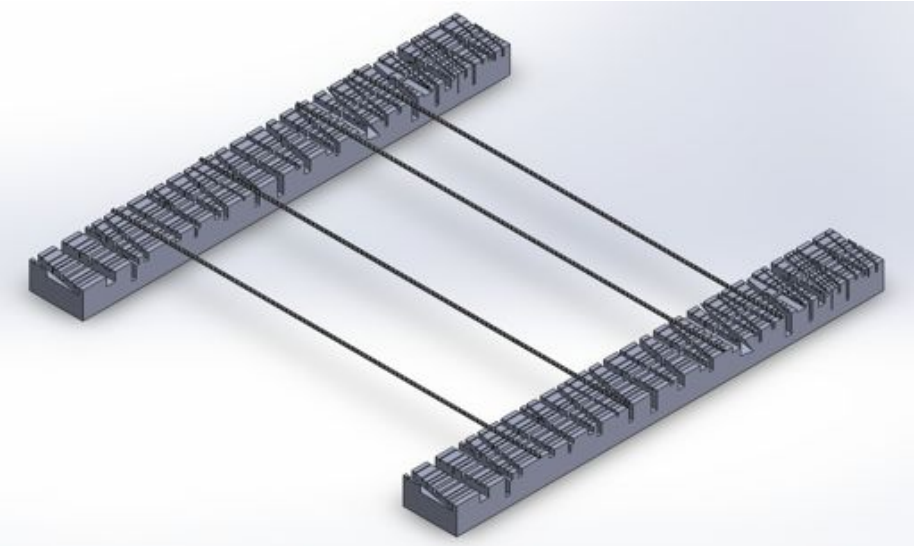


Figure 18: **Roughened surface -** Illustrates the concept of using a rough surface to attach fibrin microthreads.

Table 16: Pros and cons of roughened surface

Pros	Cons
Easy to maintain	Difficult to clean
Easy to reproduce	Questionable effectiveness
	Sharp edges could shear threads

Velcro

The Velcro design consisted of Velcro strips on which the threads could be extruded and clamped into place by another Velcro piece (Figure 19). This design was cost-effective, easily replaceable, and easily testable during preliminary testing, but could also be very difficult to clean (Table 17).

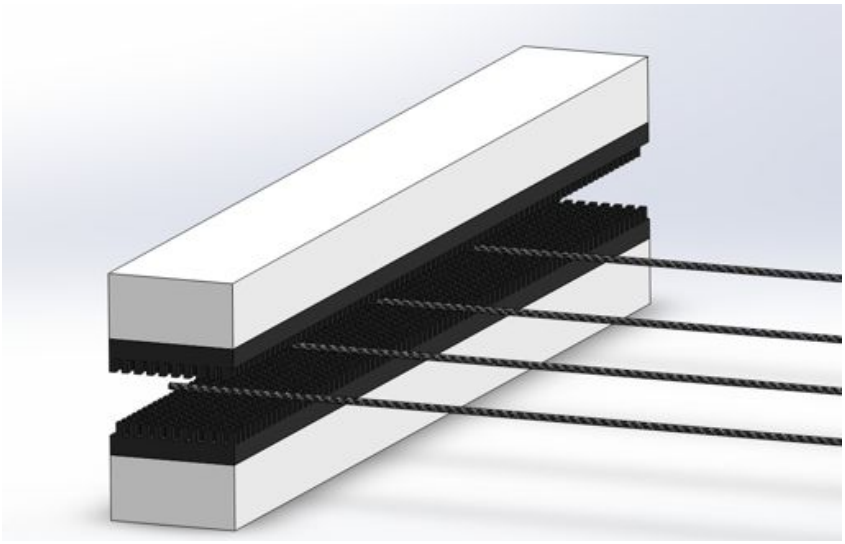


Figure 19: Velcro - This figure illustrates the concept of using Velcro to attach microthreads to the stretching frame.

Table 17: Pros and cons of Velcro

Pros	Cons
Cost effective	Difficult to clean
Easy to replace	

Seal

The seal idea consisted of Stretcher plates and PDMS sheets (Figure 20). As tested previously, new Acrylic and PDMS interface well with each other and form a tight seal. This design was ideal because it minimized potential thread damage, but without conclusive testing in HEPES baths, the seal idea needed verification (Table 18).

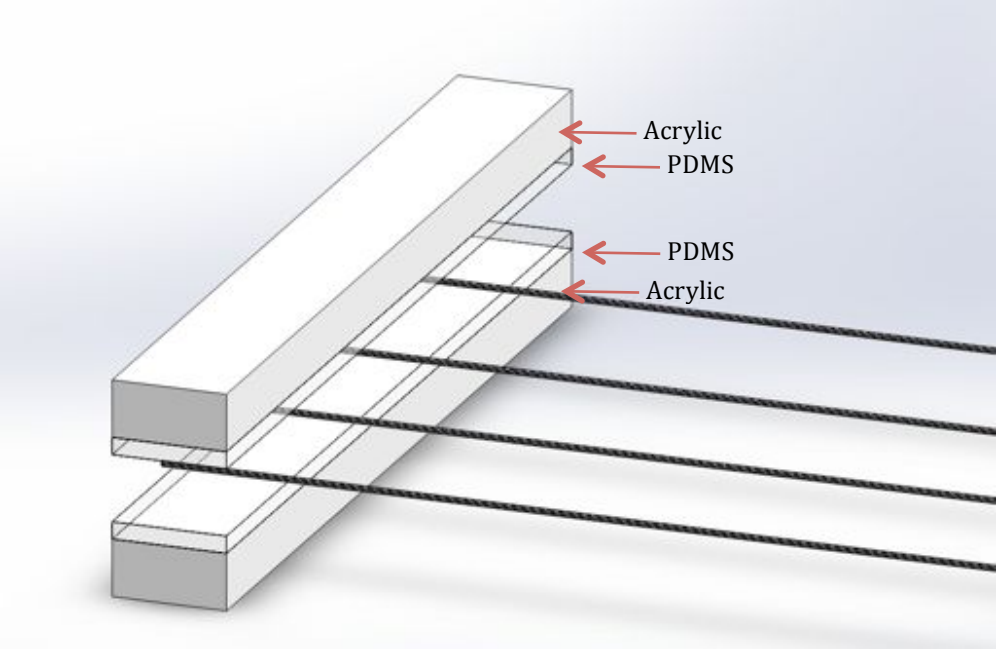


Figure 20: Seal - Fibrin threads are secured for stretching via PDMS seal on each end.

Table 18: Pros and cons of seal

Pros	Cons
Minimized potential thread damage	May not seal as well in HEPES
Easy to replace	

Slanted Gear

The slanted gear design consisted of a gear with slanted teeth (Figure 21). The gear would rotate and secure the threads for stretching. However, this design would have been difficult to implement with other design features and the teeth of the gear may also add additional stresses on the microthreads (Table 19).

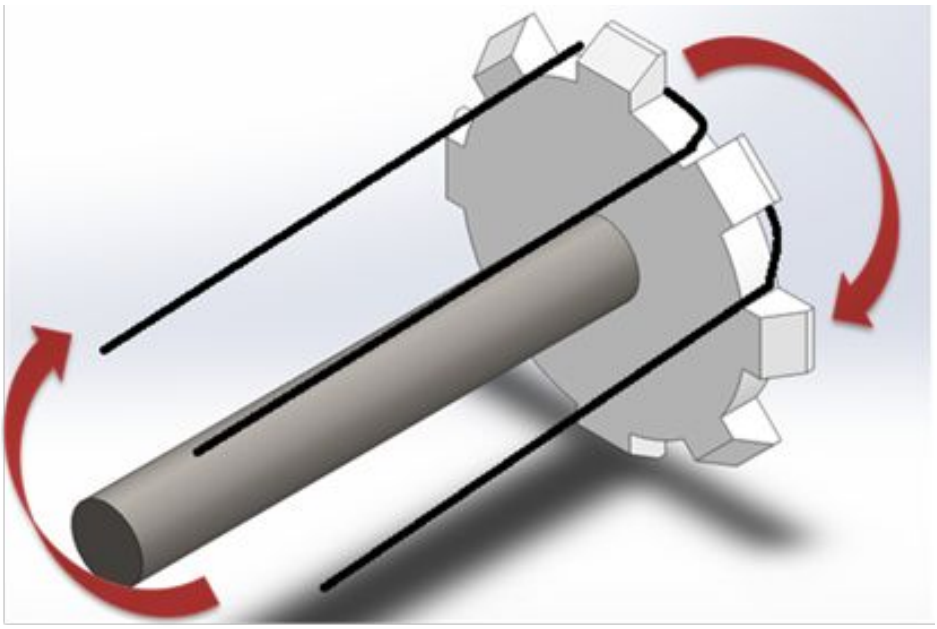


Figure 21: Slanted gear - Slanted gear rotates and threads are secured in the teeth of the gear.

Table 19: Pros and cons of slanted gear

Pros	Cons
Easy to clean	Difficult to integrate with other features
	Difficult construction
	Additional stresses on threads

4.3.2 Stretching Mechanism

One of the key components of the production process for fibrin microthreads was stretching. A stretching device must minimize thread failure while consistently stretching threads at determined rates to desired lengths. Some of the ideas for a stretching device included an accordion design, a motorized stretcher, motorized rollers, angled hydraulic lifts, and a stretching track.

Accordion

In the accordion design, angled metal bars would expand and pull the threads to desired lengths (Figure 22). This design was advantageous because it allowed for a longer life span of the device and could return to starting stretch position automatically (Table 20).

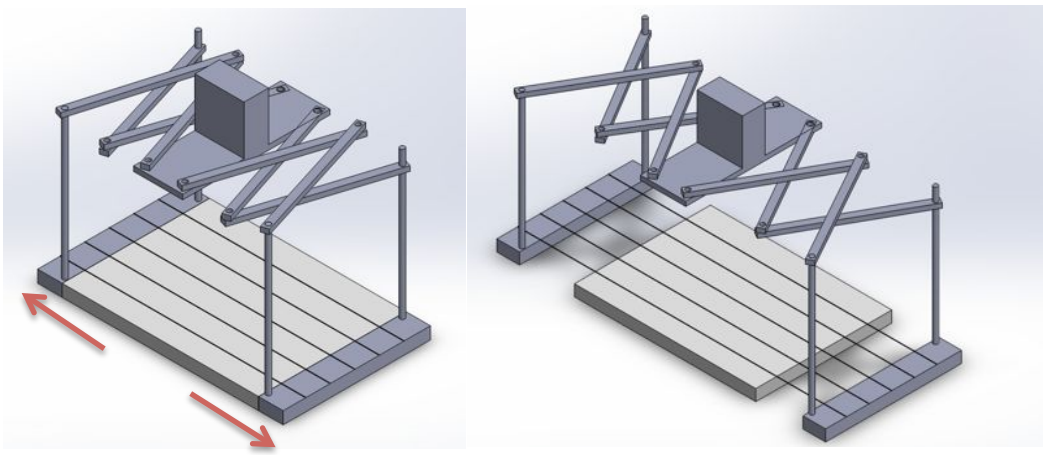


Figure 22: Accordion - This figure illustrates the accordion concept, which uses an expandable frame to translate the stretching frame to the desired stretch percentage.

Table 20: Pros and cons of accordion

Pros	Cons
Longer life span	Construction
Easily return to starting position	

Motorized Stretch

The idea behind the motorized stretch mechanism was utilizing a threaded rod to move plates back and forth to stretch the threads (Figure 23). The motorized design would have included a motor to uniformly stretch the threads. This design mimicked the manual stretching process, which would minimize cost but improve the consistency of stretch rates. One concern, however, was that the metal threaded rod would have to remain in the HEPES buffer, increasing the risk of rust (Table 21).

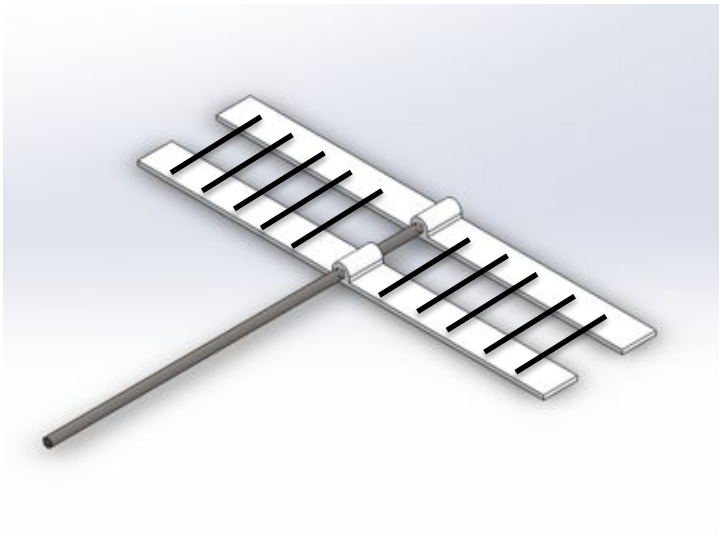


Figure 23: Motorized stretch - This figure illustrates the concept of using a threaded rod which is attached to a motor. The rotation of the motor would translate to planar movement of the middle section of the frame and stretch the threads.

Table 21: Pros and cons of motorized stretch

Pros	Cons
Mimics manual stretching	Rusting of threaded rod
Minimal cost	
Consistent threads	

Rollers

In the roller design, two rollers would pull threads from both ends uniformly and coil the threads for rapid drying (Figure 24). The challenge with this design was the lateral movement of the rollers to avoid thread overlap during rolling (Table 22).

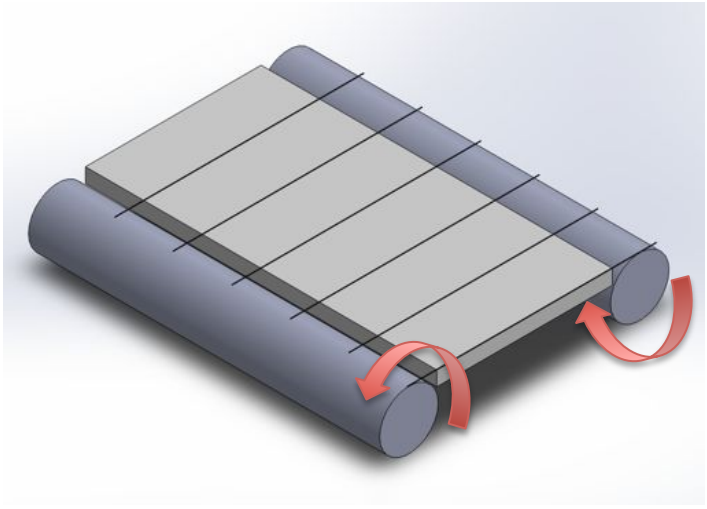


Figure 24: Rollers - This figure illustrates the rollers concept for the stretching mechanism of the system. The rollers would rotate in opposite directions and pull the threads to desired lengths.

Table 22: Pros and cons of rollers

Pros	Cons
Easy to maintain	Lateral movement of rollers to avoid thread overlap
Easy drying	

Angled Hydraulic Lifts

The angled hydraulic design consisted of two posts which would move upwards at an angle of 45° to stretch the threads (Figure 25). The role of the angled posts would be to remove and stretch the threads concurrently. However, this design would have increase vertical clearance and increased design cost (Table 23).

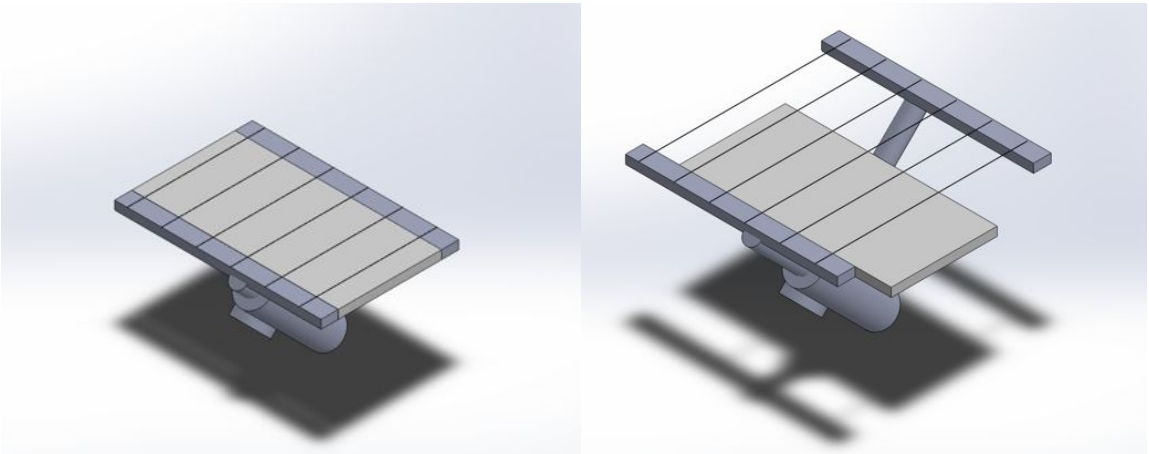


Figure 25: Angled Hydraulic – this figure illustrates the angled hydraulic concept for the stretching mechanism of the system. Then angled pistons would push to expand the frame at an angle to lift the threads off the pan and stretch them simultaneously.

Table 23: Pros and cons of angled hydraulic

Pros	Cons
Similar to manual motion	Increased vertical clearance
	Expensive

Track/Tread

In the track design, the thread frame would follow a designed track to remove and stretch threads in on path. One end of the thread adhesion frame remains stationary as the other is stretched along track (Figure 26). This design could have also increased cost because it incorporated multiple moving parts (Table 24).

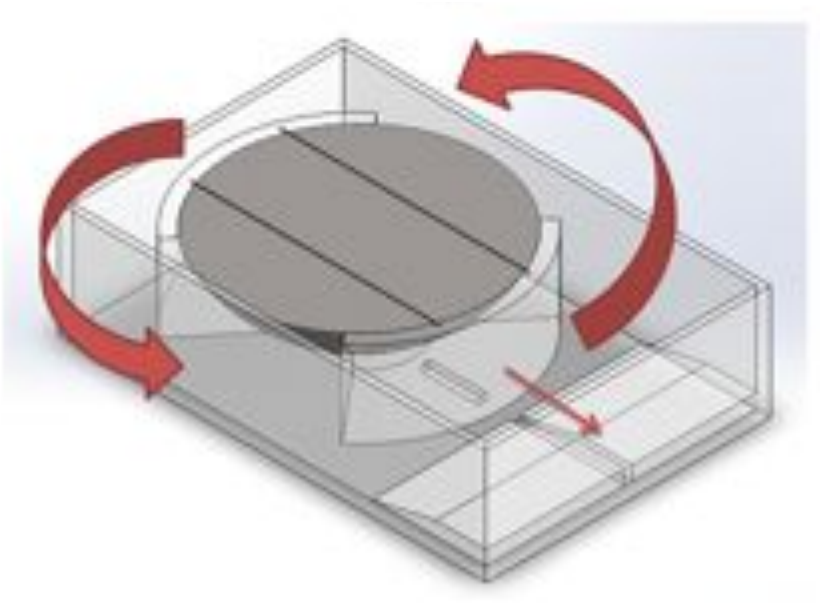


Figure 26: Track/tread - This figure illustrates the concept of using track and tread to lift and stretch microthreads.

Table 24: Pros and cons of track/tread

Pros	Cons
Reduced stress on threads	Multiple moving parts
	Expensive

4.3.3 Bath

When designing components of the fibrin processing system, the bath continued to be a major consideration because of its crucial role in the size of the system as well as its role in determining the drying step of the threads. The bath needed to be structurally sound while holding the buffered solution during extrusion and stretching, and be able to drain after completion. Some of the ideas generated for bath systems to drain buffer solution or lower buffer water-line to facilitate thread drying included an angled bath, a bottom drain, foldable walls, a humidified chamber, or a compartmentalized system.

Angled Bath

In the angled bath idea, the corner of a slanted bath would drain buffer solution for easy drainage using gravity (Figure 27). This design required larger amounts of buffer but could be incorporated into many other designs (Table 25).

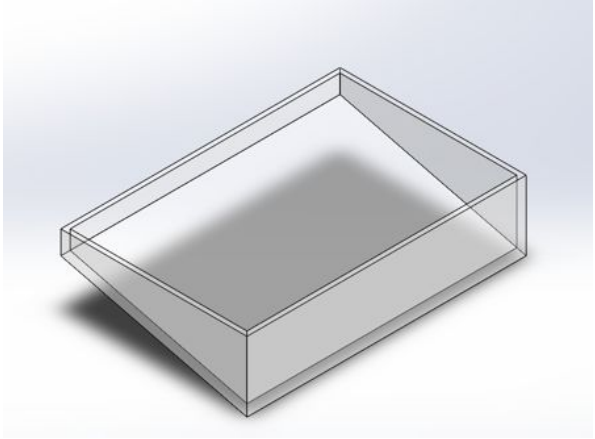


Figure 27: Angled bath - This figure illustrates the angled bath idea in which the bath would be slightly slanted. The angled bath would drain due to gravity and would eliminate the need for a pump.

Table 25: Pros and cons of angled bath

Pros	Cons
Easy to clean	Large amount of buffer
Easy to drain	
Easy to interface	

Bottom Drain

The bottom drain bath was another method that could potentially hold buffer solution, and could fully drain out of the bottom of the bath (Figure 28). It was different from the angled bath design because its slight slant in the middle would accommodate a flat surface for the stretching system to sit on. This bath required a pump, conversely, to pump the buffer back into the system before the next batch (Table 26).

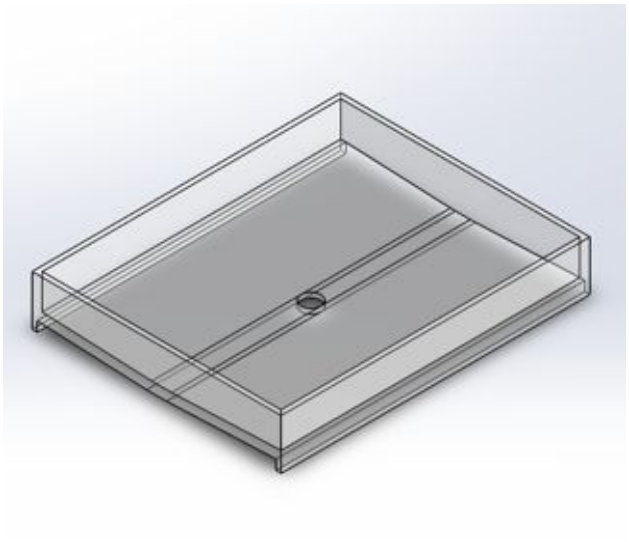


Figure 28: Bottom drain - This figure illustrates the concept of using a bottom drain located in the center of the bath. The hole would be central to the bottom, and small slants in the bottom would allow for proper drainage.

Table 26: Pros and cons of bottom drain

Pros	Cons
Easy to drain	Pump to put buffer back into system
Easy to clean	
Flatter surface	

Foldable Walls

This design consisted of a rectangular bath with foldable walls that, as threads were stretched, would fall and decrease the level of the bath (Figure 29). This would lower the buffer level and allow the threads to be above the buffer without having to raise them. This design however, had moving parts and the challenge would have been to seal the folding wall so it did not leak (Table 27).

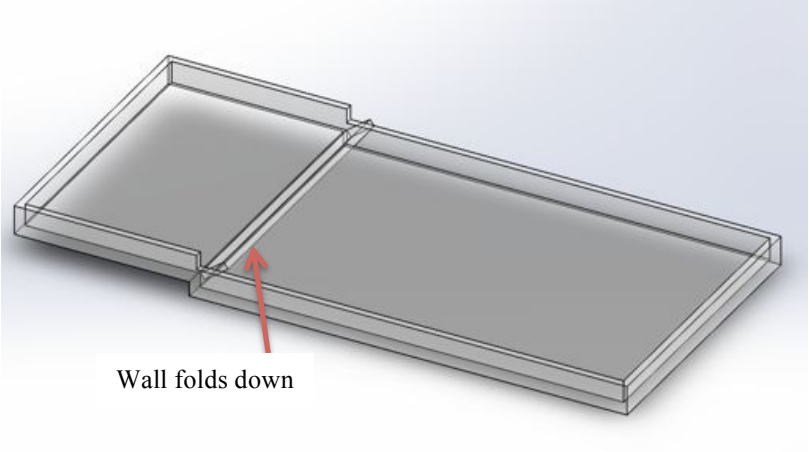


Figure 29: Foldable wall - This figure illustrates the concepts of using a foldable wall system in which the center wall would fold and the bath liquid would drain into the larger chamber. The change in fluid levels would allow for removal of the threads from fluid after stretching.

Table 27: Pros and cons of foldable wall

Pros	Cons
Allows for stretching	Challenge to seal
Promotes drying without draining	Lots of moving parts

Humidifier

The humidifier design consisted of a 100% humidified chamber in which threads could be extruded and stretched (Figure 30). With this design, threads would not be extruded into the HEPES but into a humidified chamber, eliminating the need for a controlled bath intake and outflow. However, this design proved too complex to fall within the scope of this project (Table 28).

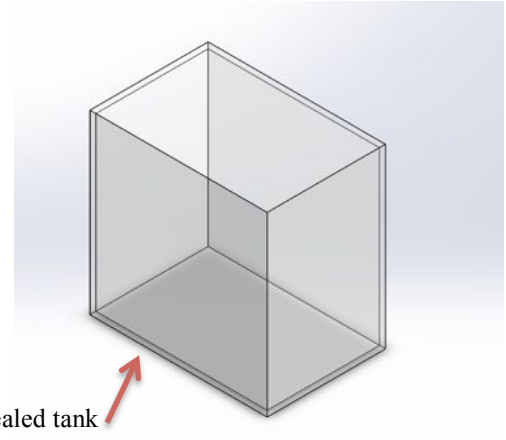


Figure 30: Humidifier - This figure illustrates the concept of a humidifier tank to hydrate threads and dry threads.

Table 28: Pros and cons of humidifier

Pros	Cons
No controlled bath intake and outflow	Complex
	Expensive

Compartments

In this design, the humidifier idea would be expanded to include non-humidified chambers to manipulate threads (Figure 31). The threads could have been extruded and stretched within a chamber and dried in a second chamber. This design proved to be difficult in terms of feasibility and was not pursued further. In order for this design to work, the box would need three separate components that could seal off from each other. The humidifying section would need to be completely sealed off from the other sections (Table 29).

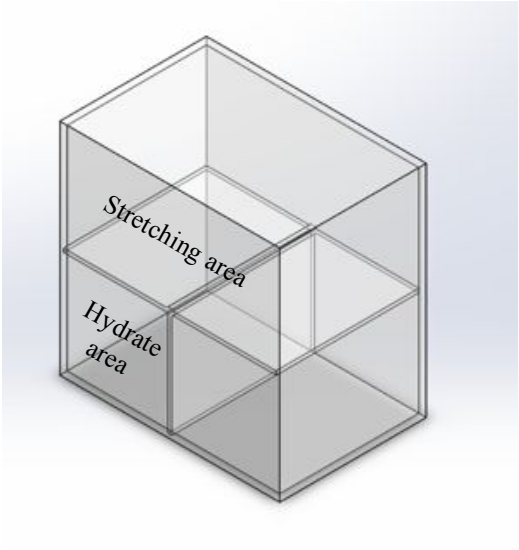


Figure 31: Compartments - This figure illustrates the concept of using compartmentalized system to hydrate, stretch, and dry threads.

Table 29: Pros and cons of compartments

Pros	Cons
All enclosed system to prevent outside interference	Complex
	Many seals, potential for leaks

4.3.4 Removal and Drying Mechanism

After threads are extruded, they must be removed from the extrusion pan, stretched, and removed to dry. The design team examined possible methods for removal of threads from the extrusion pan, as well as ideas to automate removal of threads from HEPES bath. Some of the

ideas included a 90° turn, windshield wiper, stretching threads to remove them, a track idea, and movable pan or movable adhesive sides.

90° Turn

The 90° turn mechanism was designed to mimic the manual removal process (Figure 32). In this design the threads are removed through a 90° twist in which the polymers turn and adhere to themselves and remove from the pan (Table 30).

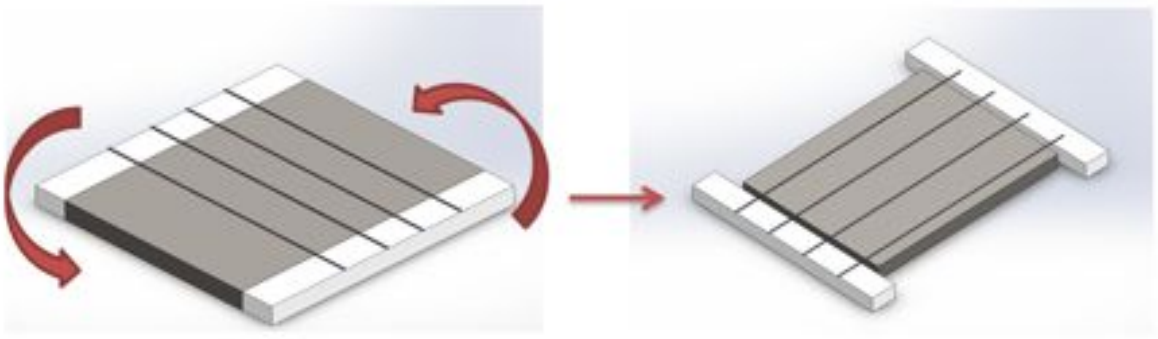


Figure 32: 90° turn - This figure illustrates the 90° rotation concept that removes the threads from the pan for stretching.

Table 30: Pros and cons of 90° turn

Pros	Cons
Mimics manual removal	Potential for threads to adhere to themselves

Windshield Wiper

The windshield wiper design would lift the threads out of the buffer bath with a motion similar to that of a windshield wiper on a car (Figure 33). This design took up vertical space, and had some moving parts, but it also was a smooth motion with dispersed forces along the threads. This would prevent the threads from failing (Table 31).

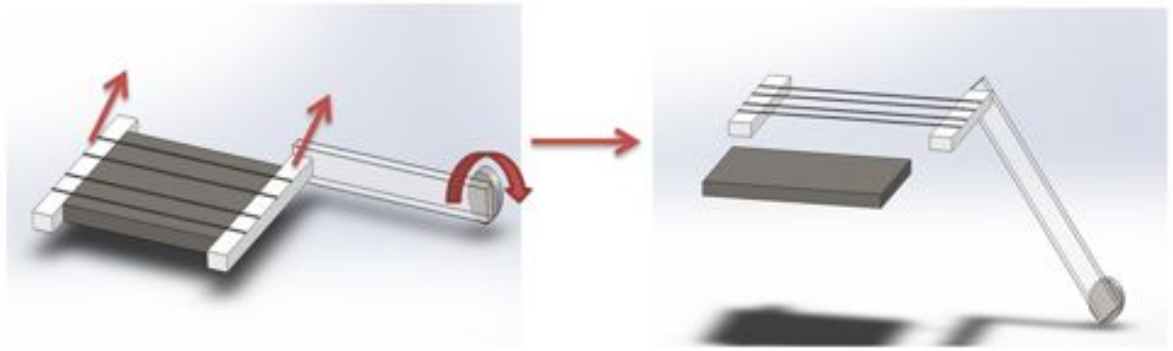


Figure 33: Windshield wiper - This figure illustrates the concept of using a windshield wiper movement to remove the threads from the pan. Once the threads are extruded the wiper arm would rotate and remove the frame system from the pan.

Table 31: Pros and cons of windshield wiper

Pros	Cons
Smooth motion	Surface tension of water could break threads
	Takes up vertical space

Stretch-to-remove

In this design, there was no vertical lift of the threads. The threads would be pulled in opposite directions and stretched until they were completely removed from the pan (Figure 34). This design dispersed the forces along the threads, and also took up very little space. However, this design needed to be tested to ensure the threads could fully detach from the pan by stretching only (Table 32).

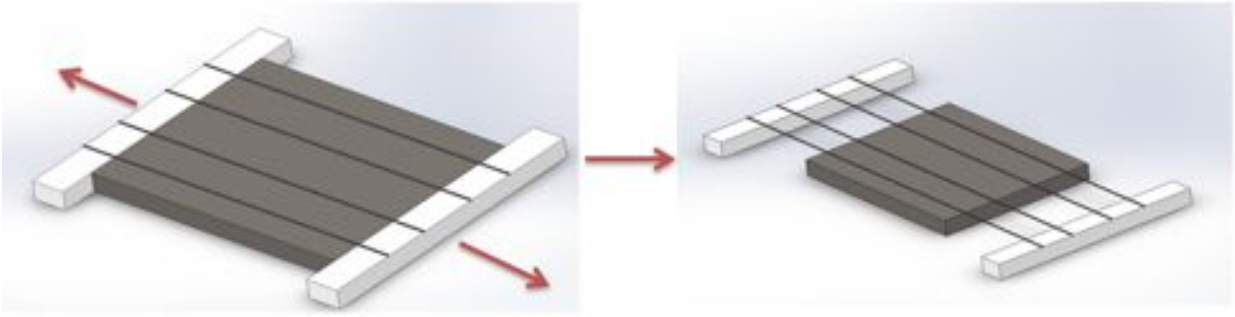


Figure 34: Stretch-to-remove - This figure illustrates the stretch-to-remove concept. Microthreads are extruded on a pan and as they are stretched the threads detach from the pan

Table 32: Pros and cons of stretch-to-remove

Pros	Cons
Takes up very little space	Further testing to determine if threads truly detach
Dispersed forces along threads	

Track

This design consisted of a track that would both turn and raise the threads (Figure 35). The track was designed to move the threads in a corkscrew motion to both lift and remove them. This design incorporated two types of movement to optimize the dispersion of forces on the threads. This design, however, required the construction of two custom made parts (Table 33).

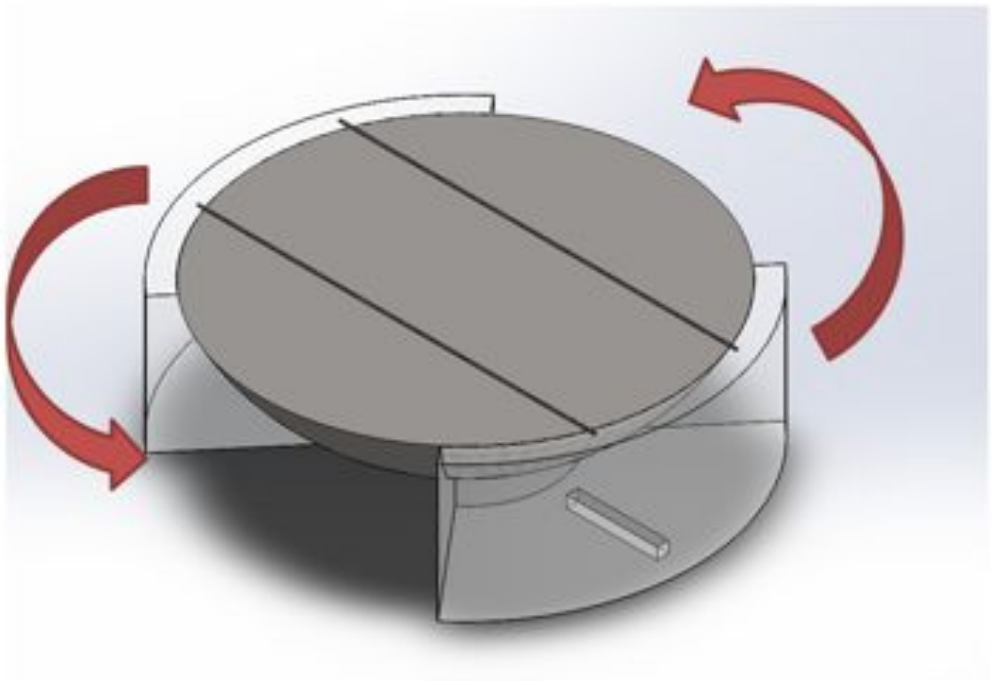


Figure 35: Track - This figure illustrates the concept of using a track to remove the threads from the pan. Threads are extruded and secured, the frame rotates up a track and the threads are removed from the extrusion pan.

Table 33: Pros and cons of track

Pros	Cons
Dispersion of forces	Custom made parts
	Difficult to clean

Drop-Down Angled Pan

The drop-down pan design incorporated a hinged pan that would drop out and allow for the threads to be removed (Figure 36). However, the sudden dropping of the pan could put unwanted stress and strain on the threads causing them to break (Table 34).

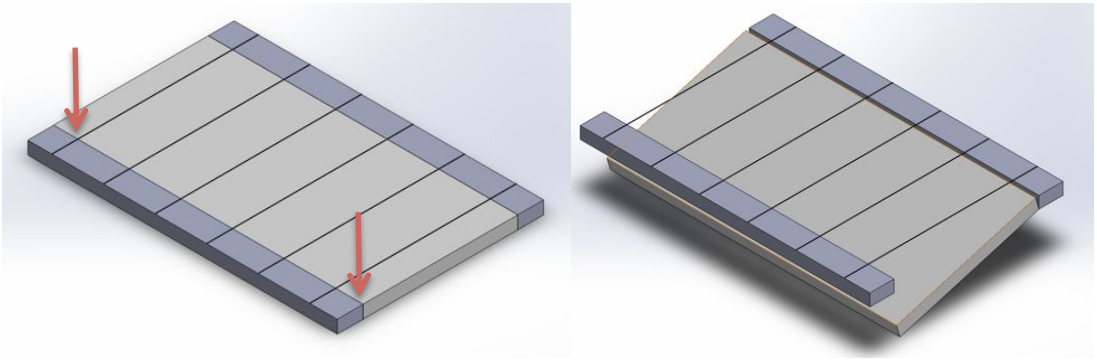


Figure 36: Drop-Down Angled – this figure illustrates the concept of the angled drop down pan. As depicted, the pan lowers on a hinged side to gradually lower from the threads, leaving the threads attached solely to the frame.

Table 34: Pros and cons of drop-down angled

Pros	Cons
Simple design (1 hinge)	Could cause thread failure
	Takes up vertical space

Drop-Down Flat Pan

The drop down flat pan design consisted of a pan that falls all at once, allowing the threads to remove from the pan (Figure 37). This idea could work with any design that had a square bath (Table 35).

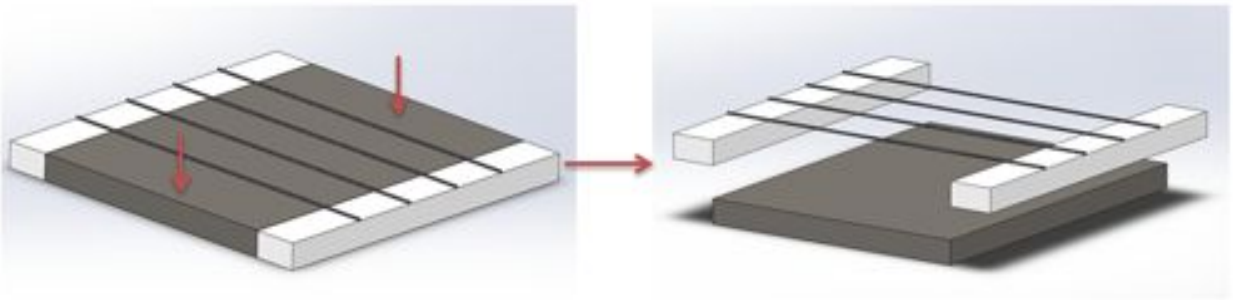


Figure 37: Drop-down flat - This figure illustrates the concept of a drop-down pan. As depicted, the pan lowers from the level of the threads, leaving the threads attached solely to the frame.

Table 35: Pros and cons of drop-down flat pan

Pros	Cons
Easily interfaced with other designs	Potential for thread failure
Easy to clean	

Lift-Away

The lift-away design incorporated the two end pieces of the frame, which lifted the threads off the pan (Figure 38). This design, however, utilized a motor or hydraulic and would increase cost of the device (Table 36).

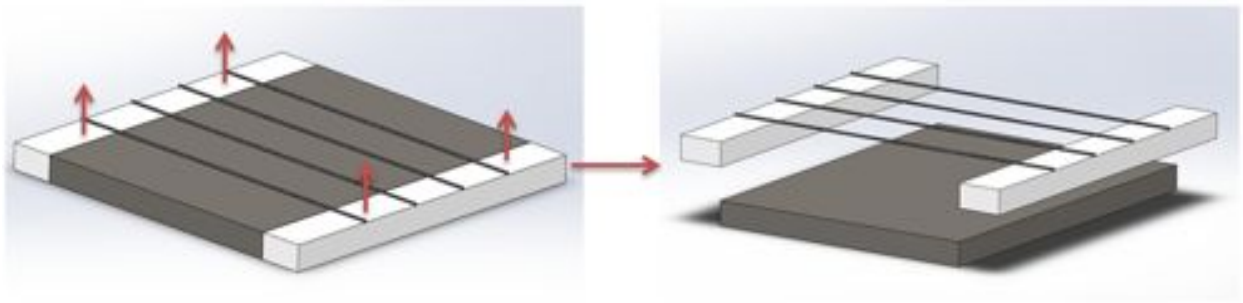


Figure 38: Lift away - This figure illustrates the concept of lifting the frame away from the pan to remove the threads. The idea is similar to the drop-down pan but the frame is instead lifted away.

Table 36: Pros and cons of lift away

Pros	Cons
Easily interfaced with other designs	Potential for thread failure
Easy to clean	Hydraulic is expensive

4.3.5 Conceptual Tentative Final Design

To come to a conclusion on the initial final design, the team took all the design ideas into consideration and compared the ideas based on the objectives and constraints. First, the team

calculated a weight for each objective and sub-objective, based on the importance assigned by the user, client, and design team's pairwise comparison charts. Each design idea was discussed and assigned a grade for how well the design would work towards the objectives. This system was used to narrow down which ideas to be tested for the final design.

To calculate the weight for each objective, the team began with level 1 objectives, which were effective, user friendly, automated, and versatile. Table 37 shows the weights for each of the objectives. These weights were calculated based on the original pairwise comparison charts completed by the client, the user, and the design team. The user and client's grades in the pairwise comparison charts were each considered 40% of the average, and the design team was considered 20%. This gave the user and the client's opinion a greater weight than the design team, because the user and client will be the primary operators of the final product and are more experienced with the microthread processing system. The total average was considered and all four objectives average scores were added together to get a final score. Each objective then received a percentage score based on their total score. For example, automated received an average score of 2.4, which was 40% of the total score, so all the level 2 objectives under automated had to add up to 40% of the total final score for the design. The sub-objectives weights were calculated in a similar manner. Once the team had determined a weight for each objective, they were able to begin comparing their designs.

Table 37: Calculated weighted objectives

Level 1 Objectives	Design Team	Client	User	Total	Modifing %
User friendly	0.5	0	0.5	0.3	0.05
Automated	2	3	2	2.4	0.4
Effective	3	2	2.5	2.4	0.4
Versatility	0.5	1	1	0.9	0.15
Level 2 User Friendly	Design Team	Client	User	Total	Modified Total
Easy to clean	0.5	0.5	1	0.7	0.035
Modifiable interface	3	2	2.5	2.4	0.12
Easy to maintain	1.5	0.5	1.5	1.1	0.055
Reliable	3.5	3.5	3	3.3	0.165
Durable	1.5	3.5	2	2.5	0.125
Level 2 Automated	Design Team	Client	User	Total	Modified Total
Stretch	2	2	1.5	1.8	0.72
Removal	1	0.5	1.5	1	0.4
Drying	0	0.5	0	0.2	0.08
Level 2 Effectiveness	Design Team	Client	User	Total	Modified Total
Accuracy	2.5	0	1.5	1.1	0.44
Precision	2.5	2.5	1.5	2.1	0.84
Reproducibility	1	2.5	1	1.6	0.64
Minimize thread failure	0	1	2	1.2	0.48
Level 2 Versatility	Design Team	Client	User	Total	Modified Total
Sterilization	2	0.5	0.5	0.8	0.12
Portability	0	2	0.5	1	0.15
Crosslinking	1	0.5	2.5	1.4	0.21
Modifiable parameters	3	3	2.5	2.8	0.42
Level 3 Modifiable Parameters	Design Team	Client	User	Total	Modified Total
Stretch speed	2.5	1	2.5	1.9	0.13
Stretch percentage	2.5	2.5	2.5	2.5	0.18
Cycle time	1	0.5	0	0.4	0.03
Thread length	0	2	1	1.2	0.08

The team created a comparison chart for each set of designs based on function. In order to be considered a possibility, a design had to meet each constraint. Each design was then scored

1 through 5 for each objective. A score of a 1 meant the design performed the task poorly, a 2 was decent, a 3 was average, a 4 was good, and a 5 was excellent. Since no preliminary design testing was done previous to idea comparison, scores were based on conjecture only, and therefore the top two designs from each comparison were selected to test further in a laboratory setting before the ideal design was chosen. Objectives that did not pertain to that specific function were given a score of 0, and were not considered. Table 38 shows an example of the design comparison chart for frame adhesion ideas. The tables for the bath, stretching and removal systems are located in **[Appendix C**: Idea Comparison**]**.

Table 38: Frame adhesion idea comparison - The chart shows quantitative analysis of each design when compared to certain parameters (User Friendly, Automated, Effective, Versatility, Modifiable Parameters). Additionally, the idea which scored the highest is indicated in blue.

Frame Idea Comparisons			Metal pegs	Weighted	Single roller	Weighted	Flat clamps	Weighted	Rotational clamp	Weighted	Roughened surface	Weighted	Seal	Weighted	Velcro	Weighted	Slanted gears	Weighted
C		Time limit (A-D term)	Y		Y		Y		Y		Y		Y		Y		Y	
C		Size (4x6x4 ft)	Y		Y		Y		Y		Y		Y		Y		Y	
C		Interface with existing system	Y		Y		Y		Y		Y		Y		Y		Y	
C		Limited budget ($524)	Y		Y		Y		Y		Y		Y		Y		Y	
O	0.3	**User Friendly**																
O	0.04	Easy to clean	3	0.11	2	0.07	4	0.14	5	0.18	1	0.04	2	0.07	1	0.035	2	0.07
O	0.12	Modifiable interface	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O	0.06	Easy to maintain	5	0.28	3	0.17	4	0.22	4	0.22	3	0.17	1	0.055	5	0.275	3	0.17
O	0.17	Reliable	5	0.83	3	0.5	4	0.66	4	0.66	2	0.33	4	0.66	4	0.66	3	0.5
O	0.13	Durable	5	0.63	3	0.38	5	0.63	4	0.5	3	0.38	3	0.375	5	0.625	5	0.63
O	2.4	**Automated**																
O	0.72	Stretch	3	2.16	3	2.16	5	3.6	5	3.6	4	2.88	4	2.88	3	2.16	2	1.44
O	0.4	Removal	3	1.2	2	0.8	3	1.2	3	1.2	3	1.2	4	1.6	3	1.2	2	0.8
O	0.08	Drying	3	0.24	3	0.24	5	0.4	5	0.4	4	0.32	4	0.32	3	0.24	2	0.16
O	2.4	**Effective**																
O	0.44	Accuracy	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O	0.84	Precision	2	1.68	1	0.84	4	3.36	4	3.36	4	3.36	4	3.36	3	2.52	2	1.68
O	0.64	Reproducibility	2	1.28	1	0.64	4	2.56	4	2.56	4	2.56	4	2.56	3	1.92	2	1.28
O	0.48	Minimize thread failure	1	0.48	1	0.48	3	1.44	3	1.44	2	0.96	5	2.4	1	0.48	1	0.48
O	0.9	**Versatility**																
O	0.12	Sterilization	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O	0.15	Portability	4	0.6	2	0.3	3	0.45	4	0.6	5	0.75	3	0.45	3	0.45	2	0.3
O	0.21	Crosslinking	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O	0.42	Modifiable parameters	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O	0.42	**Modifiable Parameters**																
O	0.13	Strain rate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O	0.18	Stretch percentage	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O	0.03	Cycle time	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O	0.08	Thread length	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Total		**9.47**		**6.57**		**14.7**		**14.7**		**12.9**		**14.7**		**10.6**		**7.5**

Once the chart was completed, the weighted objective was multiplied by the team score and then a modified score was calculated. These modified scores added together to get a total. The design with the highest total scores were considered the best, these designs will undergo testing to determine the effectiveness of each design, once testing is complete a final design will

be chosen. In this case, there was a three-way tie; flat clamps, rotational clamps, and the seal idea would all need to be tested in the lab during preliminary testing.

After completing all of the idea comparison charts, the team chose two ideas from each category that would be valid finalists and could be used in a final design. For frame adhesion, both the clamps and seal ideas would be tested. In terms of a stretching mechanism, the team scored the motorized stretcher as the clear winner but chose to test the accordion idea as well. In terms of a bath, both bottom drain and angled drain bath ideas would be considered. Lastly, in terms of a removal and drying mechanism, the track was the clear winner, but the stretch-to-remove design would also be tested because it was an easier concept to test and design. These final ideas were then tested through preliminary testing and a final idea was chosen based which idea performed best within the objectives during testing.

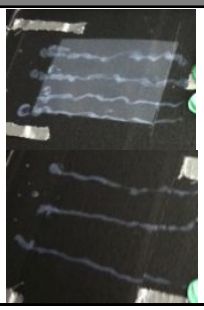
4.3.6 Feasibility Study & Experiments

Before the team could begin constructing a working proto-type, feasibility was completed first. The objective of the team's feasibility testing was to prove fundamental concepts of the components of the microthread processing system. While brainstorming, the team came up with many ideas that would conceptually work well, but could prove to be unfeasible. Before choosing a final design, the relative effectiveness of each of the highest ranked ideas was tested by the design team in the feasibility study. Table 39 and Table 40 show a summary of the tests performed. Threads that sheared or slipped at the adhesion interface were marked as failing for the adhesion tests, and threads that were not fully removed from the pan surface without breaking were marked as failed for the removal tests.

Table 39: Summary of adhesion test results

Test 1 - Adhesion test, manually extruded threads			
Thread #	**Adhesion Type**	**Result**	
1	Squeegee clamp	Pass	
2	Squeegee clamp	Pass	
3	Squeegee clamp	Pass	
4	Hook and loop velcro	Fail	
5	Hook and loop velcro	Fail	
6	Hook and loop velcro	Fail	
7	Hook and loop velcro	Pass	
8	Hook and loop velcro	Fail	
9	Beaded velcro	Pass	
10	Beaded velcro	Fail	
11	Beaded velcro	Fail	
12	Beaded velcro	Fail	

Table 40: Summary of removal test results

Test 2 - Removal test, manually extruded threads			
Thread #	**Removal Method**	**Result**	
1	Stretch-to-remove	Pass	
2	Stretch-to-remove	Pass	
3	Stretch-to-remove	Fail	
16	Porous surface	Fail	
17	Porous surface	Fail	
18	Porous surface	Fail	
19	Porous surface	Fail	

The main testing the team completed was to test the different adhesion concepts (Table 39). From the various pairwise comparison charts, automation and effectiveness were the two highest ranked objectives. To ensure efficient automated stretching and removal of threads, a secure thread fixture method was required. First, the team tested several adhesion methods. Thread fixture was an important component of the device because it was essential to thread stretching and removal. In the first round of testing, the team tested the different clamps, including the squeegee clamp and several Velcro clamps. The purpose of each clamp test was to determine an effective method to secure the fibrin microthreads. The squeegee clamp method uses a strip of flexible plastic to clamp down the threads and secure them for removal, stretching, and drying. Velcro is fabric that utilizes hooks and loops to attach to each other. Two types of Velcro were used to fasten microthreads during preliminary testing, including the hook and loop and the beaded hooks, because they were simple and feasible to test. Theoretically, the Velcro clamp would be a simple and effective method for thread fixation, but because it had never been tested before, the team had to test it to determine its effectiveness.

The second test performed was extrusion and removal testing. The purposes of these tests were to evaluate the effectiveness of a few concepts including extruding onto a porous surface and the stretch-to-remove concept. The porous surface used was a 50µ CellMicroSieve™ from BioDesign Inc. The current method of fibrin microthread extrusion involves extrusion onto Teflon pans. Teflon has been used because of its low friction coefficient, which leads to less thread adhesion. The team proposed extruding microthreads onto a porous material to further minimize thread adhesion. Extruding onto a porous extruding surface might have led to less thread adhesion and the surface could be pulled away leaving the threads. The porous extruding surface concept could have been a useful addition to the microthread processing system.

Another method the team tested during the proof of concept testing was the stretch-to-remove concept. In the design team's experiments, once the threads were extruded onto the stretch-to-remove surface in the HEPES bath, there was a small amount of thread adhesion to the stretch-to-remove surface. When creating fibrin microthreads by hand, each thread was individually manipulated, rotated, and stretched to be removed from the stretch-to-remove surface. The idea behind the stretch-to-remove concept was that stretching alone will provide enough force to remove the threads from the stretch-to-remove surface.

The feasibility study and preliminary testing were used to test some of the basic ideas conceptualized during brainstorming. The primary goal of these tests was to determine the relative feasibilities for the tested ideas. The results from this initial testing were important for the team because they gave validation to some of the initial ideas and provided the base from which the design team can build a working prototype.

4.3.7 Preliminary Results

The preliminary results include the findings from the feasibility results. Velcro clamps, a squeegee clamp, the stretch-to-remove concept, and the porous extruding surface were the components tested in the team's preliminary testing.

4.3.7.1 Thread Anchorage

The first Velcro clamps tested were the hook and loop design. The hook and loop Velcro consists of a set of hooks that attach to a set of loops to provide a clamp. Figure 39 is an image of the hook and loop Velcro used in the team's preliminary testing. This Velcro worked poorly as a clamping method for three main reasons. First, there was a relatively large change in height from the surface of the Velcro to the surface of the stretch-to-remove surface. The microthreads were extruded over this lips and prior to polymerization, the threads snapped under their own weights. The second problem was the microthread adhesion to the hook and loop Velcro. The microthreads were extruded on the Velcro surface, but because of the mesh of hooks and loops (Figure 39), the microthreads did not have a stable surface to polymerize on and before the threads could polymerize, the un-polymerized materials seeped into the mesh. Because the ends of the microthreads were now in the Velcro, these portions of the threads broke off, leaving no adhesion surface between the thread on the stretch-to-remove surface and the Velcro. The last problem encountered with the hook and loop Velcro was the cleaning after the test was completed. Portions of the microthread had seeped into the Velcro and made cleaning very

difficult. As depicted in Figure 39, the Velcro adhered to the threads sufficiently but because of the multitudes of hooks and loops, it would prove difficult to clean. Because of these main problems, the hook and loop Velcro will not be used as an adhesion method in the final design.

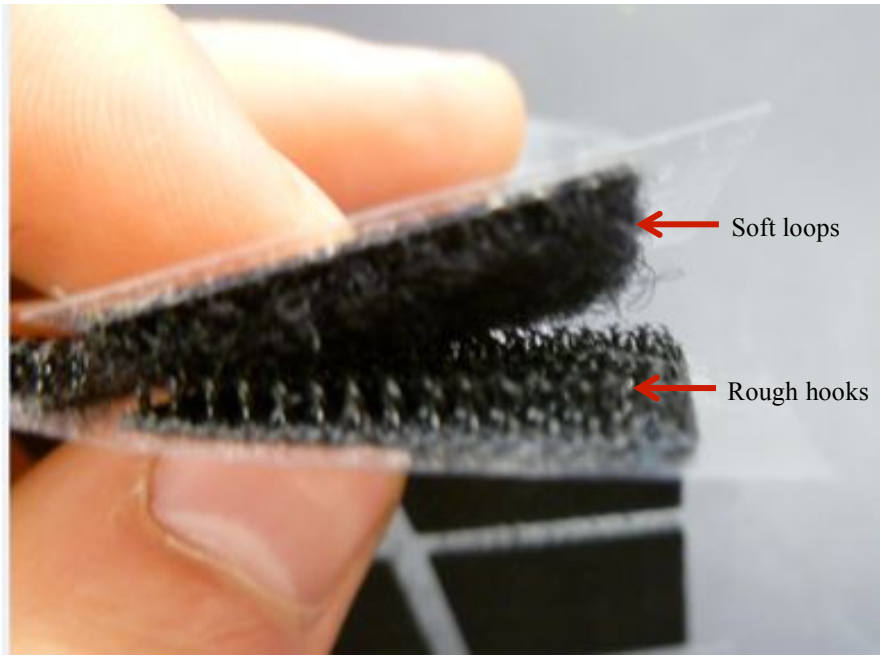


Figure 39: Hook and Loop Velcro – Illustrates the main source of adhesion in the hook and loop Velcro. The mesh of hooks and looks proved difficult for thread adhesion and will not be pursued in terms of adhesives.

The second adhesion method tested was the beaded Velcro. This Velcro had tiny strands with beads on the end and when pressed against another beaded Velcro strip, the strips attached firmly. Figure 40 illustrates the "beaded" Velcro. This Velcro worked well as an adhesion method. In the "hook and loop" Velcro, the ends of the threads seeped into the Velcro. In the beaded Velcro this problem also occurred, but because the number of beaded strands was much less than the hook or loop meshes, the microthread did not tear as a result of seeping into the beaded strands. The threads were able to polymerize within the channels between the beads and prevent the thread from tearing. Cleaning of the beaded Velcro was much easier than the hook and loop Velcro, requiring only brushing the surface of the Velcro to remove the leftover fibrin. The beaded Velcro was much thinner when compared to the hook and loop Velcro and proved to be more effective in terms of thread adhesion.

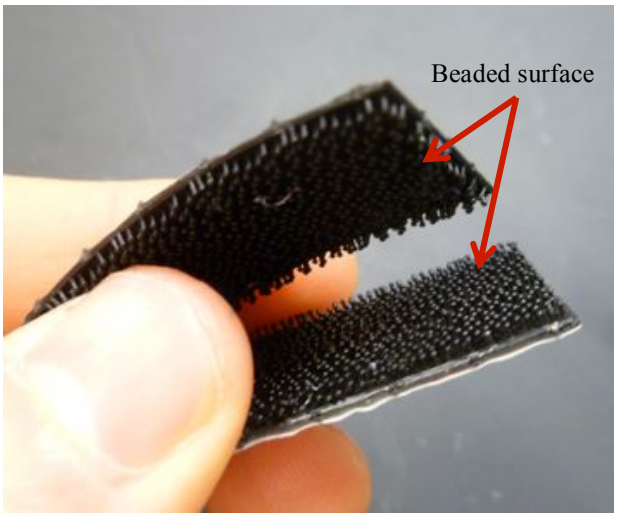


Figure 40: Bead Velcro – Illustrates the beads present on the ends of Velcro used in adhesion testing. These beads provided a firm attachment for threads while maintaining thread stability and polymerization.

The squeegee clamp method was another adhesion method tested. This method used a plastic squeegee like material to clamp down and secure the microthreads in place to be stretched. The squeegee clamp worked well and resulted in no thread failure at the clamp sites, which was defined as a complete shearing of the thread at the adhesion site. It was very easy to clean, only requiring wiping it with a Kim wipe after use to remove any attached threads. The squeegee clamp method proved to be an effective method for securing threads and will be considered in future designs.

4.3.7.2 Thread Removal and Stretching

The porous extruding surface was tested in the preliminary testing. For this portion of the test, threads were extruded on top of the porous material and allowed to polymerize for 20 minutes. Once the threads had polymerized, attempts were made to remove the threads but after polymerization, the threads attached more to the porous material instead. At first the stretch-to-remove method was attempted using the squeegee in order to remove the threads from the stretch-to-remove surface, but this was unsuccessful. Instead, the team attempted to scrape and salvage the threads from the mesh with forceps, but this removal method was also unsuccessful. The team theorized that once the fibrin was extruded and still in the initial liquid phase, it became incorporated into the pores in the porous mesh. Once polymerization had completed, the threads were bound to the mesh and could not be removed without significant damage to the threads.

The stretch-to-remove concept was the last concept tested in the preliminary testing. The stretch-to-remove concept was based off the idea that as the threads are stretched, the stretching will provide enough force to remove the polymerized microthreads from the floor of the bath. The team extruded microthreads on a Teflon pan by hand. Five microthreads were extruded onto a Teflon pan and stretched to observe if the threads detached from the stretch-to-remove surface during the process of stretching. The only problem observed was that when there were uneven threads, there was often thread breakage and failure. The stretch-to-remove concept successfully removed 3 microthreads out of 5, but the microthreads were extruded by hand and had inconsistencies in the thread diameters, which may have accounted for the thread breakages. The team believed that if the threads were extruded automatically with a controlled head rate, this problem would have been eliminated. Future tests would include the stretch-to-remove method with an automated extrusion head in order to eliminate the thread inconsistencies due to manual extrusion.

The team's preliminary testing showed that several theorized concepts were useful and applicable to the fibrin microthread processing system, while other ideas and concepts did not work. The hook and loop Velcro encountered several problems including difficult cleaning and minimal thread adhesion. Beaded Velcro showed optimal thread adhesion and clamping ability, while maintaining a cleanable system. The squeegee clamp provided an easy method to secure the threads and was also easy to clean. The porous extruding surface had excessive thread adhesion and proved difficult when removing threads. The stretch-to-remove method proved to be a promising method for thread removal that would be considered in the final design of the fibrin micro processing system.

5 Design Verification

After deciding on a final preliminary design to pursue, the design team quickly began work constructing and testing different aspects of the design. It was important to test each concept of the device individually, in order to demonstrate that each part of the device met the design criteria, and that design concepts not previously tested would work in the final design. In order to keep accurate record of all expenses and prototyping efforts, a budget and breakdown of all prototype costs can be found in **[Appendix D**: Budget and Cost Analysis**]**. This allowed the team to accurately access the amount spent on each aspect of the design.

5.1 Motorized Stretcher Construction

The ideal stretching mechanism for the system, as chosen by idea comparison charts and preliminary validation testing, was the motorized stretching system. This system was chosen based on its comparative simplicity, relative low cost, and ability to minimize thread damage. Although similar hand-driven systems have been used in the past to stretch threads, it was important to construct and analyze the system fully before use in the final prototype.

5.1.1 Motorized Stretcher Design

In order to create a system that worked within the design constraints, the design team used the manual hand-cranked stretching system used currently in Professor Pins' lab (Figure 41) and modified some of the concepts for ease of use. The hand-cranked system consisted of two pieces of ½ inch Lexan with one threaded hole in the center and two guide holes on the ends. As the threaded rod was turned, the first piece would remain in place as the second piece would move slowly and steadily away from the other piece allowing the threads to be stretched. This initial concept was evaluated by the design team and modified to fit the project's unique needs.

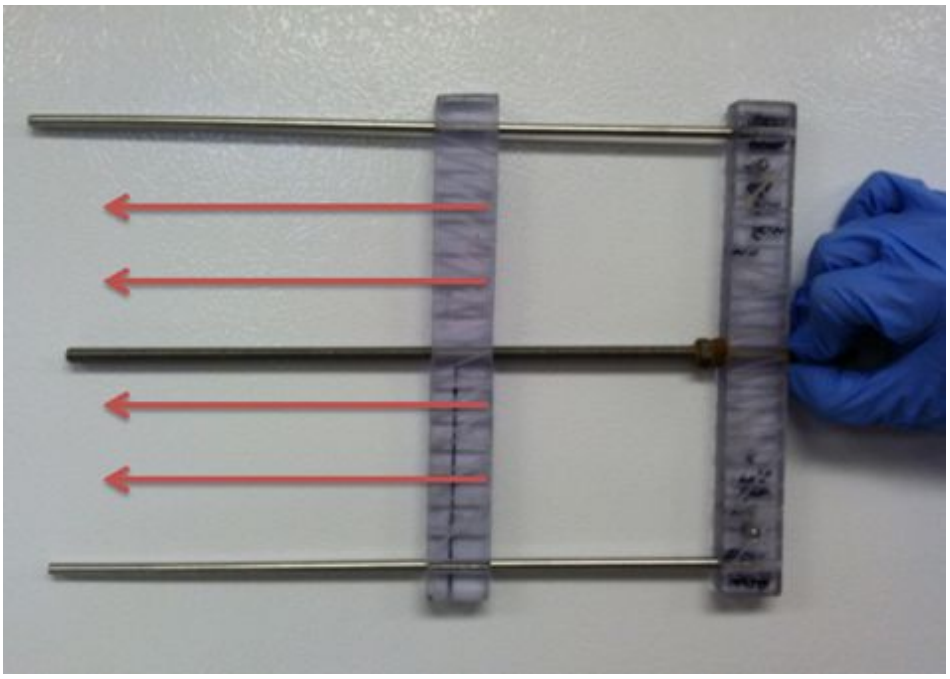


Figure 41: Hand-cranked stretching system made from Lexan.

The first concern when designing the motorized stretcher was the width dimensions of the adhesion plates. The width dimensions of the motorized stretcher would drive the width of the entire system, so it was important to consider scale and design criteria. The system had to integrate with an automated extrusion system previously built. The maximum thread extrusion width, as driven by the construction and design of the previous system, was measured to be 14.5 cm, as depicted in Figure 42. Given this information, the team decided that the thread extrusion plane would measure 14 cm by 8 cm, and that there would be an additional 2.5 cm on each side of the plates for fixtures. The total width of the motorized stretcher plates would be 20 cm. A top view drawing of the final dimensions for the stretching plates, in addition to the hole specifications for the adhesion system and other fixture components can be seen in Figure 42. The ¼-20 sized tapped and clearance holes were used for posts to attach the top stretcher plates to the bottom stretcher plates, explained below, and the 10-32 sized holes were designed to secure the squeegee clamps to the stretcher frame.

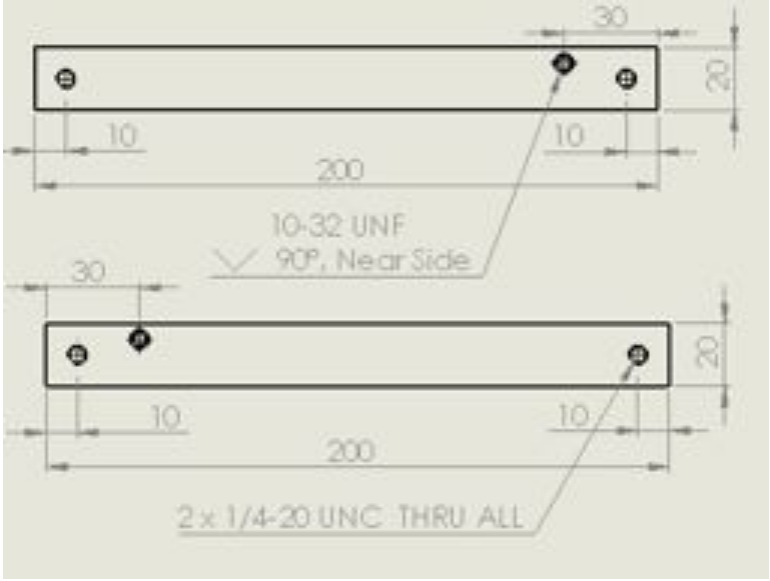


Figure 42: Motorized stretcher plate dimensions - This figure illustrates a top-down drawing of motorized stretching plate dimensions (mm).

The stretching frame comprised of two acrylic cut pieces, two stainless steel rods, and a threaded rod was an ideal design to stretch fibrin threads effectively, but because the threaded rod extended beyond the bath wall to the motor, the stretching frame could not be removed as it was. To accommodate the need for removal of the stretching frame, the team manufactured an additional frame set of stretcher plates that would sit on top of the original frame, as seen in Figure 43. As illustrated, the bottom frame incorporates the threaded rod for stretching and the top frame allows for removal. The top frame contained the steel rods for guidance and stability during stretching and removal. The logic behind creating a two part system was that users would be able to remove the frame from the bath with the threads still attached and allow the threads to be dried in a different location. Additionally, a third piece was added to the end of the motorized stretching system for stability and removal purposes. In order to connect the two pieces, holes were drilled in the stretcher plates on the exterior of the extrusion plane.

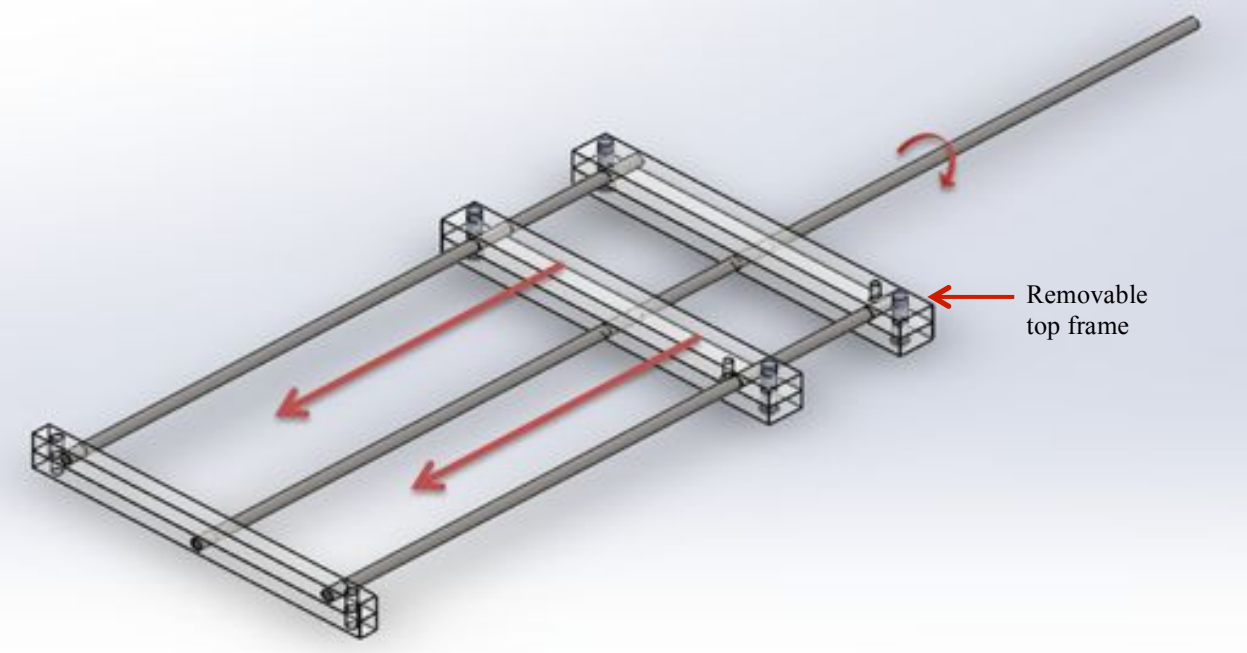


Figure 43: Motorized stretcher final design CAD model.

5.1.2 Motorized Stretcher Construction

The first part of the motorized stretcher that was constructed was the plates. The original hand-drawn system used Lexan plates, but after some experimentation, the team chose to use acrylic because it had similar thread adhesion properties but was easier to manufacture and cheaper to purchase. The team purchased all acrylic used in this project in ten pound supply from Peidmont Plastics, selecting ½ inch thick pieces for the plates and ¼ pieces to be used in the bath design. These thicknesses were similar to those used in previous models. The team also purchased a threaded and a coated steel rod from Home Depot, but for the final design, medical grade stainless steel guide rods were purchased to ensure the biocompatibility of the system. Additionally at Home Depot, the team purchased fixture pieces, including ¼ - 20 1-1/2 inch screws, which would be screwed into the bottom plate and serve as a post for the top plate to slide on and off.

All pieces of the motorized stretcher were machined by the design team in Worcester Polytechnic Institute (WPI) labs. The plates and pilot holes for the fixtures were cut by a Versalaser VLS-4.60 laser, manufactured by Universal Laser Systems. After being cut, the pilot holes were used to ensure the accuracy of the drill press. The holes on the side of the stretcher plates were drilled using a milling machine for increased accuracy. The threaded rod and guide rods for the system were easiest to manufacture, and were cut to correct size using a hack saw.

One of the problems the design team faced during construction was aligning the hole correctly so that the plates moved with minimal effort but the rods had little to no ability to move within the holes. After the first round of manufacturing, one of the holes did not line up with the others, despite careful milling. To solve this problem, the design team drilled out the guide hole on the static plate, reasoning that the play could be taken out of the equation by added extra glue when adhering the stainless steel rod to this area. Once drilled out, the prototype moved well and the design team was confident in its ability to perform needed tasks.

5.2 Stretch-To-Remove Construction

Chosen mainly for its simplicity, the stretch-to-remove system was selected by the design team as way to remove the threads from the extrusion pan. The stretch-to-remove concept was evaluated in earlier feasibility studies, and the design team was confident that this system would allow for ease of use, decrease the complexity of the entire system, and minimize thread damage. Although preliminary testing showed promising results, it was important to construct the entire removal system for further testing and incorporation with the rest of the components.

5.2.1 Stretch-to-remove Design

By nature of its design, the stretch-to-remove system was relatively simple to design, but incorporation of the system with the other components of the design required more work. As mentioned previously, the width of the system was driven by the existing extrusion head and the motorized stretcher, but the overall length of the system was driven by the stretching system. In practical use, threads used for experimentation can range in length from less than 1 cm to approximately 3 cm. To reduce the amount of wasted material, the design team used 8 cm as a total extrusion length, allowing for 1 cm of clamping waste at each end of the threads. As a whole, the system measured 35 cm in length, which allowed for a maximum stretch percentage of 400%.

Considering that the extrusion thread length was 6 cm, the extrusion pan from which to stretch the threads off of would need to be 6 cm long and a minimum of 14 cm wide to accommodate all of the extruded threads. Because of the two part design of the motorized stretcher, it was critical the extrusion pan did not interfere with the removable frame. The stretch-to-remove surface was designed to fit in between the guide rods (in the top frame) and was adhered to the bottom of the bath with enough room for the rotation of the threaded rod. The dimensions and lay out for the stretch-to-remove system are found in Figure 44.

In theory, threads would be extruded onto the extrusion plate and removed during stretching, allowing for easy removal. At first, the extrusion pan was designed to be flush with the motorized stretching plates to reduce shear stress on the threads, but after initial testing, which is detailed in Section 5.6, the extrusion pan was placed approximately 1 mm below the motorized stretching plates. The 1 mm recession was created by reducing the height of the feet for the stretch-to-remove surface. Once this concept was tested, however, the design team realized that this method did not reduce the shear stress on the threads. The final decision made by the design team was to micro machine the stretch-to-remove surface such that it was completely flush with the stretcher plates. The figure below (Figure 44) depicts the final design for the stretch-to-remove system.

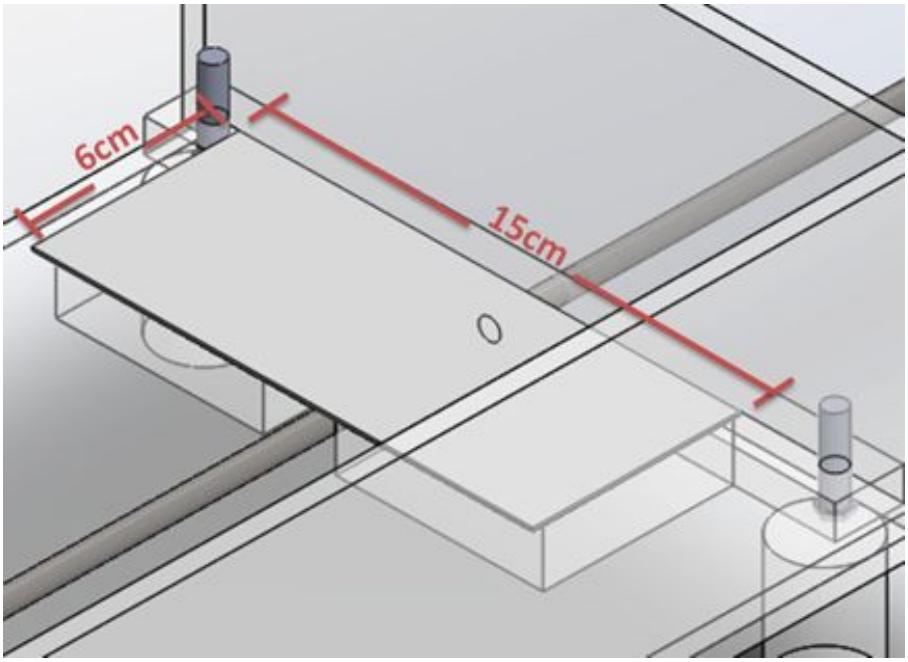


Figure 44: Extrusion pan set up for the stretch-to-remove system – This figure illustrates a view of the set up for the extrusion pan and feet with the entire system in Solidworks®.

5.2.2 Stretch-to-remove Construction

The stretch-to-remove system was constructed using a Teflon coated baking sheet and ½ inch thick acrylic pieces as mounting feet to create the 1 mm recession. The Teflon coated baking sheet was used because it is the same material used in the manual extrusion process. Using the same pan material would ensure that the threads would not stick to the stretch-to-remove surface.

All parts of the stretch-to-removed system were machined at WPI in the Washburn shops. The first part of the system that needed to be constructed was the extrusion pan. As designated

previously, the extrusion pan length and width were 6 cm by 15 cm respectively. The Teflon pan used for the extrusion pan was cut using both a plasma cutter and a shearing device. After the stretch-to-remove surface was marked with the direct dimension, the plasma cutter was used to cut the edges of the stretch-to-remove surface off. To refine the cut edges, the shearing device was used to produce uniform, straight edges. The corners were filed down to prevent damage to the threads or other parts of the system. The mounting feet for the extrusion pan were made by gluing together two ½ inch pieces of acrylic to ensure a pan height of 1 inch off the bottom of the stretch-to-remove surface.

The initial design, which required a 1 inch height for the stretch-to-remove surface to be flush with the stretcher plates, was modified to create a recession of the stretch-to-remove surface below the stretching level. However, in the machined prototype, the fixtures and added parts to the motorized stretcher increased its height, and the 1 inch mount plates left the extrusion plate slightly recessed.

5.3 Thread Adhesion System Construction

It was essential that the automated system secure the threads to the extrusion pan as well as motorized stretcher plates. In choosing a thread adhesion system, the team took into consideration minimization of thread failure, reproducibility and precision of threads, portability, and ease of use. The team chose to build a rotational clamping device to adhere the threads to the plates. Although preliminary testing was performed, it was important to design and construct the entire adhesion system in order to validate its effectiveness before use in the final design.

5.3.1 Thread Adhesion System Design

The team chose to build a squeegee clamp system based off of the adhesion system currently used in the lab, which consists of thin pieces of polydimethylsiloxane (PDMS) sealed to Lexan plates in a dry environment. Using this information, the team chose to create a squeegee clamp that would rotate on a hinge and secure the clamps horizontally across the plates. The team purchased a 14 inch squeegee from Home Depot and removed the rubber insert. It was decided that the team would use the outer aluminum frame and create a new PDMS insert, because the team knew PDMS was a material which interfaced well with the threads. The squeegee insert, would be 15 cm long to span the length of the space in which threads would be

extruded, and would secure the threads uniformly. The circular end would fit inside the aluminum frame with the angled part directly over the threads.

The end was tapered with a 15 degree angle so as to mimic the original squeegee design from Home Depot. The design team decided that a tapered end would interface well with the threads by minimizing the amount of shear stress on the edge of the threads. It was important to take this into consideration to limit the breaking of the threads between the motorized stretcher plates and the stretch-to-remove surface. Once the design team had the dimensions for the squeegee, a mold could be created in order to cure the PDMS and make the final product.

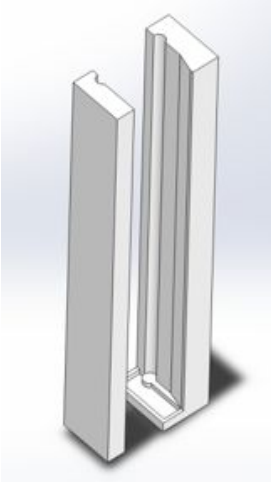


Figure 45: **Solidworks model of the initial squeegee mold**.

The design team chose to make the mold for the squeegee out of ABS plastic, because it could easily be manufacture on a rapid prototyping machine for approximately twenty dollars. The team designed the mold to come apart into two pieces so that removing the part would not be difficult. The two pieces fit together and locked in place on the bottom, leaving the top as an exposed channel through which the PDMS could be poured. The Solidworks design for the mold can be seen in Figure 45.

The initial mold design was designed to allow for air removal during the vacuum step in the PDMS process. However, during the curing of the PDMS within the mold, the team realized it was extremely difficult to remove the air bubbles through the opening in the top of the mold. In addition, the mold did not form a proper seal, and PDMS leaked out of the bottom of the mold. A second mold was constructed, following the previously described method for ABS plastic, but it allowed for efficient vacuuming of the PDMS prior to curing. The new mold consisted of a bottom piece and top piece that, when compressed, formed a cylindrical mold with

a rectangular part that would be used to secure the threads, as seen in Figure 46. The new mold incorporated all of the features of the previous mold but eliminated the angled cut in the securing edge because the team thought this would eliminate the potential for tearing and bubble formation.

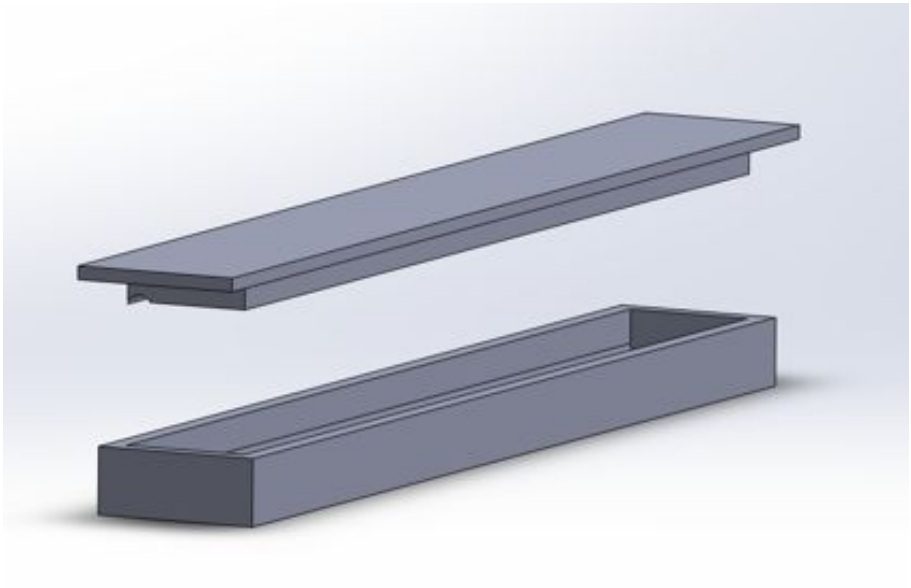


Figure 46: Solidworks model of the newly designed squeegee insert mold.

After use with the new mold, the team realized that after application of the top piece of the mold, the PDMS formed large bubbles between the two mold pieces because of the volume of trapped air. After further inspection, the team decided to just use the bottom piece of the mold to construct the PDMS. With the appropriate final volume of 7 cc of elastomer base and curing agent in solution at a ratio of 10:1, the PDMS clamps were cured and fit into the aluminum squeegee frame. The team also decided that the rotational clamp mechanism would not fit the needs of the system and drilled additional holes in both the PDMS and aluminum frame. This allowed for flat clamping of the threads.

5.3.2 Thread Adhesion System Construction

The PDMS was constructed from a silicone elastomer and a curing agent at a ratio of 10:1. The circular piece of the sheet allowed for easy placement and removal within the commercial metal squeegee clamp. The angled extension allowed for angular placement and adhesion to fibrin threads within the bath. The ratio of 10:1 allowed for easy adhesion to the stretcher plates under bath conditions. The combination of desired ratio and angled extension created constant force applied to the threads and minimized shear stress to the threads. To construct the PDMS, 3

grams of curing agent were added to 30 grams of elastomer base, within the PDMS mold, stirred, and vacuumed until all bubbles were removed. The mixture was cured at 60^0C for one hour.

The team designed a locking mechanism to hold the squeegee clamps in place while the threads were stretched. This locking mechanism consisted of two ½ in. screws that ran through the squeegee and were secured by the user using two wing-nuts. The screws allowed for easy clamping of the threads using the PDMS sheets.

Using PDMS as a clamping mechanism for fibrin microthreads allowed for easy, controlled clamping force, distributed evenly across the threads. The PDMS formed a seal when applied to the stretcher plates, indicating a complete interaction between the two surfaces. This was necessary to secure the threads because it maximized the contact area and reduced the possibility of stress concentrations at the clamping sites.

5.4 Angled Bath Construction

When designing a bath for the automated stretching system, it was important that it drain properly and interface with the rest of the system. The bath system was important because it both holds the polymerization solution for the threads and determines the size of the entire system. The angled bath design was chosen because it was relatively cheap, would ensure full drainage of the HEPES, and most importantly, would interface with the rest of the system. Because the angled bath design was simple in concept, no preliminary testing was conducted. It was important to build and test the angled bath system quickly in order to be able to test other aspects of the system within the HEPES polymerization solution.

5.4.1 Angled Bath Design

In initial design concepts, the angled bath consisted of a four walled bath with a completely angled bottom. One of the drawbacks of this design was that there was no support system for the stretch-to-remove surface or the motorized stretcher because there was no flat surface to extrude on. The design team modified the concept of the angled bath slightly, in that only the end right side of the bath was slanted. The slant of the corner was a 5° degree angle, bringing the corner of the bath down vertically a total of 2.5 cm. This ensured that the slant would not take a lot of extra HEPES solution or disrupt the function of the rest of the bath by becoming too cumbersome. Figure 47 below shows the Solidworks model of the bath design.

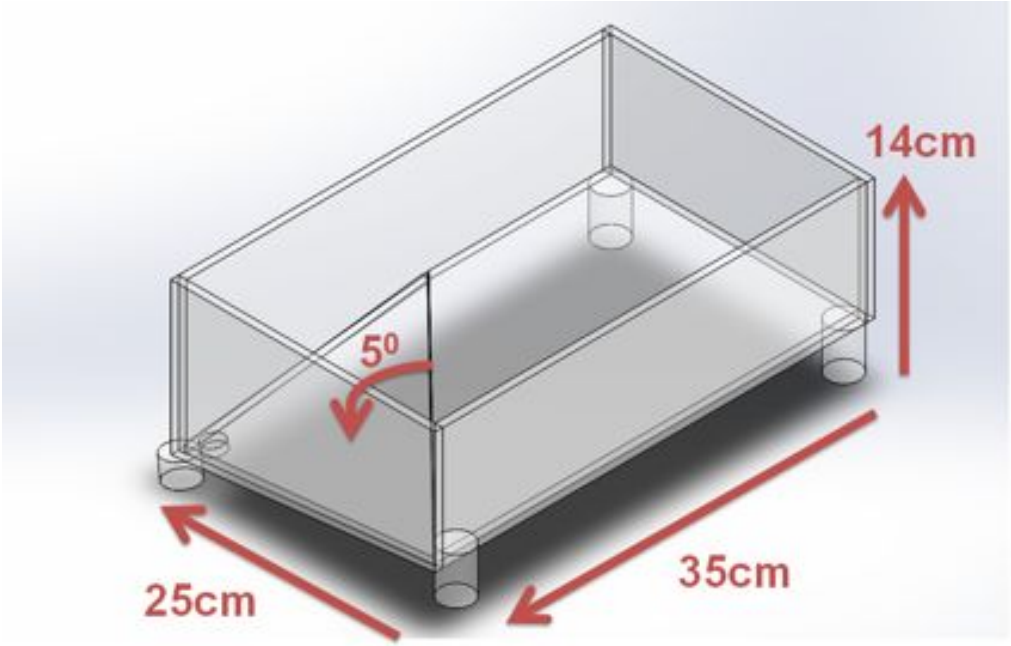


Figure 47: Solidworks model for the angled bath system.

The dimensions of the bath were an important design consideration because the bath would almost completely encompass the system, with exception of the motor and the extrusion head. The length of the bath was the most important feature because it determined the amount of HEPES needed and the maximum stretch percentage of the threads. The final length of the bath was 35 cm, which accommodated all the components of the motorized stretcher, and allowed for the threads, of initial length 6 cm, to be stretched by 400 %. The width of the bath was designed to be 25 cm, so as to accommodate the width of the 20 cm stretcher plates and leave room on the sides for HEPES solution to flow through evenly. The depth of the bath, without including the angled corner, was designed to be approximately 10 cm, which would give sufficient clearance of the motorized stretcher components. The end pieces of the bath were designed to overlap the end face of the side pieces, in order to create a tight seal, and thus were approximately 25.5 cm. Figure 48 below shows the final dimensions for the angled bath design.

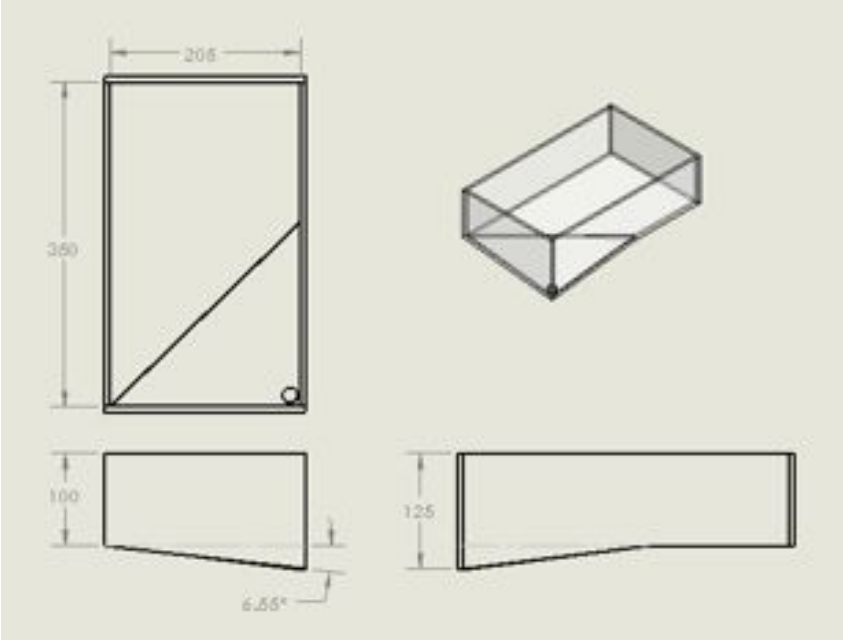


Figure 48: Drawing of the final angled bath dimensions (mm).

5.4.2 Angled Bath Construction

Of all of the components of the stretching system, the angled bath was the most difficult to construct. The sides of the bath were cut from ¼ inch acrylic on the same Versalaser VLS-4.60 laser used to machine the motorized stretcher plates. The holes needed for the bath drain and the threaded rod were cut after most of the other components were completed. The end piece and the angled piece were the last parts of the bath to be added and cured. Initially, the design team had bought an all-purpose glue to adhere the sides of the bath. Unfortunately, this glue did not properly glue the sides of the bath. After the failed attempt at putting the bath together, the design team purchased acetone-based glue from the acrylic supplier, which worked by chemically bonding the acrylic pieces together.

In order to construct the bath, the design team used four corner clamps and completed the curing in four steps. The first step was to glue one of the sides of the bath to the flat bottom piece and the other two sides together. This was done so that the pieces could then be glued together with multiple seals at once to save time. After each gluing step, the pieces were left to cure and dry over twenty four hours. After three of the sides and the flat bottom piece were glued together, the angled piece was glued in. This piece had to be added after all of the other sides, excluding the last end piece, because it needed to rest on the other pieces of the system so that it would not fail when glued.

The reason that the final end piece was left until last was because it needed to have a hole in it for the threaded rod to connect to the motor. Making this interface between the threaded rod and the outside of the bath water-tight was the one of the biggest challenges of the bath construction. In order to keep the system water-tight, the design team decided to use a stuffing box, which is used in sinks and boat propellers to drive water-tight shafts. Figure 49 depicts the set up for the stuffing box on the outside wall of the bath. In order to accommodate the large size of the stuffing box and ensure a water-tight seal, the design team machined an exterior plate out of the ½ inch acrylic, which the stuffing box could be threaded through. Along with the bath feet, this was the last component that was added to the bath before completion.

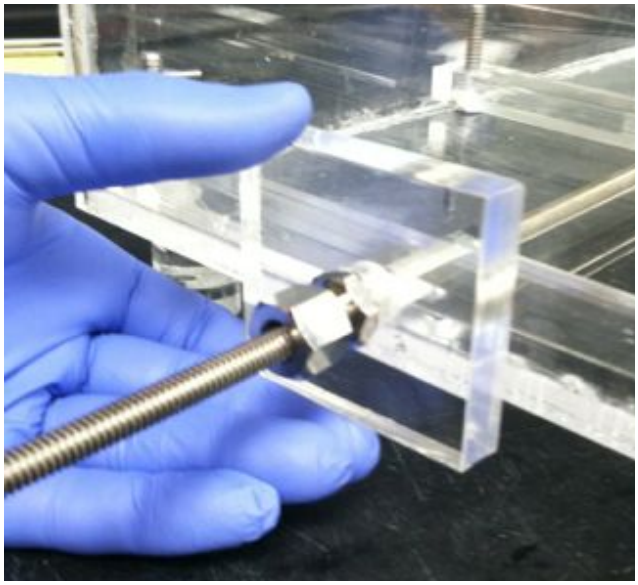


Figure 49: Threaded rod and stuffing box set up.

After the bath was constructed, it was critical to seal the edges of the bath to make sure the system was water-tight. The design team applied auto/marine sealant to the outside edges of the bath. After each application, leak testing was conducted, and additional sealant was applied to the outsider edges of the bath as needed. The leak testing involved filling the bath and observing accumulations of water at the base of the bath for 24 hours. The leak testing protocol can be found in **[Appendix F**: Protocols**]**.

5.5 Motor

To automate the stretching device, the team placed a motor at the end of the threaded rod. The team also felt that the consistent automation would ultimately reduce the variability between threads. The team acquired a Vex high torque motor, typically used for robotics, and repurposed

it to drive the threaded rod. For the team's purposes, a quadrature encoder was added because it is able to determine location and direction of the motor which allowed the team to make the stretch speed and stretch percentage adjustable, as well as program the motor to return to its initial position after stretching, making it easier for the user.

5.5.1 Motor Capabilities

The motor utilized by the design team was a Vex high torque motor, which operates as a stepper motor. The motor has a maximum power of 100 rpm at 7.5 volts, and a stall torque of 6.5 in-lbs. The motor is easily programmed in EasyC Pro using a setting between 0 and 127, where 0 is full power and 127 is stop (2-Wire Motor 393). For the design team's purposes, it was critical to understand the precision, torque, and other unique properties of the motor.

The motor precision was determined by calculating the number of counts registered by the quadrature encoder and then multiplying that by the threaded rod to understand how accurately the motor would be able to stretch the threads. Using 90 steps per revolution, and a 20 threads-per-inch threaded rod, the design team determined the motor to be accurate to 0.1 inches, or 0.24 cm which is more accurate than manually created threads.

In terms of torque, the motor needed to turn the threaded rod, which was 18 inches long and ¼ inch in diameter. The chosen motor exhibited appropriate torque (1.67 N-m) for the threaded rod, however in order to avoid damage to the motor, it was suggested that the team create a gear box for the motor and include a clutch for appropriate protection in case the motor got stuck (2-Wire Motor 393).

Using EasyC Pro as a programming platform, the team programmed the motor to move to the desired stretch percentage at a modifiable stretch speed, then would return to its original location after a user pressed a continue button.

5.5.2 Wiring

The motor was wired into a Vex PIC microcontroller, where the first peg was a control signal, the middle peg was a voltage signal (+5 V) and the last peg was a ground signal (PIC Microcontroller V0.5). This microcontroller was attached to the 2010 MQP and so the motor was programmed to run in conjunction with a previously written program for the extrusion head. The circuit utilized a battery power source. The PIC microcontroller was easy to plug wires into as it had labeled ports. The design team's motor was connected to a controller which (2-Wire Motor 393) allowed it to be driven appropriately by the microcontroller. The quadrature optical shaft

encoder was then plugged in as well, and wired as both an interrupt and a digital reader. This means when the encoder read a certain amount of counts it would shut off the motor according to a registered value (Quadrature optical shaft encoder). The wiring of these two parts would be according to Figure 50: PIC microcontroller wiring for .

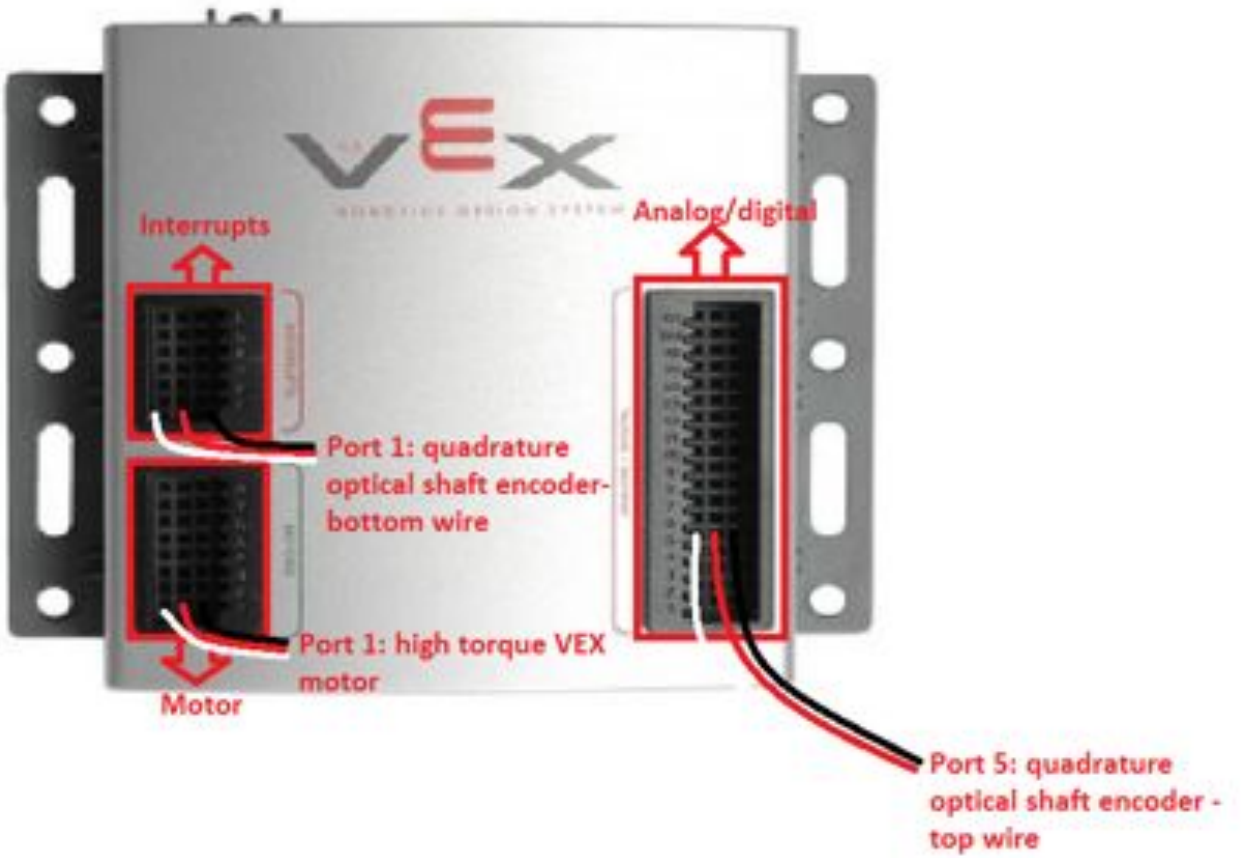


Figure 50: PIC microcontroller wiring for stretching system include motor and quadrature optical shaft encoder.

5.6 Initial Design Verification

The team needed to prove that the design would reproducibly, precisely, and automatically extrude, stretch, and remove the threads. Testing included leakage in the bath, extrusion pan sticking, and the stretching mechanism to ensure all aspects of the device successfully worked the way they were originally intended.

5.6.1 Bath Contamination Testing

The team performed a UV absorbance analysis to check for any leeching into the HEPES buffer from the bath itself. The team used a batch of HEPES that had never touched either bath as a control and tested HEPES that had been soaked in the old bath compared to

HEPES from the new bath. The UV absorbance analysis was then completed after the HEPES had soaked for an hour. The control batch of HEPES was used to zero the machine, and results are exhibited in Figure 51.

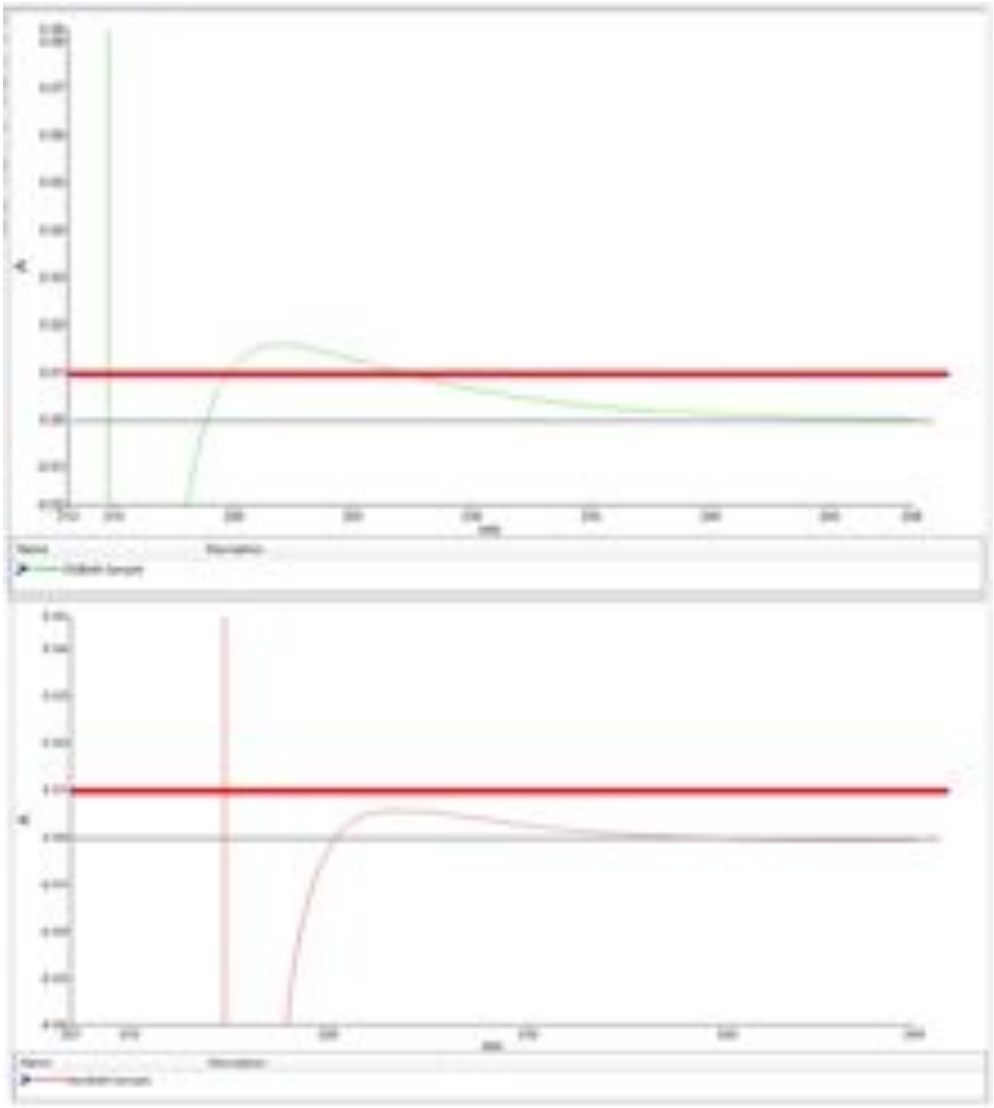


Figure 51: UV Absorbance Results – The top diagram depicts the absorbance of HEPES from the old bath system, and the bottom diagram depicts HEPES from the new bath. Note that normal HEPES absorbs around 215 and 225 nm.

These results depict a spike in the absorbance levels in the old bath at 222nm to a 0.01 A, which is not seen in the new bath. The red line depicts an absorbance that is statistically significant above normal noise in the spectra. The team was able to hypothesize that it could be from either an old marine sealant that was used, or from the acetone leeching through the sealant. The new bath however depicts no such feature, so the team continued further experimentation and thread batches using the new bath, which shows no spike in absorbance.

5.6.2 Hand Cranked Stretching Mechanism

Before the motor and the extrusion head were added to the stretching system, it was important to determine whether or not the prototype concept would work in practice. In order to do this, the team combined all of the functional components of the system together so that the motorized stretcher could be driven by hand, with all other components fully developed. These tests were termed hand cranked stretching mechanism tests. Table 41 shows a summary of the results from the hand cranked stretching mechanism tests. Out of the total number of threads extruded, each was given a number and, if it broke, its failure mechanism was recorded. Shearing indicated that the thread broke at the stretcher plate and pan interface, whereas stretching indicated that the thread failed during the stretching process.

Table 41: Summary of results from hand cranked stretching mechanism tests

Test #	# threads	Stretch %	Failed threads	Failure mechanism
1	9	50	9	(1,2,3,4,5,7,8,9) shearing, (6) stretching
2	9	50	9	(1,2,5,6,7,8,9) shearing (3, 4) stretching

The team tested the hand crank stretching mechanism to ensure it would equally stretch the threads and perform all other expected function. It was important that the system stretch the threads at a constant rate, without shearing or breaking the threads, and without causing interruptions in the stretching rate through bumps or interference from other components. The team used the hand crank system to observe both the tendency of threads to detach from the stretch-to-remove surface and the stretching mechanism in terms of fluid movement. Tests were performed by turning the hand crank attached to the threaded rod and stretching the threads off the stretch-to-remove surface. For this testing, the threads were secured with PDMS strips. Figure 52 shows the setup of the experiment.

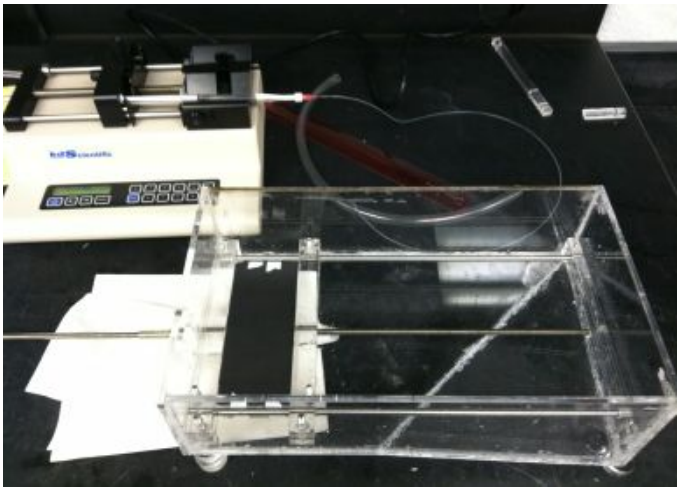


Figure 52: Hand crank testing with manual extrusion.

Initial testing with this system appeared to be promising; however, the threads appeared to break soon after stretching off of the stretch-to-remove surface. It was determined that there could be two causes for the problems associated with the stretching of the threads. One problem associated with this system could have been friction on the bottom of the bath caused by the sealant. This caused a problem for the fluid running of the stretching mechanism, and additionally caused the dynamic stretching plate to rock back and forth as it moved. This not only increased the variability of the threads, but could have caused enough vibrations to cause the threads to fail and break. To resolve this problem, the team scraped away much of the sealant on the bottom of the bath and resealed the bath from the outside. This procedure was repeated until the friction was removed from the track in which the stretching device would move.

The second problem with this system was determined to be the interface between the stretch-to-remove surface, the motorized stretcher plates, and the squeegee adhesion system. Although most of the threads broke before stretching, the stretch-to-remove system was proven in concept, and threads which were picked up and re-adhered to the stretching plates stretched easily off the stretch-to-remove surface, as seen in Figure 53.

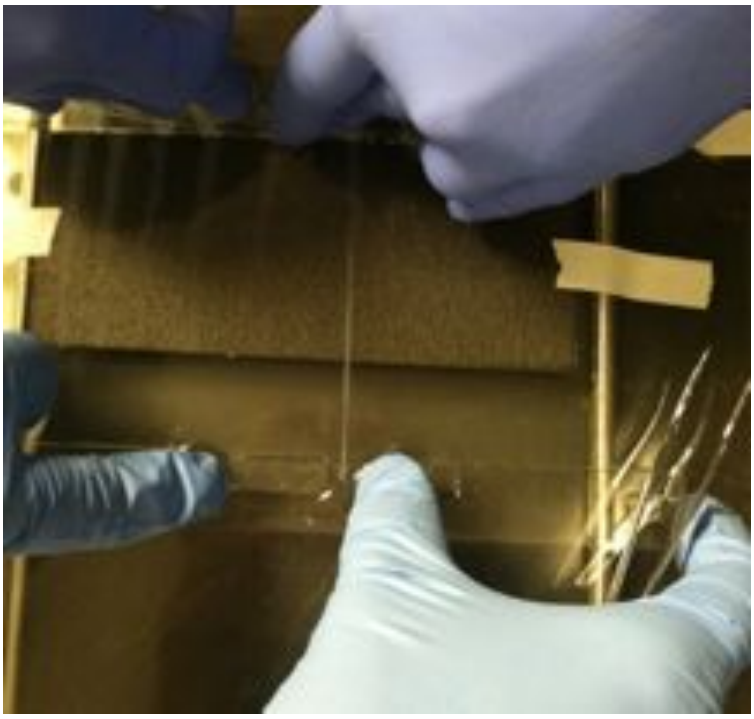


Figure 53: Stretch-to-remove testing after re-adhering broken threads to stretch-to-remove system.

The design team determined that stretching the threads to remove them would work as long as the interfaces between the extrusion pan, the motorized stretcher plates, and the squeegee adhesion system were precisely machined. Initial design verification testing was not sufficient to fix this interface problem because the design team felt that incorporation of the automated extrusion head would be necessary to ensure completely uniform threads for stretching. Since the theory was proven in concept, the design team chose to make additional modification to the stretch-to-remove system in order to correct this problem.

Stretch to remove could have worked during preliminary testing because the small gap between the acrylic and the extrusion pan was not a factor in the initial preliminary testing, and allowed for a continuous thread to be extruded. In the preliminary testing, the continuous thread was stretched and removed from the stretch-to-remove surface.

5.7 Additional Modifications

Because of the complex nature of fibrin microthread manufacturing and the effects of subtle changes in production processes, the team implemented several changes to various parts of the system. Of the changes made, modifications to the bath in terms of new sealant and additional feet were needed. Other changes include modifications to the top piece of the stretching frame and to the stretch-to-remove surface. Changes to system parts were attempts to

rectify the problems identified with the production of fibrin threads prior to machine validation testing.

5.7.1 Angled Bath

In terms of the bath, additional feet were glued to the bottom of the existing feet, to raise the bath system ½" to provide more room for a valve drainage system. The additional feet were cut from the ½" acrylic plate obtained from Plastics Unlimited, Worcester, Massachusetts, in the same method as the initial feet. Once the feet were added, a level was used to ensure that the feet of the bath were even and did not allow for rocking of the system. The criteria for pass or fail of the test were whether or not the bubble inside the level was between the left and right vertical lines. Figure 54 shows the locations and results of the level test.

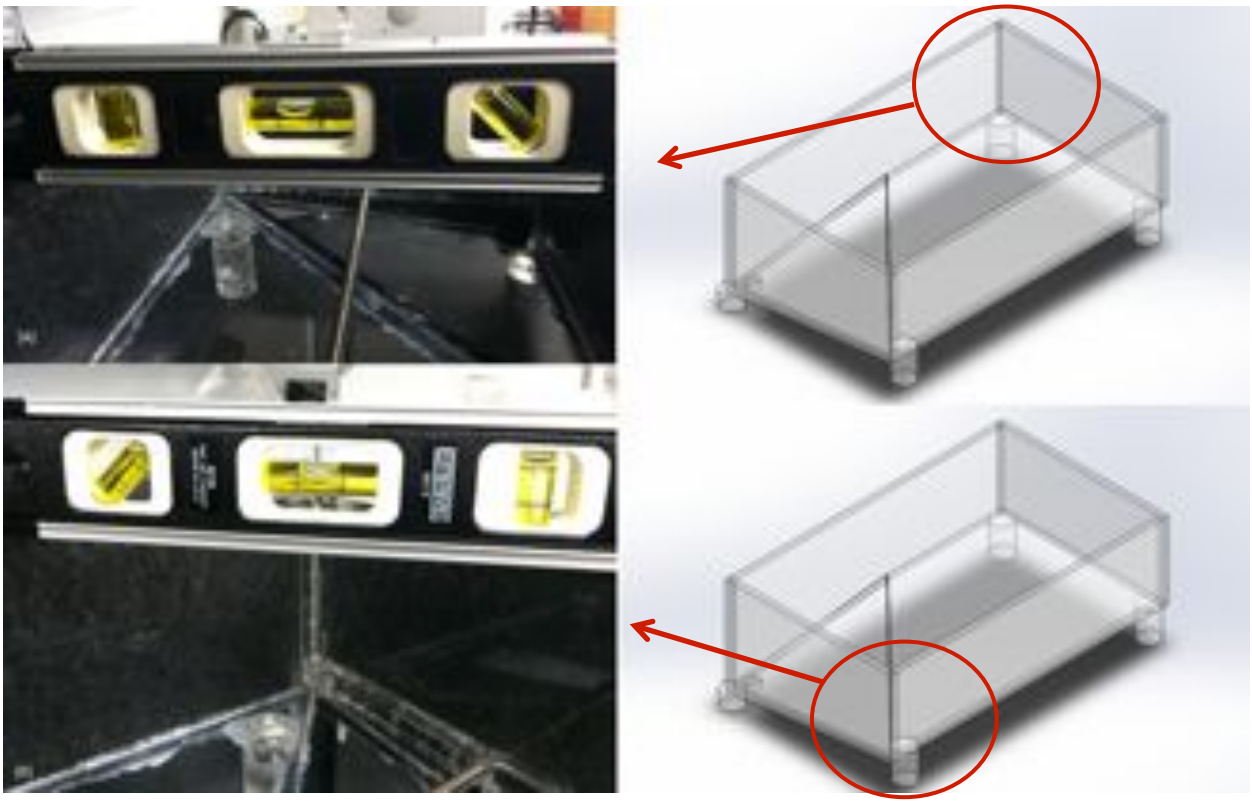


Figure 54**: Level test** - The level test was conducted to make sure the bath was flat and that the fibrin microthreads would be extruded on a level surface.

Additionally, a valve system was added to the final design of the angled bath in order to accommodate easier drainage without the user having to reach into the bath and pull out a cork. The system that was added consists of 10 mm silicone tubing with an inner diameter of 6mm, connected to the bath by a modified plastic adapter so it could be glued to the hole in the bath

floor. Once sealed, the valve system allowed the design team to be able to empty the HEPES from the bath into a container so that it could either be reused or disposed of.

While additional modifications were made to the system as a whole, the team performed leak tests, approximately 2 per week for a period of 10-20 minutes with 2 L of water, to test if the bath was completely sealed. Because the bath leaked during each test, additional coats of sealant were added to the junctions between side-pieces and the bottom. Additional acetone was added to the inside of the junctions to form more cohesive bonds between the walls. After a sufficient seal was achieved, the team performed 2 additional leak tests of longer durations (30-45 minutes) to measure leakage for longer durations. The 45-minute time limit is the approximate time for the HEPES to be in the bath during extrusion and stretching. In addition to these test, the design team performed a final 24-hour leak test to further confirm the long-term seal of the bath and affirm its durability. Details of the leak testing conducted and the results can be found in **[6.1.1 Leak Testing Validation]**.

5.7.2 Stretch-to-remove

During initial testing, the team found that the stretch-to-remove technique was effective in removing threads from the Teflon pan and stretching them to the desired lengths. After construction of the stretch-to-remove surface and stretching frame, additional modifications were made to ensure proper removal of the threads from the stretch-to-remove surface. Initial observations with hand-drawn threads included bulbous, non-uniform threads that frequently sheared on the interfaces of the stretch-to-remove surface with the stretching frame. To combat this problem, the team sanded the edges of the acrylic and where the stretch-to-remove surface met the stretcher plates, to create a smooth surface that would eliminate thread shearing. Table 42 shows the resulting number of sheared threads from each test and the measured height of the stretch-to-remove surface off-set at the time of the test. Additionally, the surfaces of the stretcher plates were roughened in order to prevent the threads from being dragged by the extruder head, seen in Figure 55.

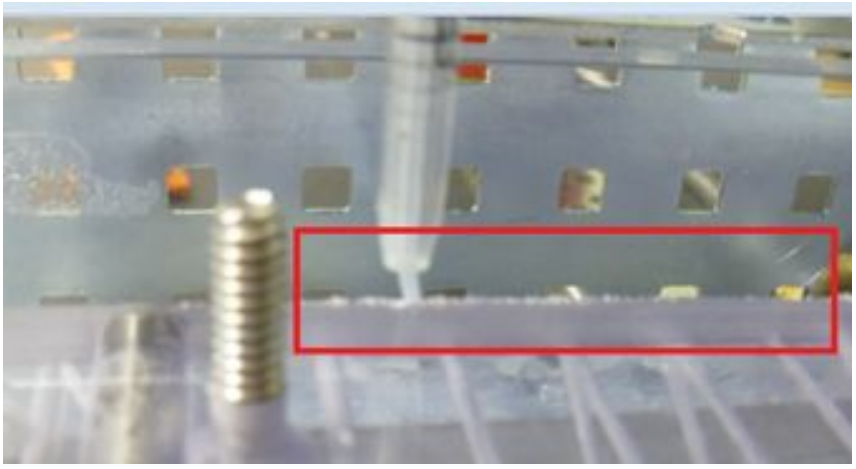


Figure 55: Roughened surface picture - the roughened surface provides a surface for the threads to adhere to (roughened surface is indicated by the red box)

Table 42: Summary of tests correlating to the stretch-to-remove surface off-set height

Test #	Pan off-set height (mm)	# threads	# sheared threads
1	2	8	8
2	2	6	5
3	2	8	8
4	2	7	7
5	0.1	11	8
6	0.1	11	9

After 4 tests with varying stretch speeds and modifications, the threads were still shearing and unable to be stretched. It was determined that the interfaces between the stretch-to-remove surface and stretcher plates were still problematic, and the team sanded the stretcher plates and edges of the stretch-to-remove surface to be flush (within 0.1 mm). To do this, caliper measurements were taken at three edges on each acrylic plate and the stretch-to-remove surface to measure the heights. From the averages of these values, the lowest point was set to the depth of the plates and the stretch-to-remove surface. The stretch-to-remove surface feet and stretcher plates were sanded with a belt sander and measured for precision. The final dimensions of the pan with feet and stretcher plates were: height = 23.4 ± 0.1 mm for the stretcher plates and pan (dimensions taken from the base of the bath).

Once the pan and acrylic plate heights were set to within 0.1 mm of each other, further thread tests showed a more connected formation of threads across the interfaces (Figure 56). The red arrows indicate where the recession between the Teflon pan and acrylic frame was, note how

the fibrin microthread (in blue), was polymerizing around the recession. Tests with automated extrusion and automated stretching revealed a decrease in thread shearing, but also highlighted problems with the stretch-to-remove technique.

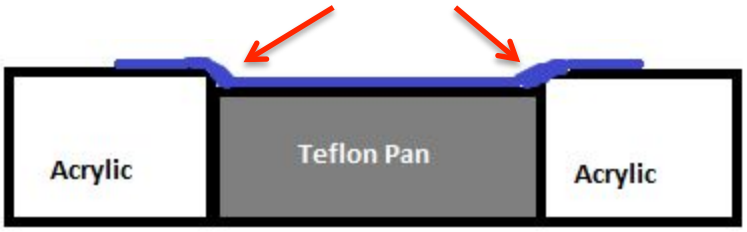


Figure 56 Diagram of pan / acrylic interface – the idea behind this design was that the small lip (indicated by the arrows) would mimic the upward lift of tweezers in the maual method, in actuallity, the lip created in consistent threads that became stuck in the stretch-to-remove surface-stretcher plate interface.

During stretching, the threads were pulled from the stretch-to-remove surface, but the thread was not completely removed from the stretch-to-remove surface, causing it to break (Figure 57). The threads remained attached to the acrylic as well as the Teflon pan and the threads stretched unevenly as the acrylic moved away from the stretch-to-remove surface. The team chose to manually remove the threads from the stretch-to-remove surface using forceps, and reattach them to the stretcher plates for automated stretching. After completion of preliminary tests, stretching frame modifications, and further thread tests, the team began tests to validate the consistency of both the machine and properties of the threads produced by a process that included manual extrusion and transfer of the threads, with automated stretching.

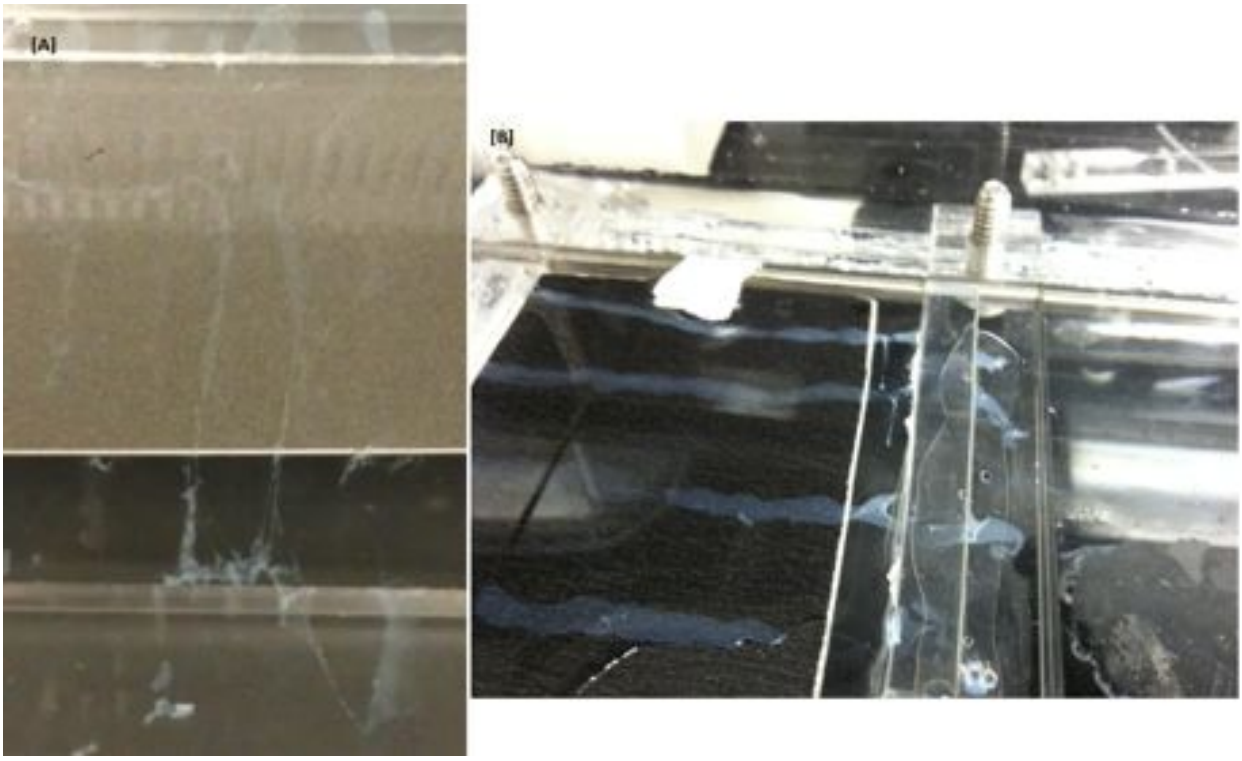


Figure 57: Picture of threads on extrusion pan during stretching - note of parts of the thread are removed and other parts are stuck on the stretch-to-remove surface in both A and B. Additionally in B you can also see parts of the threads had fallen through the gap between the Teflon pan and acrylic and polymerized in a tear drop like formation

5.7.3 Extrusion Head Modification

The original extrusion head the team received from the 2010 MQP team was designed and programmed specifically according to parameters needed to extrude collagen microthreads. As such, many modifications were made to ensure it would interface well with the additional bath and stretching device, as well as effectively extrude fibrin microthreads.

The first modification the team made was to the extruder itself. The head appeared to get stuck on its path due to some warped gears and a separating track gear. The team replaced the warped gears with high strength gears of the same size to prevent further warping over time and promote longevity of the machine. The track gear was removed and replaced but the pieces were fitted together more precisely to eliminate gaps between pieces. After testing, it was shown that the machine no longer stalled, and ran more smoothly.

Next, the team noticed that the extruder head would sometimes run slowly or not at all. It was determined that the previous solders were becoming weak and breaking. The team soldered these wires, and any potential issues foreseen with other wires. Afterwards the team used

electrical tape to secure each solder point and ensure they would no longer be exposed then secured them to the underside of the base of the extrusion head system for safety.

The last modification made was to the speed of the extruder. The optimal speed was previously set to 0.816 cm/sec, which was optimal for collagen but was theorized to be contributing to the shearing issue with the threads at the edge of the stretch-to-remove surface-stretcher plate interface. Measurements made for manual extrusion speeds showed that something close to double that speed may help the threads polymerize correctly and therefore exhibit better mechanical properties. The team chose to experiment and set the speed to 2.0 cm/sec, which they determined was closest to that of manually extruded threads. The threads made at this speed polymerized into threads that were more easily stretched, and showed a better resilience to shearing. However, there are some problems with the torque required by the motors to move the extrusion head at this speed, and therefore the user must now guide the rail with their hand to ensure it does not stall, and make thick non-uniform threads.

5.7.4 Final Bath Design

Since it was determined that the initial bath was contaminated, based on the contamination section **[5.6.1 Bath Contamination Testing]**, it was necessary to create a second, uncontaminated bath to ensure that the threads were not harmed by contaminates during the process. The design team took advantage of the need for rebuilding the hydration system to redesign certain components of the bath. The bath was lengthened by 2 cm in order to accommodate moving the stretcher plates and extrusion pan away from the bath wall to allow for easier extrusion of threads and easier cleaning of the system as a whole. Additionally, the bath was widened by 1 cm so that the bath could be sealed from the inside without hindering the motion of the stretcher plates. Sealing the bath from the inside was advantageous because it guaranteed a cleaner system long term because HEPES could not seep into the cracks of the bath. The last modification that was made to the bath system was the lower the walls by 5 cm for easier access to threads and increased portability. Figure 58 shows the final dimensions of the bath system.

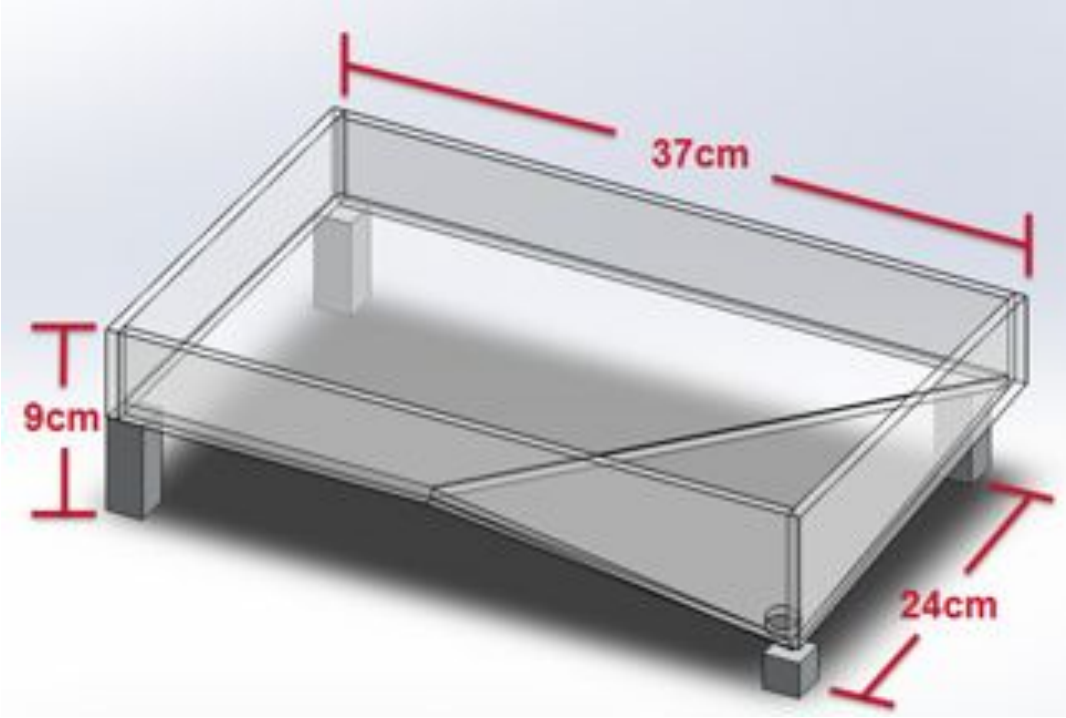


Figure 58: Dimensions for new bath system - illustrates the increase in the footprint of the bath and the decrease of the wall height.

5.7.5 New Frame Construction

After UV spec data indicated the old initial bath showed leeching, a new bath was required. The team decided to construct a new removable frame for the bath as well. The new removable frame was constructed that was used for manual stretching of fibrin microthreads. The new stretching frame was of similar dimensions to the previously constructed frame. Additionally, another end piece was laser cut from ½ inch acrylic, this piece secure the guide rods at the end farthest from the stretching motor. For incorporation into our system, this frame needed some modifications. The stainless steel guide rods, needed to be removed from the hand stretching frame and replaced with longer ones. The longer guide rods were press fit into ¼ inch holes in both the Lexan and acrylic ends. For the middle piece, two holes were drilled slightly larger than ¼ inches (0.257 inches) to allow movement of this piece. The lower stretching frame was the same frame used in the previous bath system. Additionally, the removable frame was roughed along the end closest to the extrusion system and the middle piece to allow the fibrin microthreads to attach to the removable frame.

5.8 Hardware System Development

Initially, the design team had conceived a LabVIEW interface to use in conjunction with the system. However, this plan was modified when the design team learned that VEX did not interface with LabVIEW and therefore would not work with the system. Instead, the design team chose to make a hardware system that could be attached to the front of the extruder head and still provide the modifiable parameters required for a successful system. The design, manufacturing, and programming for the hardware system are detailed in the following sections.

5.8.1 Hardware System Design

The hardware system was designed to be easy to use, allow the user to change the parameters of the system, and inform the user as it went through the different processes. The system was designed to have two dials to change the stretch percentage and stretch speed, six LEDs to alert the user as to what the system was doing, and both a continue and an emergency stop button. The system was designed to attach to the side of the extruder head and set before each system run.

The two dials were designed using VEX potentiometers, which register an angle between zero and two hundred and fifty degrees. By attaching them to shafts with handles on them, they would allow the user to be able to set different settings using a rotation knob. The six LEDs were designed using VEX LEDs, which come in yellow, green, and red. A green LED was used in steps where the machine was running, a yellow LED was used in situations where the system was waiting, and a red LED was used in situations where the system was stopped because the user needed to manually do something before the system could continue.

The steps of the system that were designed to have LEDs were: extruding, polymerizing, secure threads, stretching, remove threads, and done. The “secure threads” LED and the “remove threads” LED were designed to be red, because they were interrupts in the program. When the program reached that point in the system, the red LED would turn on, signaling to the user that they needed to do something before the program could continue. Once he/she had secured or removed the threads, the user could press the continue button, and the program would resume. The continue button and the emergency stop button were both designed using VEX bumper switches. The continue button would only continue the process when pressed during the “secure threads” or “remove threads” points in the process. The emergency stop, however, could be pressed at any time in the process and immediately stop the process.

The design team designed the front panel so that it could be cut and engraved with the laser cutter. The holes and labels for each of the different components were designed in SolidWorks. Figure 59 below shows the details of the front panel. The two circular holes, 6 center rectangular holes, and the outer rectangle would be cut out by the laser cutter, with the rest of the design being engraved on the surface.

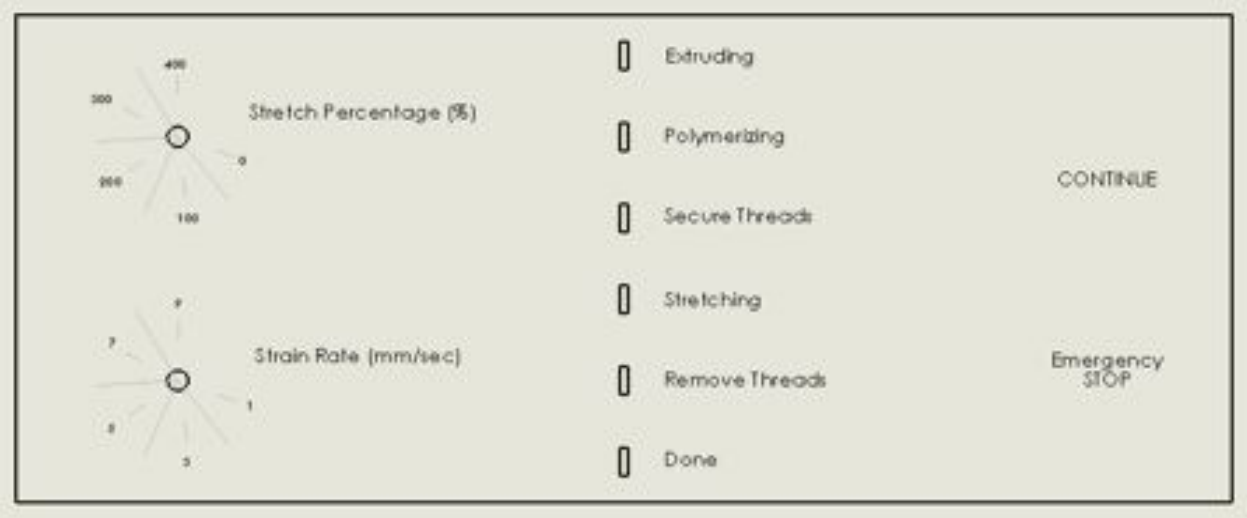


Figure 59: SolidWorks model of front panel user interface

Each of the dials has nine incremental markings that cover a rotational angle of two hundred and fifty degrees. For stretch percentage, the dial markings go from 0 to 400 in increments of 50 %. For the stretch speed, initially the dial was set to run from 0.1 to 0.9 mm/s, and is marked as such. During validation testing, however, the desired speeds were found to not be within the capabilities of the motor, so the team modified the mean of the settings to be simply numerical from 1 to 9, with 9 being the highest stretch speed for the machine. The values for these settings can be found in the machine validation section of this paper as well as **[Appendix G**: Motor Parameter**]**.

5.8.2 Hardware System Manufacturing

The hardware system was manufactured using a Versalaser VLS-4.60 laser out of black acrylic to match the extruder head. Additionally, small knobs for the dials were cut out of black acrylic using the laser cutter. The holes for the bumper buttons were cut separately using a 1 ½" drill bit. Once everything was cut, a hot glue gun was used to attach the components to the front panel. Triangular pieces attached to Velcro were also created and attached to back side of the front panel. These triangular pieces served as a way to attach the front panel to the extruder head

at an angle so that the user could easily see it. The wire connection and interface programming is detailed in the next section. Figure 60 depicts the finished front panel after manufacturing.

Figure 60: Front panel hardware interface

5.8.3 Hardware System Coding

The hardware system was set into the motor programming according to **[Appendix E**: Vex Code**]**. Each component of the hardware system as described above was programmed using EasyC Pro, and plugged into the PIC microcontroller used for the motor according to Figure 61. The program was written with conditional statements according to the user's entry on the potentiometer dials. For each of the nine conditions set, the code would perform the stretching to the specified stretch speed (mm/sec) and stretch percentage. These two dials are programmed potentiometers that register 1023 points along their 255 degree turn radius which can be individually programmed. These points were programmed to change parameters measured by both the digital encoder and the motor.

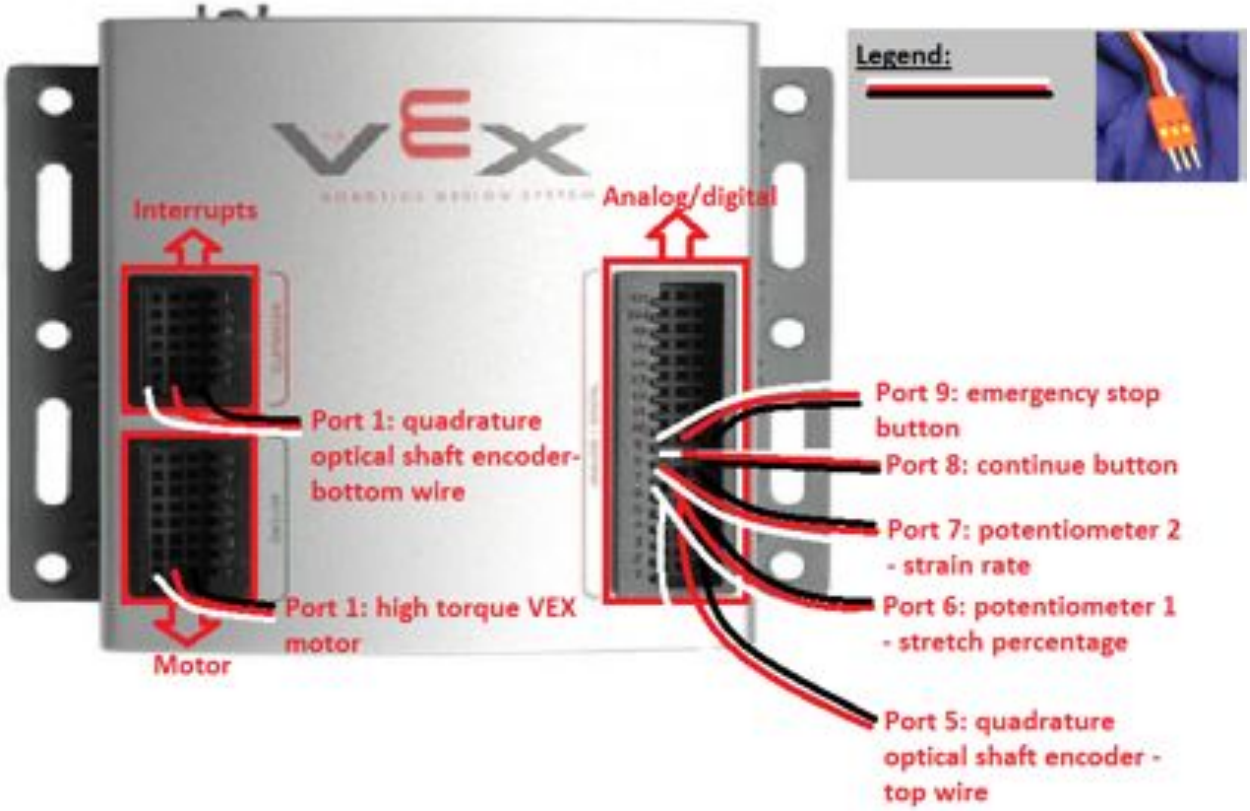


Figure 61: PIC microcontroller wiring of all components for hardware system.

The coding was designed such to allow for human error in the setting of the dials. If the dial was accidentally set in between two of the settings, like in Figure 62, the code would read the setting which the dial was closest to. The dial works increments of degrees, with 255 possible degree readouts. In order to avoid having the code read the wrong setting, the user would want to place the dial as close to the setting as possible, but as long as it was not set in the middle of two lines, the user could be sure that the system would identify the correct setting. The specific calculations that led to the final predicted values of stretch percentage and stretch speed are included in **[Appendix G**: Motor Parameter**]**.

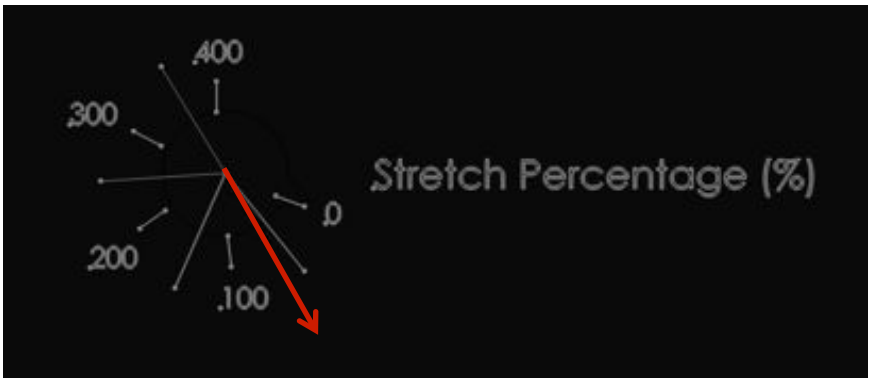


Figure 62: Diagram of dial setting for the hardware system - illustrates the problem with setting the dial in between two settings

The continue button was only programmed to register a press during certain phases of the production process to ensure an accidental bump of the switch would not allow the program to skip crucial steps such as polymerization. If the emergency stop button is pressed, it will automatically skip to the end of the program, no matter where in the program the user presses it.

Initially the team planned on running the complete program at once, but the battery could not to last the 25 minutes the system needed to run. The team decided to program a switch into the system to solve this problem. Now, after the extrusion process, the user will unplug the battery and turn the system off and replace the battery to the charger. When the threads have polymerized and are ready to stretch, the user will plug the battery back in, turn on the system, and hold the continue button for 5 seconds. This will send the program to the stretch phase of the process and allow the stretch to occur at parameters input by the user.

5.9 Incorporation of Extrusion Device

One of the crucial design objectives was to interface the team's new system with old components of another extrusion device. This would allow the team to design a system which automatically extruded and stretched the threads. In order to accomplish this goal, the design team combined the motor from the stretching system with the two motors from the extrusion system, and needed to incorporate them to work together to form a functioning machine. The extrusion device was the extrusion head from the 2010 MQP team project. This utilized two motors moving laterally and longitudinally. In order to interface the two systems, the team needed to modify the programming of the extrusion device and develop a cohesive interface.

The extrusion head utilized two motors to move the extrusion head in a precise pattern that drew the threads upon the extrusion surface. The motors were programmed in EasyC Pro,

and they created 15 threads, about 29 centimeters long, extruded at 0.225 mm/sec, and 1 cm apart (Ellis, 2010). The team reprogrammed the code to create 14 threads, 6 centimeters long, extruded at 0.225 mm/sec, and 1 centimeter apart. This was done in order to meet the specifications of the design team's stretching system.

Once programmed, the extrusion device was incorporated into the system by placing it at the end of the bath. Fortunately, the extrusion system was tall enough so that it did not need to be modified to fit above the stretching system. To incorporate the two systems together, the hardware interface described in the previous section was used to allow for the input of modifiable parameters. The coding for all of these sections can be found in **[Appendix E].**

5.10 Assessment of Final Design

The purpose of building a full prototype was to assess the functionality of each of the components and the system as a whole. After each component was built and tested, it was critical to assess how well the system met the initial objectives of the project. If the system met the objectives and was within the constraints set by the design team, the project would succeed. After all these criteria were met, the design team could move forward with the final design and validation testing.

The high-level objectives for this project were that the device must be automated, extrude threads, stretch threads, be user friendly, be reliable, and be durable. The team used the extrusion head from the previous MQP team to automate the drawing of the threads and motor to control the stretching of the threads. One of the considerations that the team had to make was making the device fully automated or having some human interaction required. Originally, the team, client, and user envisioned a device that with the push of a button would extrude, stretch, and hang the fibrin microthreads to dry. At first this is what the design team tried to produce. One component of this device that important to this device, not listed in the objectives was a removable frame. The client and user stated that once the threads were stretched, they would like to be able to remove the frame that was securing the threads, which was accomplished with the two-part frame. As for user-friendly, the fibrin microthread processing system integrates with the hardware interface and has user imputed parameters of stretch percentage, stretch speed and extrusion head rate for user specific, customizable batches of microthreads. The device was manufactured with components that will resist rust and corrosion, so the system can be used for extended periods of time.

After establishing that the system met the high-level objectives of the project, it was important to consider if the functional requirements had been met using preliminary validation testing. The testing described previously established that the system was capable of performing all functions with minimal failure to the threads. Preliminary validation testing allowed the design team to separate each of the functions and evaluate them individually. Once each of the functions was validated individually, it was possible to establish whether or not the system as a whole would be successful. From preliminary validation testing, the design team felt that the system met all functional requirements needed to move forward.

Finally, it was important to consider the constraints of the project before moving forward to the final design. One of the main constraints of the project was the budget, which was determined to be $524. After the prototype was built, the total budget used was $278.41 [**Appendix D].** This total included all of the parts for each component of the system, including the motor. This total was well under the design team's total budget, and thus the system met the budget constraint, leaving room for cost associated with validation testing. Figure 63 shows a breakdown of the relative cost spent on each component of the prototype. As expected from preliminary validation testing and design, the stretch-to-remove system was the most inexpensive part of the stretching system. The motorized stretching component was the most costly, but the team designed the system to only run on one motor. This motor was acquired at no cost, which greatly reduced the overall cost of the system. Another important constraint the design team needed to consider was the time for completion of the project. The project needed to be completed within the 2012-2013 academic year. The design team was able to complete an initial prototype by the end of December 2012 and felt that there was an adequate amount of time remaining to finalize the prototype, validate the final system and compare the threads created to the manual production process. After the design team ensured that the constraints of the project were being met, the final design could be built and validated.

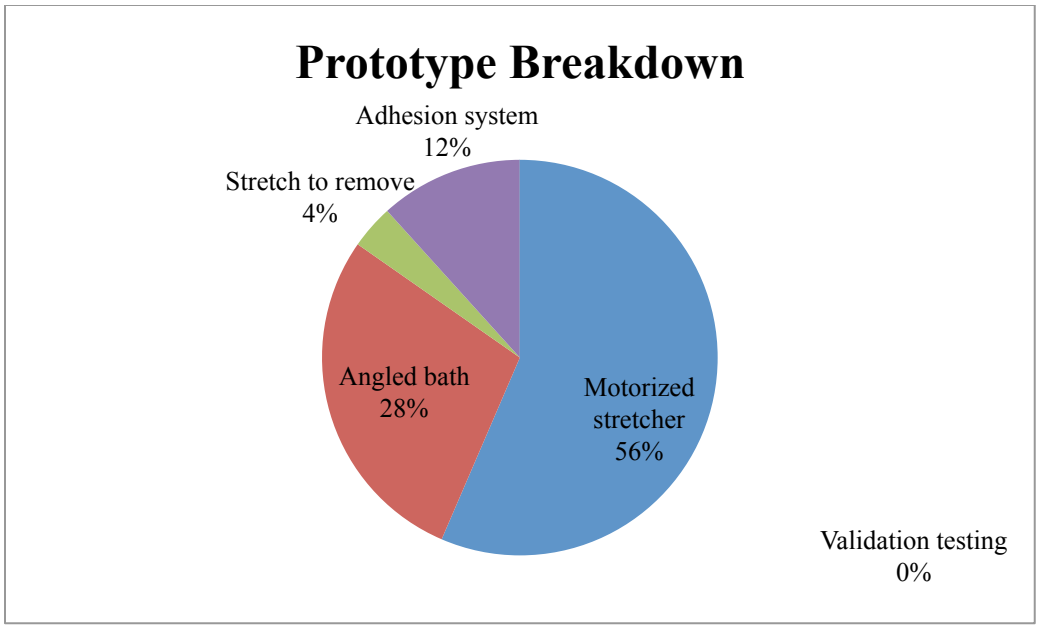


Figure 63: Breakdown of cost based on functional components.

It was important for the design team to validate the final prototype based on the objectives, functions and constraints in order to establish that all of the goals for the project had been met before moving to final design and validation of the system. Although parts of the system needed to be modified, the design team was able to come up with a prototype that met each of the necessary criteria. The system allowed for automated extrusion and partially automated stretching and removal of the threads, while remaining user-friendly, reliable, durable and precise. Additionally the design team was able to develop a prototype within the constraints of the project. Overall, the design team felt that the final design was verified and that the team could move forward to machine and thread validation.

6 Final Design and Validation

Once the design team had reached a decision on the final design for the system through initial concept validation testing, it was important to conduct machine validation and thread validation on the final design as a whole. Machine validation would serve to affirm that all components of the machine were performing to certain standards, which the design team defined as performing within ± 10 % of each set parameter and a maximum thread failure rate that is less than that of the manual method, which is about 25%. Thread validation would serve to confirm that the automated processing system produced threads which were superior to manually produced threads in structural and mechanical properties. The controls that were used to test the threads created by the system were manually hand drawn and stretched threads. After successful validation of the design, the team would confidently be able to determine how automating the system had improved microthread production.

6.1 Machine Validation

It was important to conduct machine validation testing on the final design in order to ensure that all components were performing to their specifications. Machine validation included leak testing of the bath, as well as testing of the stretch speed, stretch percentage and extrusion head rate, and finally computing the failure rates of the system and calculating the expected thread yield of the system. This data not only validated that the system was accurate to within 5%, but also would be used to alert the user as to what he could expect when operating the system.

6.1.1 Leak Testing Validation

Leak testing of the bath is essential to ensure that no HEPES is lost during fibrin microthread production. Leak tests were performed with water. The bath was filled with water for 30 minutes, 1 hour, or 24 hours. During leak tests, no stretching or extrusion was performed, but leaking was noted if it occurred. Table 43 summarizes the data collected from the leak tests. The bath filled with water was left a certain amount of time (30 mins, 1, or 24 hours), after the time had passed, any water that had leaked was collected and the volume was measured.

Table 43: Old (First Constructed) Bath Leak Testing Results

Test	Time	Leakage (mL)	Success/Failure
1	30 min	0	Success
2	1 hr	0	Success
3	24 hrs	5	Fail

The team constructed a second bath, the final bath, which was also leak tested (Table 44). As mentioned before this bath was sealed in a different manner, which consisted of applying the silicone sealant on the interior of the bath. The leak test data showed no leaks in any of the 30 minute, 1 hour or 24 hour leak tests.

Table 44: New (Second Constructed) Bath Leak Testing Results

Test	Time	Leakage (mL)	Success/Failure
1	30 min	0	Success
2	1 hr	0	Success
3	24 hrs	0	Success

6.1.2 Parameter Verification Testing

The design team conducted parameter validation testing on the stretch speed, stretch percentage and extrusion head rate parameters, expecting them to pass the standard of being within 5% of the desired value for each run. Although extrusion head rate was not a parameter that could be modified by the front panel, each of the jumpers was tested for accurate extruder head rates. During verification testing, the design team found that some parameters were inaccurate, and thus they were modified and retested.

The first parameter that was tested was stretch percentage. To run the test, the design team attached a ruler to the side of the bath and ran the system at each stretch percentage. To speed up the testing process, the extrusion and polymerizing sequences of the code were commented out to allow for the stretching motor to run immediately without modifying the code completely. Figure 64 shows the set up for the stretch percentage testing.

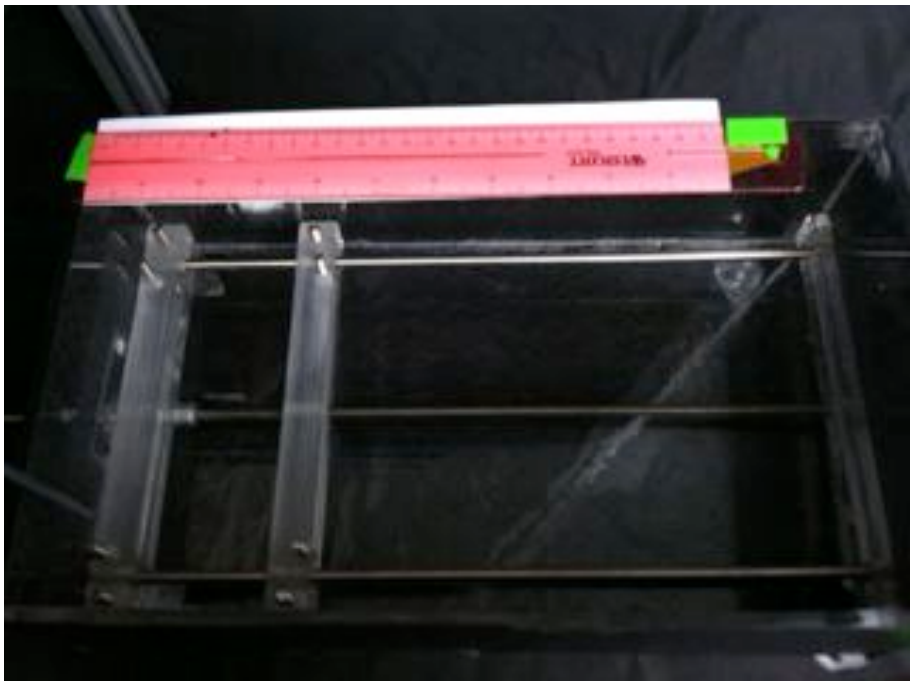


Figure 64: Stretch percentage parameter testing - this figure depicts the set up for the parameter testing, which included a ruler on the far side of the bath in order to measure the movement of the middle stretcher plate during each run.

During testing, the stretch percentage parameter was set at zero to start then modified incrementally by 50% at each of the subsequent tests. Each stretch percentage was tested five times and averaged to exhibit the reproducibility of the system. In order to calculate the accuracy of each setting, the initial and final locations of the center stretcher plate was recorded and then the resulting stretch percentage and percentage deviation was calculate using the following equation:

$$Calculated\ stretch\ percentage\ = (Final\ location - Initial\ location) \div (Pan\ length)$$

$$Deviation = Calculated\ stretch\ percentage - Set\ stretch\ percentage$$

In total, the design team ran stretch percentage parameter tests at nine different settings. The average results of the testing are seen in Table 45, full data set can be seen in **[Appendix G**: Motor Parameter**]**. As confirmed by the data in Table 45, each of the stretch percentage settings performed to the standard of ±10%.

Table 45: Stetch percentage parameter testing data - this table displays the average results from the parameter testing along with all calculated values and a pass/fail rating for each setting.

Set Value (%)	Encoder Setting	Average Initial Location (mm)	Average Final Location (mm)	Average Actual Value (%)	Average Deviation	Pass (within 10% of set)
0	0	77.0	77.0	0	0	yes
50	2500	77.0	113.6	47.5	2.5	yes
100	5500	77.0	153.6	99.5	0.5	yes
150	8000	77.0	193.6	151.4	-1.4	yes
200	11000	77.0	235.0	205.2	-5.2	yes
250	14000	77.0	271.8	253.0	-3.0	yes
300	17000	77.0	301.8	292.0	8.1	yes

The next parameter that was tested was the stretch speed setting. In order to test this parameter, the ruler setup was left on the bath to continue to measure the initial and final location of the stretcher, but additionally a stopwatch was used to calculate the total stretch time for each of the settings. In order to consistent, the stretch percentage for the system was set at 100% for each run. The resulting stretch speed and percent deviation for each run was calculated using the following equations:

$$Calculated\ strain\ rate = (Final\ location - Initial\ location) \div Total\ run\ time$$

$$Deviation\ percentage = (Calculated\ strain\ rate - Set\ value) \div Set\ value$$

In total, the design team ran a total of nine tests at set stretch speeds from 0.1 to 0.9 mm/s. During these tests, the design team ran into several problems. First, the motor did not have sufficient torque to run the system at the settings of 0.1, 0.2 and 0.3 mm/s. Additionally, the settings that did work, did not perform to the expected values. Each stretch speed had a deviation percentage of at least 80%. The team hypothesized that they had done the torque calculation for the motor incorrectly and needed to re-evaluate each of the settings. Additionally, the design team decided that the motor would have to be geared down in order to create sufficient torque to run at the speeds desired for the system, one of which being 0.2 mm/s, which was reflective of the stretch speed generated from manual stretching. The data from the initial stretch speed parameter test can be found in Table 46, and led to design team to redo the test after modifications to the system were made.

Table 46: Stretch speed parameter testing data - Below is the data that resulted from the stretch speed testing and led to the re-evaluation of the system in order to pass each setting. An X represents a stretch speed that did not have enough torque to turn the threaded rod.

Trial #	Calculated stretch speed (mm/sec)	Initial location (mm)	Final location (mm)	Total run time (sec)	Actual stretch speed (mm/sec)	Deviation from calculated	Pass (Y/N)
1	0.1	X	X	X	X	X	N
2	0.2	X	X	X	X	X	N
3	0.3	X	X	X	X	X	N
4	0.4	77.0	137.0	56.7	1.1	-165%	N
5	0.5	77.0	137.0	44.2	1.4	-171.0%	N
6	0.6	77.0	137.0	39.4	1.5	-154.0%	N
7	0.7	77.0	137.0	36.8	1.6	-133.0%	N
8	0.8	77.0	137.0	37.7	1.6	-98.9%	N
9	0.9	77.0	137.0	35.8	1.7	-86.2%	N

After reevaluation, the team determined that friction was going to be a factor, and the friction affected the amount of torque needed by the motor, therefore the power settings that were expected to output consistent stretch speeds in a linear fashion, were actually going to output stretch speeds logarithmically. The team then tested every three motor power settings from 82 to 109 to generate a compilation of accurately tested stretch speeds. These settings were chosen because they exhibited enough torque to turn the threaded rod, and they are capable of producing threads without breaking them. The team ran two trials of each stretch speed, and instead of calculating the expected outcome simply determined the stretch speed experimentally for each power setting at full battery power. A sample of the average data is provided below in Table 47, full data can be found in **[Appendix G**: Motor Parameter**].**

Table 47: Stretch speed parameter validation - Below is the data that resulted from the stretch speed testing based purely on motor power. All of our tests for mechanical threads utilized power setting 100, as seen below.

Motor power setting (0-127)	Average initial location (mm)	Average final location (mm)	Average run time (sec)	Average stretch speed (mm/sec)	% Error	Pass (± 10%)
109	84.0	160.0	366.0	2.1	-0.01	Y
107	84.0	160.0	159.0	4.8	0	Y
103	84.0	160.0	110.0	6.9	0.13	Y
100	85.0	161.0	90.5	8.4	0.09	Y
97	84.0	160.0	73.5	10.3	0.14	Y
94	84.0	160.0	64.5	11.8	0.18	Y
91	84.0	160.0	57.5	13.2	-0.23	Y
88	85.0	161.0	54.5	13.9	0.26	Y
85	85.0	161.0	49.5	15.4	0.31	Y

This final test validated the reproducibility of the stretch speeds based on the power setting of the motor. While there are only nine options for power settings on the hardware interface, a user may go into the program and change values based on the chart provided in **[Appendix G**: Motor Parameter**]** to obtain a more accurate power setting for their need.

6.2 Thread Validation

Thread validation was conducted to ensure that the system created consistent threads, at an acceptable failure rate. After machine testing ensured that the machine performed to desired parameters, the team began testing the system as a whole with the automated extrusion system and the stretching system.

6.2.1 Initial thread production

Initially, the team began tests with the automated biopolymer extrusion head, the angled bath, the stretch-to-remove surface, the squeegee clamp system, and the stretching system. Although the device had performed well during machine validation, creating threads with the whole system proved difficult. The results of the initial thread tests showed thread shearing and ribboning, which were defined qualitatively as the presence of two strands within single threads. Consistent threads, which are defined as cylindrical threads with constant properties, were not visible during initial tests, and resulted in increased thread failure. From this information the team began testing potential variables within the system, which could have contributed to the presence of ribboned threads. Initial tests focused on parts within the system including the

stretch-to-remove surface and stretcher plate interface, the length of the polyethylene tubing, the extrusion pump rate, the angle of extrusion of the polyethylene tubing, and the bath itself for contamination.

6.2.2 Variable Testing

The automated extrusion variables that were tested were the length of polyethylene tubing, the automated extrusion head rate, the angle of the polyethylene tubing, and the extrusion pump rate. These variables were defined by the design team as the machine variables for the system. the full results can be found in **[Appendix I**: Thread Validation Results**].**

The polyethylene tube length was used to transport and initially polymerize the fibrin materials prior to their incorporation in the HEPES bath. This variable was changed from 54 cm (the approximate length used in the current manual production) to 74 cm to increase the initial polymerization time of the fibrin microthreads prior to extrusion on the stretch-to-remove surface. The team hypothesized that the additional polymerization time would result would decrease the incidence of ribboning. The increase in tube length exhibited a decrease in thread ribboning, but an increase in thread waste production, due to the larger volume of fibrinogen and thrombin present in the tube at the end of the extrusion. Table 48 shows the results in terms of percent yield.

Table 48: Results from polyethylene tubing variable testing

Length (cm)	Results (Yield %)
54	17
74	54

The next variable that was modified was the extrusion pump rate, which increased the initial polymerization time of the fibrin microthreads. The original extrusion pump rate was 0.225 ml/min as defined by the manual process. Initial qualitative results showed an increase in thread polymerization, but also an increase in thread shearing during stretching and removal. The extrusion pump speed was modified to 0.11 ml/min (half of the original extrusion speed), but the team noticed increased residual clotting within the tubing, which resulted in further clotting and eventual extrusion failure. In two tests using 0.11 ml/min extrusion pump speed, 12 threads were produced, but all threads failed due to shearing at the stretch-to-remove surface interface during stretching. The team concluded that the decrease in extrusion pump speed did not produce viable

threads for stretching and discontinued further tests with variations in the pump speed relative to the manual pump speed of 0.225 ml/min. Table 49 shows the results in terms of percent yield.

Table 49: Results from extrusion pump variable testing

Rate (mL/min)	Results (Yield %)
0.11	6
0.225	31

The biopolymer extrusion system was used to automate the extrusion of the fibrin microthreads. Initially used for collagen microthreads, the extrusion head needed to be modified to adjust for fibrin microthreads. The extrusion head rate was revisited as a variable in order to decrease thread thickness and ribboning. The extrusion speed of collagen microthreads (0.3mm/s) is much less than the manual extrusion rate for fibrin microthreads. The team increased the extrusion head rate to various speeds to find a speed that was comparable to the manual extrusion speed and evaluated the results on a thread failure basis. The extrusion speed was tested at varying power settings, which translated to varying extrusion head speeds. Of the power settings tested, 1.75, which resulted in an extrusion speed of 2.0 cm/s, was proven to be the most effective because it produced the least ribboned threads. The increase in the speed of the biopolymer extrusion head rate resulted in thinner threads with less ribboning, still did not produce consistent threads at an acceptable yield percentage. The extrusion speed was kept constant for other tests to ensure proper isolation of variables.

After it was concluded that neither the tube length, extrusion pump rate, nor the extrusion head rate caused the ribboning of the threads, the team tested the angle of the polyethylene tubing during extrusion. Originally, the polyethylene tubing dropped the thread materials onto the surface of the pan, but after further examination of the manual process, the team decided to drag the tubing in an attempt to lay the thread materials (Figure 65). Prior to dragging, threads produced via dropping produced 8 threads, of which all showed ribboning and failed to remove from the stretch-to-remove surface. After dragging was incorporated, during two tests 8 threads were produced, of which 5 showed ribboning, 2 ripped during stretching, and 1 did not attach to the end plate for stretching. Table 50 shows the results in terms of percent yield.

Table 50: Results from angle of polyethylene tubing during extrusion testing

Method	Results (Yield %)
Dragging	0
Dropping	37

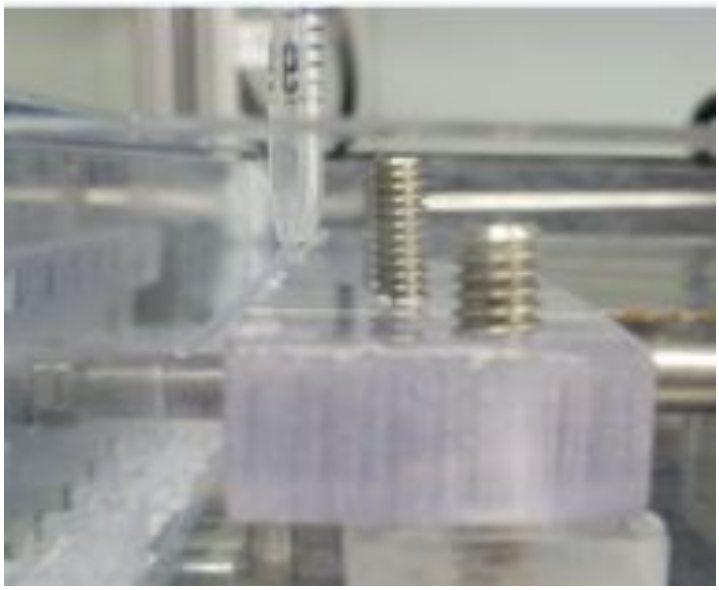


Figure 65: The interface between the polyethylene tubing and the stretcher plate - illustrates the dragging method for automated extrusion as opposed to the dropping method, where no tubing sticks out of the pipet tip

After each machine variable was isolated and eliminated as the independent cause of the ribboning, the team decided to test other process variables that could have negative effects on the thread properties. In comparison to the tests conducted with the biopolymer extrusion system, manually extruded threads, which were transferred onto the automated stretching system, exhibited much lower failure rates. Because of time constraints, the design team decided to validate a partially automated process and move forward with mechanical validation.

6.2.3 Manual Extrusion with Machine Stretching

The team decided to end the pursuit of an automated extrusion system for fibrin microthreads on the basis that increased variables within the system contributed to further complications and could not be solved within the time-frame. Additionally, threads were removed manually from the stretch-to-remove surface as explained in [**5.7.2 Stretch-to-remove]**. The stretching system was the most essential portion of the project and needed to make

consistent threads to produce mechanical data and ensure complete validation of the stretching system.

Since the design team had already determined that the stretch-to-remove technique would not work, two alternative removal methods were tested. First, the team attempted to slightly remove the frame at an angle, in an attempt to reduce the shear stress on the threads from the water tension, in order to pull the threads off the stretch-to-remove surface. Next, the team attempted to manually remove the threads individually from the stretch-to-remove surface using forceps and replace them on the frame for stretching. Finally, the team attempted to extrude threads in an external pan and manually transfer the polymerized threads to the stretching frame. The manual transfer method combined with manual extrusion of the threads resulted in a yield of 72%, which was the closest to the design team's acceptable yield of 75%. The results of these tests are found in Table 51.

Table 51: Results from the removal method validation testing

Method	Results (Yield %)
Frame removal	6
Manual removal	25
Manual transfer	72

Since the failure rate from the manual transfer process was closest to the specified failure rate, thread production for mechanical testing and validation was done using this system. Mechanical tests were performed on threads which were manually extruded, manually transferred, and automatically stretched to varying percentages.

6.2.4 Mechanical Testing

Mechanical testing was conducted on the fibrin microthreads to validate the consistency of the structural and mechanical properties. Dry and wet diameters were collected and mechanical testing was performed to validate that the partially automated system produced more consistent threads, when compared to threads produced in manual production. Once this was validated, the team could conclude the effectiveness of the automated stretching system in reducing thread variability.

To prepare the threads for mechanical testing, each thread was cut to approximately 3.5 cm and mounted on vellum frames. Each frame contained an elliptical hole that measured 2.0 cm

across at its longest point. The threads were mounted to ensure that the testing area was exactly 2.0 cm during tension tests. Dry diameter measurements were taken using an upright confocal microscope with a 10x magnification and recorded. The threads were hydrated in phosphate buffered saline solution for 45 minutes and measured again for wet diameter measurements. Following wet measurements, each thread was mounted on custom grips within an Instron machine. Each thread was pulled to failure using a 1 N load cell, and a recording frequency of 10 Hz. All data was collected and analyzed using MATLAB **[Appendix H**: Matlab Code for Mechanical Testing**]**, and transferred to a working document with stretch percentage averages. A full protocol can be found in **[Appendix F**: Protocols**]**. In terms of thread inclusions for mechanical data, for each thread segment, the averages for all properties were calculated along with an IQR based on the average wet diameters. From the IQR, outliers were removed and the data was finalized.

The test groups for the machine-stretched threads were 50%, 100%, 200%, and a secondary 100% stretch group to test the batch-to-batch variability within the automated system. Prior to the mechanical testing of machine-stretched threads, 100%-manually-stretched threads were produced and tested mechanically using the same protocol to serve as a control group. The 100% machine stretch groups were compared to those produced using manual production. The mechanical data for all test groups is presented in Figure 66 and Figure 67. The minimum number of thread segments per test group was 11, and the number of thread segments per test can be found in Table 52.

Table 52: Test groups for mechanical testing - shows the number of fibrin microthread segments per test group.

Test groups	# of thread segments
Unstretched control	39
100% manual	30
100% machine	28
50% machine	12
200% machine	16

6.2.4.1 Structural Analysis

The machine stretched thread groups had similar variability in the average standard deviation when compared to the manually stretched threads. The dry and wet diameters for 100% manual stretched threads were 69.6 ± 13.7 μm and 161.3 ± 39.1 μm respectively (Figure 66A &

B). The deviations for dry and wet diameters were 19.7 % and 24.2 % when compared to the mean values. The dry and wet diameters of manually extruded, 100% machine stretched threads were 55.3 ± 10.2 μm and 94.3 ± 23.9 μm respectively, which correlated to 18.4 % and 25.4 % deviations from the means. The variations between machine stretched threads and manual stretched threads in terms of diameters could have been greatly affected due to the manual extrusion of both processes. In terms of swelling ratio, manual stretched threads (1.4 ± 0.55) had a greater deviation (39.3 %) from the mean when compared to machine stretched threads (1.7 ± 0.32) which had a deviation of 18.8 % (Figure 66C).

While the team's goal was to create a fully automated extrusion and stretching system for fibrin microthreads, the ultimate decision to use manually extruded threads resulted in relatively the same variability in thread diameters. Additional groups tested using the machine stretching were 50% and 200% stretch. For 50% machine stretched threads, the dry and wet diameter averages were 49.2 ± 13.5 μm and 93.0 ± 36.6 μm, which indicated 27.4% and 39.4% deviations from the respective means. For 200% machine stretched threads, dry and wet diameter measurements averaged 28.4 ± 6.45 μm and 42.1 ± 17.2 μm, or 22.7% and 40.8% deviations from the means.

The team produced threads with smaller diameters when comparing the dry and wet diameters of the 100% machine stretched threads to the 100% manual stretched threads. The thread diameters may be highly dependent on the extrusion method of the threads. When the team compared the variations in the dry and wet diameters of the 100% machine stretched threads and the 100% manual stretched threads, it was concluded that the variations in the diameters may have been caused by the manual extrusion used in both cases. The differences in the mean dry and wet diameters of the machine and manual stretched threads could have also been caused by residual stretching that occurs when the threads are removed from the pan and placed on the stretching frame.

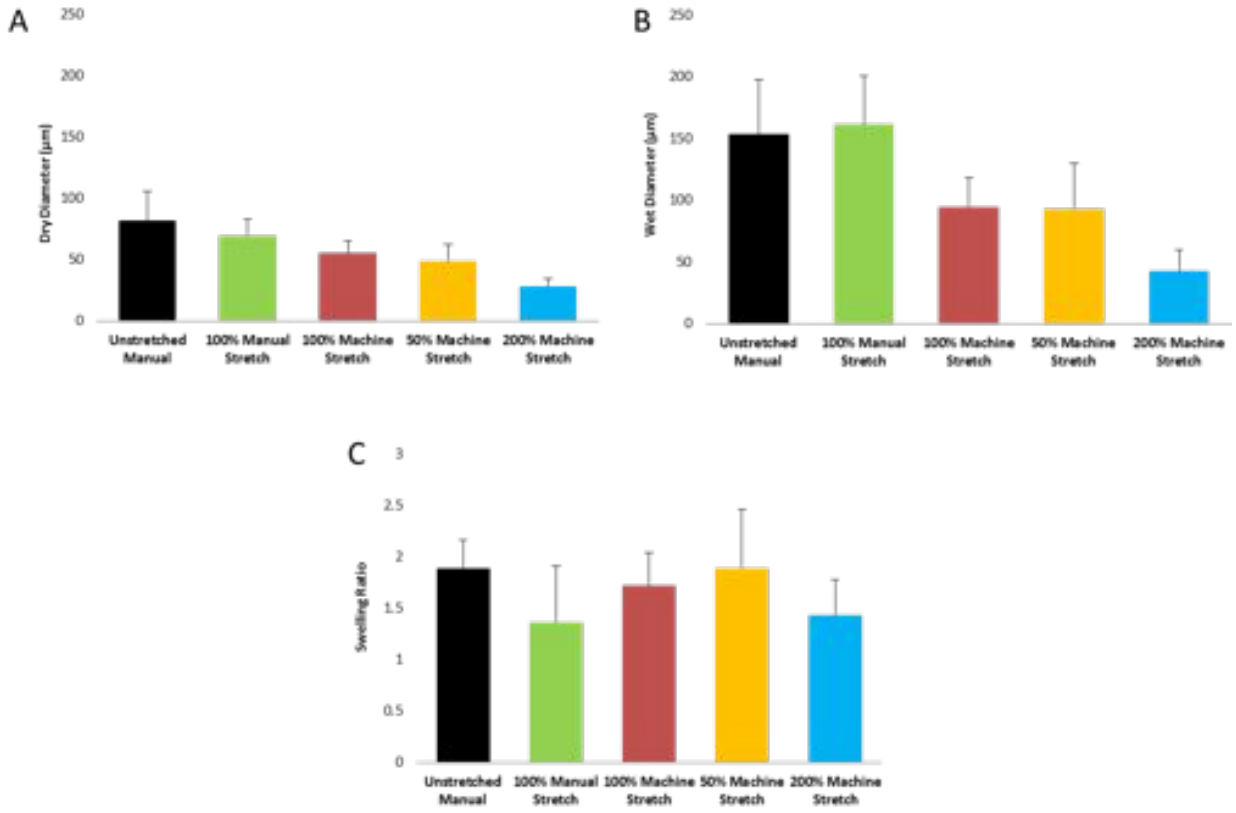


Figure 66: Thread Structural Data - Illustrates the differences in dry and wet diameters and swelling ratio

6.2.4.2 Mechanical Analysis

There were differences between manually stretched threads and machine stretched threads. The average ultimate tensile strength (UTS) and strain at failure (SAF) for 100% manually stretched threads were 1.43 ± 0.76 MPa and 0.59 ± 0.16 mm/mm, which translated to 53.1% and 27.1% deviations from the means. The average UTS and SAF for 100% machine stretched threads were 3.6 ± 1.26 MPa and 0.31 ± 0.13 mm/mm, which correlated to deviation percentages of 35.0% and 41.9%. The differences in standard deviations when compared to the means of both UTS and SAF for 100% manual and 100% machine stretched groups indicate a decrease in the UTS variability, but an increase in the SAF variability (Figure 67A & B). When compared against the unstretched control for UTS and SAF (1.69 ± 0.86 MPa and 0.59 ± 0.27 mm/mm), which had deviations of 50.9% and 45.8%, the 100% manual and 100% machine stretched threads exhibited less variability in terms of deviation percentages. While the 100% machine stretched threads had a higher deviation when compared to the SAF deviation of 100%

manual stretched threads, the machine stretched threads had decreases in UTS deviations, and therefore less variability.

In terms of other stretch percentages, the team tested and analyzed the UTS and SAF of 50% and 200% machine stretched threads. The team observed an increase in the UTS variabilities of 50% and 200% stretched threads (3.12 ± 1.69 MPa and 6.55 ± 4.68 MPa), which translated to deviations of 54.2% and 71.5%. The increases in deviation percentages in the 50% and 200% stretch groups could indicate the variability in manual extrusion, but could also indicate residual stretching that occurs when the threads are removed from the pan. The team did notice initial increase in thread lengths as the threads were removed from the pan, but attempted to minimize the residual stretching prior to controlled machine stretching.

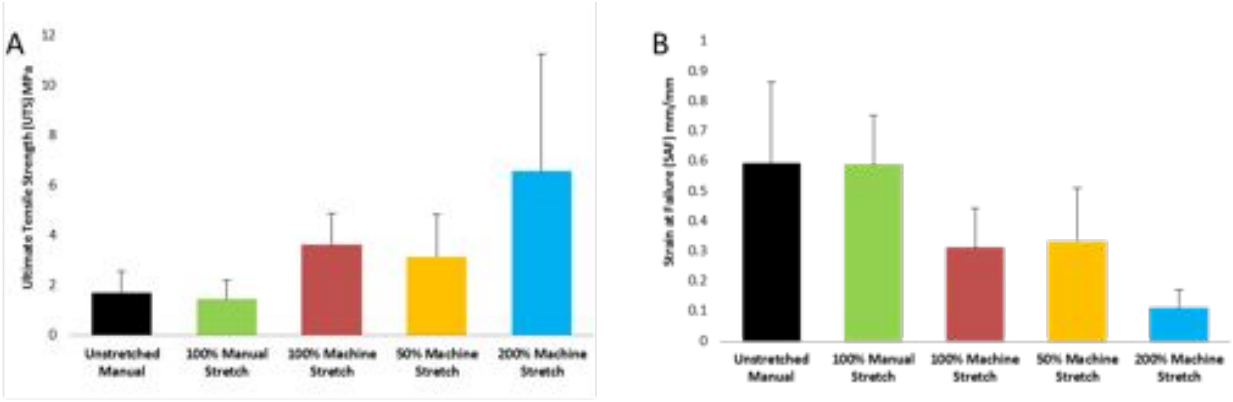


Figure 67: Mechanical properties of threads (UTS and SAF) – Illustrates the trends in the UTS and SAF between stretch percentage groups

Other mechanical parameters measured included load and maximum tangent modulus (MTM), which can be directly related to the stiffness of the threads during thread failure. Figure 68A and Figure 68B highlight the changes in average load and stiffness experienced in each stretch group. Figure 68C removes the highly variably 200% machine stretched threads to more clearly define the deviations in the other stretch percentage groups. The team witnessed changes between stretch percentage groups when calculating the average load registered during thread failure.

The highest average load that was registered was in the unstretched control group (31.1 ± 22.8 mN, a deviation of 73.3%). For the 100% manual and 100% machine stretched threads, the registered loads decreased along with decreases in deviations (24.6 ± 7.8 mN and 23.7 ± 9.9 mN, or 31.7% and 41.8%). The 50% and 200% machine stretched groups also contributed decreases in average load deviations (18.7 ± 9.2 mN and 5.6 ± 1.5 mN, or 49.2% and 26.8%). When compared to the unstretched group with a deviation of 73.3%, the threads that were stretched to

varying stretch percentages using the machine, the deviations decreased; indicating an increase in precision of microthread properties.

For the average stiffness of the 100% manual and 100% machine stretched threads, the team observed a decrease in stiffness deviations (83.9% manual deviation vs 52.2% machine deviation), as illustrated in Figure 68C. The team also observed deviations in the 50% and 200% stretch groups of 64.7% and 123.6%.

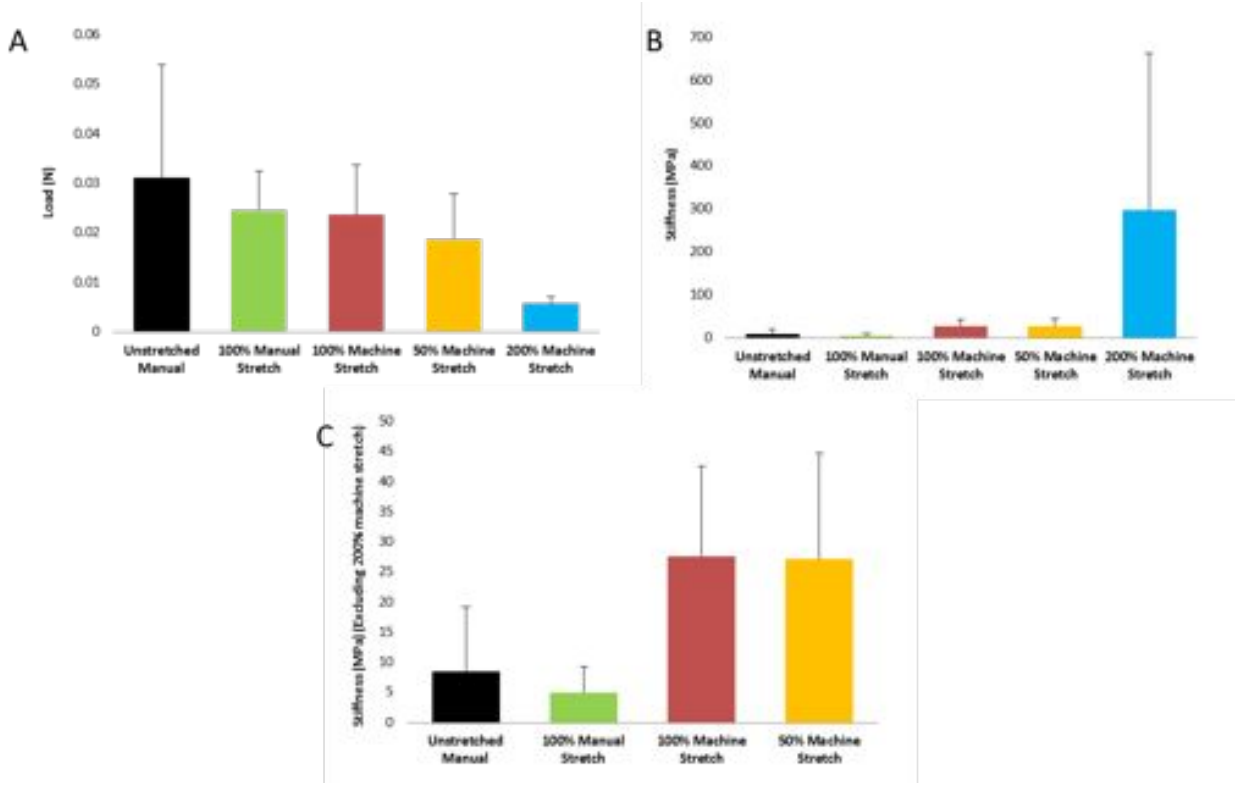


Figure 68: Mechanical properties of threads (Load and Stiffness) – Shows the differences in average load and stiffness of microthreads between stretch percentages

The team needed to ensure batch to batch variability to within 10% of the thread properties to indicate a precise stretching device. To measure the batch to batch variability, the 100% stretched threads were previously marked according to batch production number. Threads were separated into batches 1 and 2, and compared between batches and with 100 % manual stretched threads. As illustrated in Figure 69A, the average UTS values for 100 % manual, 100 % machine batch 1, and 100 % machine batch 2 threads were 1.44 ± 0.76 MPa, 3.35 ± 1.36 MPa, and 3.73 ± 1.32 MPa respectively. The difference between the average UTS values of the 100 % machine batches 1 and 2 was 0.38 MPa. The difference between the deviations of batch 1 and batch 2 for UTS (40.6% and 35.4%) was 5.2 % between batches (Figure 69A), which fell within

the desired ± 10 % range of acceptable variability. The average 100 % manual UTS was 1.44 MPa, which was 2.29 MPa less than the average UTS for batch 2; indicating that the automated stretching system was not accurate with regards to the 100 % manual stretched thread mean UTS value. However, the variation of only 5.2% between batches was indicative of a precise system by our team's standards, and the differences between the manual and machine stretched threads could be caused by manual extrusion or residual stretching. Threads stretched in the machine could have different properties when compared to manual stretched threads because the varied stretching was controlled and uniform.

The other parameters examined for batch to batch variability included SAF and thread stiffness (Figure 69B & Figure 69C). The average SAF values of 100 % machine stretched batch 1 and batch 2 threads were 0.39 ± 0.14 mm/mm and 0.26 ± 0.10 mm/mm. The difference in the deviations for both batch 1 and batch 2 (35.9% and 38.5%) fell within the 10 % range of acceptable batch to batch variability with a difference of 2.6 %. The deviation of the 100 % manual stretched threads was 27.1 %, which further indicated the limited accuracy of the automated system, but emphasized the precision. Figure 70 illustrates the variations in the stress-strain curves of the 100 % manual and 100 % machine stretched threads. The thread curves are typical, but still exhibit noise due to the increase in sampling frequency and sensitivity of the tests.

The last parameter the team used for batch to batch variability calculations was MTM, also known as the thread stiffness (in MPa). The average stiffness values for 100 % manual and 100 % machine batches 1 and 2 stretched threads were 5.02 ± 4.21 MPa, 19.14 ± 10.18 MPa, and 33.28 ± 16.16 MPa, respectively (Figure 69C). The deviations translated to 83.9%, 53.2%, and 48.6%; with a difference of 4.6% in the deviations of the threads stretched in batches 1 and 2. While the variability in terms of deviations from the mean stiffness values is close to 50 % for the machine stretched threads, the batch to batch variability is kept under 10 % for 16 thread segments.

According to the data collected, the team concluded that the machine had performed to desired specifications with regards to the precision and full automation of the stretching system. Threads were created manually, but stretched automatically to varying stretch percentages. The deviation percentages of the thread properties generally decreased when compared to threads produced using the manual production method.

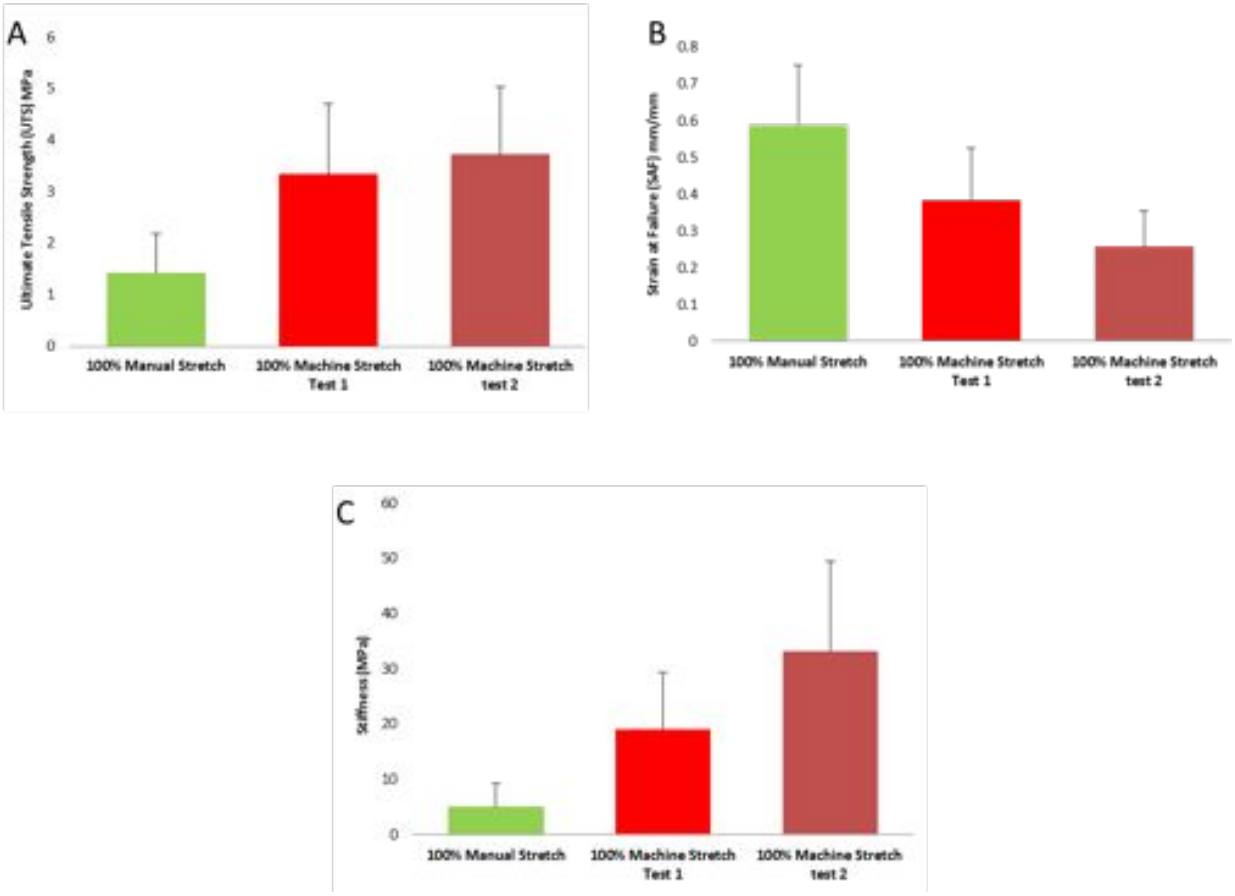


Figure 69: Mechanical data of batch to batch variability - Illustrates the differences between batches of 100% machine stretched threads

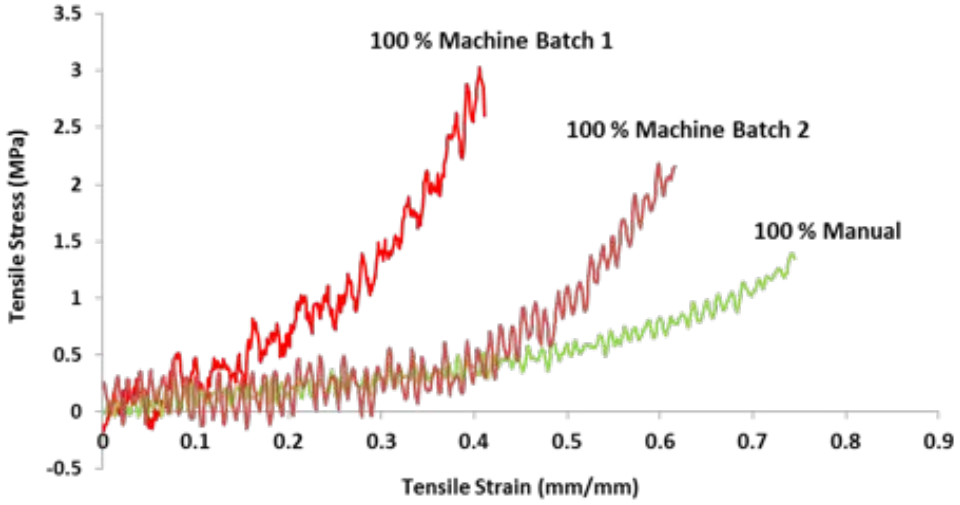


Figure 70: Machine vs manual stress / strain curves – this figure illustrates the differences in the characteristic curves of the manual and machine stretched threads

7 Discussion

After completion of a prototype for automated microthread extrusion and stretching, the team conducted validation testing to ensure the accuracy of the system. Complications with variable factors involving the automated biopolymer extrusion system led to manually extruded threads and further testing of the automated stretching system. The change from automated threads, which proved problematic, to manually extruded threads that were transferred to the automated stretching system resulted in viable threads. These threads were tested in uniaxial tension tests to verify the effectiveness of the device. The following sections discuss the effectiveness of the stretching device, as well as the potential impacts the device could have on society.

7.1 Automated Microthread Properties

The team conducted uniaxial tension tests on 5 test groups and 1 control group to determine the effects of the automated stretching on the structural and mechanical properties. For each stretch group, the average values for structural and mechanical parameters were recorded and analyzed for deviations. According to the data presented, the 100% machine-stretched groups showed a decrease in thread variability in terms of structural properties, such as dry and wet diameters, and mechanical properties, such as ultimate tensile strength, strain at failure, and stiffness.

The reduced, but still present variability of the threads produced within the automated system can be contributed to the fact that they were still extruded manually. Specifically, the data received from the 200% machine stretched threads was concluded as mostly noise, as the threads ripped 10 – 20 seconds in to the Instron tests. The threads provided very noisy data, which did not indicate variability in thread properties, but a large decrease in thread diameters when compared to other stretch groups. The stiffness of the 50% machine stretched threads was similar to that of 100% machine stretched threads. This may have occurred because threads were unintentionally stretched residually before the controlled machine stretching. The threads produced in the 50% machine stretched threads may have been stretched unintentionally, and produced similar data to the 100% machine stretched threads. With the addition of the automated stretching system, the stretch percentage and stretch speed of fibrin microthreads during production can be strictly controlled with reliability and accuracy.

An automated extrusion system that can produce consistent microthreads, can control the extrusion rate and drawing accuracy and limit the variability within the structures of the threads. The variability can be contributed to the extrusion step of the procedure because the dry and wet diameter measurements were still variable. The inclusion of an automated extrusion system that can interface with the automated stretching system has the potential to further reduce thread variability but was out of the scope of this project.

7.2 Impact Analysis

This design is a prototype, but still has the potential to impact society. The main function of this device is to produce microthreads for small-scale, laboratory production. However, the potential for microthreads to become mass produced is possible, and the device represents the first automated stretching system for fibrin microthreads. This device represents a critical step in the full automation of microthread production. The following is the team's analysis of the societal impact of the device.

7.2.1 Economics

Economics deals with the manufacture, consumption, and distribution of products. The design team constructed an automated fibrin microthread stretching system. If the stretching system were to be used in a fully automated production system, it could easily be scaled up and interface with other systems using the removable frame. The ability of this system to interface with other systems would make it more useful for different labs and different uses. It represents a significant step in the increase of the production ability of consistent fibrin microthreads, which increases the distribution potential and research scope of this scaffold material.

7.2.2 Environmental Impact

The stretching system has a minimal environmental impact. This system is made primarily of acrylic. The manufacturing process of acrylics involves toxic chemicals. Acrylic polymerization must be carefully monitored and results in fumes that are toxic to humans and the environment (Tomenson, 2000). Acrylic is also not easily recycled and has extremely long degradation times (Tomenson, 2000). From an environmental perspective, the harmful effects of production and difficult disposal techniques make acrylic a less than ideal material to be used in the microthread processing system. Acrylic is resistant to the corrosive HEPES bath necessary

for fibrin microthread production. More importantly acrylic is biocompatible, which is essential when creating biological implants. Ultimately, the production process for the stretching system would not result in a significant environmental impact if proper precautions were taken.

7.2.3 Societal Influence

This device has the potential for a large societal influence, within the clinical space of tissue engineering. This automated stretching system, combined with a fully automated extrusion system, could be manufactured on a larger scale, resulting in increased consistent fibrin microthread production for research. Clinically the societal impact for fibrin microthreads is that they could be used as a scaffold to facilitate the restoration of muscle, tendon and ligaments in large scale injuries (Page, 2011). If fibrin microthreads could be made at a scaled-up, consistent production level, more applications could also be explored.

7.2.4 Political Ramifications

The scaled-up production of consistent fibrin microthreads has the potential to impact the clinical applications of tissue engineering world-wide, decreasing the need for autografts, allografts, and other solutions in musculoskeletal injuries. The increase in medical relevance of fibrin microthreads would increase their global impact, making them relevant in the political sphere. Politics on government funding for their research, as well as wide spread availability and healthcare ramifications, would need to be discussed and debated.

7.2.5 Ethical Concern

Since this product will be used in the medical field, there are ethical concerns with the device, but they are minimal. One ethical concern may be the sourcing of blood products from bovine for implantation in the human body. However, fibrinogen and thrombin are blood productions that could ultimately be patient specific. This limits the ethical concerns with respect to cell sourcing from human cadavers, stem cells, and animals.

7.2.6 Health and Safety Issue

Health and safety issues are always involved in any medical device or medical device production method. The fibrin microthread processing system was created to create biological implants. The device was constructed out of biocompatible materials and is able to be sterilized with isopropyl alcohol or ethylene oxide. The only concern with the sterilization of this device is the electronic VEX components and wiring for the system. These parts of the system would need

to be sterilized more carefully, or isolated from the system with a compartment or medical drape so that only the thread contacting components would need full sterilization.

7.2.7 Manufacturability

The fibrin microthread processing system is easy to manufacture. Once the acrylic pieces of the bath are cut to the desired size, they are glued together (Figure 48). The frame was made from machined acrylic and consists of the threaded rod through the frame components that remain in the bath, and the removable frame which has the stainless steel rods. The device consists of few moving parts to reduce the complexity of the device and is easy to manufacture.

7.2.8 Sustainability

Currently, sustainability is considered the ability and capacity to maintain or endure. Sustainability is often related to environment and the long-term effect of a product. The main components of the microthread processing system are made of acrylic and stainless steel. These materials would last through many uses of the device. Once the device is no longer needed, acrylic is difficult to recycle and dispose of. Once the device is no longer needed the acrylic should be disposed of at proper recycling centers.

8 Conclusion and Recommendations

The purpose of this project was to design, construct, and validate an automated production system for the fabrication of fibrin microthreads. Although the designed device performed within desired parameters, validation of the machine with fibrin microthreads was limited to a semi-automated process. The biopolymer extrusion system was previously designed to extrude collagen microthreads. It was predicted that the system would also work with fibrin microthreads, requiring only minor modifications. However, during validation, it was necessary to use a process that eliminated the biopolymer extrusion system and included manual extrusion and removal to produce consistent threads, with decreased variability, at an acceptable yield percentage.

Acknowledging that thread validation was not completed on the fully automated system, the device performed to desired specifications and produced partially automated threads. Of the machine functions, the stretch-to-remove technique, which was designed and used to minimize manual manipulation, failed in terms of practical use, resulting in the need for manual removal.

Future recommendations for this device would be to automate the manual processes of this device, including a more effective removal system and the modification of the biopolymer extrusion system to accommodate fibrin microthread production. Once the automated extrusion head has been modified and validated for fibrin microthreads, it is recommended that it be reintegrated with the system.

One way that the automated extrusion head could be modified to produce more consistent threads would be to add high torque motors to the system. This would ensure that the automated extrusion head would be able to move at the appropriate speed of 2.0 cm/s without stalling or jumping. Another modification needed would be to perfect the way that the threads are laid on the stretch-to-remove surface. Initially the threads were designed to be dropped from the polyethylene tubing, but the team decided that extending the tubing out of the pipet tip so the threads are laid instead of dropped would work because it more closely mimicked the manual process. However, the design team was unable to perfect this system by decreasing the angle that the tubing bends at on the stretch-to-remove surface within the project constraints. These modifications would likely lead to a higher yield percentage of consistent threads from the extrusion system.

The removal system would also need to be modified for the full success of the automated processing system. Modifications to this system could include creating a completely flat interface between the stretcher plates and the stretch-to-remove surface or a redesign of the adhesion system to pinch the threads in the same manor as forceps would. Additionally, the forces involved in pulling the thread from the pan need to be investigated to make sure that the stretch speed is based on this force.

Full automation of the stretching process during fibrin microthread production has the potential to create more consistent threads. Threads properties can be tailored by varying the stretch percentage and the stretch speed. With the incorporation of the fully automated stretching system, the stretch percentage and speed can be performed in a controlled setting. This allows for decreases in production variability and increases in control of microthread properties for different applications in tissue engineering.

Although full automation of the fibrin microthread processing system was not achieved, the design team developed a semi-automated production system, with a fully automated stretching system, to create fibrin microthreads at an acceptable yield percentage, with decreased variability in their mechanical properties. This work represents significant progress in the automation of fibrin microthread production to include stretching. In combination with a fully automated extrusion and removal system, this stretching system has the potential to eliminate human handling of fibrin microthreads during the fabrication process.

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10 Appendices

10.1 Appendix A: Pairwise Comparison

Table A - 1: The design team's pairwise comparison chart

Pairwise Level 1

	User Friendly	Automated	Effectiveness	Versatility	Totals
User Friendly		0	0	0.5	0.5
Automated	1		0	1	2
Effectiveness	1	1		1	3
Versatility	0.5	0	0		0.5

Pairwise of User Friendliness

	Easy to Clean	Modifiable Interface	Easy to maintain	Reliable	Durable	Totals
Easy to Clean		0	0.5	0	0	0.5
Modifiable Interface	1		1	0	1	3
Easy to Maintain	0.5	0		0.5	0.5	1.5
Reliable	1	1	0.5		1	3.5
Durable	1	0	0.5	0		1.5

Pairwise of Automation

	~~Automated Extrusion~~	Automated Stretch	Automated Removal	Automated Drying	Totals
~~Automated Extrusion~~					
Automated Stretch			1	1	2
Automated Removal		0		1	1
Automated Drying		0	0		0

Pairwise of Effectiveness

	Accuracy	Precision	Reproducibility	Minimize Thread Failure	Totals
Accuracy		0.5	1	1	2.5
Precision	0.5		1	1	2.5
Reproducibility	0	0		1	1
Minimize thread failure	0	0	0		0

Pairwise of Versatility

	Sterilization	Portability	Crosslinking	Modifiable Parameters	Totals
Sterilization		1	1	0	2
Portability	0		0	0	0
Crosslinking	0	1		0	1
Modifiable Parameters	1	1	1		3

Pairwise of Modifiable Parameters

	Strain rate	Stretch percentage	Extrusion head rate	Cycle time	Thread length	Totals
Strain Rate		0.5	0	1	1	2.5
Stretch Percentage	0.5		1	1	1	3.5
Extrusion head rate	1	0		1	1	3
Cycle time	0	0	0		1	1
Thread length	0	0	0	0		0

Table A - 2: The client's pairwise comparison chart

Design of an Automated Fibrin Microthread Processing System (GXP-1201)
Pair-wise Comparison Charts

Pairwise Level 1

	User Friendly	Automated	Effectivness	Versatility	Totals
User Friendly		0	0	0	0
Autorated	1		1	1	3
Effectiveness	1	0		1	2
Versatility	1	0	0		1

Pairwise of User Friendliness

	Easy to Clean	Modifiable Interface	Easy to maintain	Reliable	Durable	Totals
Easy to Clean		0	0.5	0	0	0.5
Modifiable Interface	1		1	0	0	2
Easy to Maintain	0.5	0		0	0	0.5
Reliable	1	1	1		0.5	3.5
Durable	1	1	1	0.5		3.5

Pairwise of Automation

	~~Automated Extrusion~~	Automated Stretch	Automated Removal	Automated Drying	Totals
~~Automated Extrusion~~					
Automated Stretch			1	1	2
Automated Removal		0		0.5	0.5
Automated Drying		0	0.5		0.5

Pairwise of Effectiveness

	Accuracy	Precision	Reproducibility	Minimize Thread Failure	Totals
Accuracy		0	0	0	0
Precision	1		0.5	1	2.5
Reproducibility	1	0.5		1	1.5
Minimize thread failure	1	0	0		1

Pairwise of Versatility

	Sterilization	Portability	Crosslinking	Modifiable Parameters	Totals
Sterilization		0	0.5	0	0.5
Portability	1		1	0	1
Crosslinking	0.5	0		0	0.5
Modifiable Parameters	1	1	1		3

Pairwise of Modifiable Parameters

	Strain rate	Stretch percentage	~~Extrusion head rate~~	Cycle time	Thread length	Totals
Strain Rate		0		0.5	0.5	1
Stretch Percentage	1			1	0.5	2.5
~~Extrusion head rate~~						
Cycle time	0.5	0			0	0.5
Thread length	0.5	0.5		1		2

→ Extrusion head rate should match current design parameters. Not a design parameter

Table A - 3: The user's pairwise comparison chart

Design of an Automated Fibrin Microthread Processing System (GXP-1201)
Pair-wise Comparison Charts

Pairwise Level 1

	User Friendly	Automated	Effectivness	Versatility	Totals
User Friendly		0	0	0.5	0.5
Automated	1		0.5	0.5	2
Effectiveness	1	0.5		1	2.5
Versatility	0.5	0.5	0		1

Pairwise of User Friendliness

	Easy to Clean	Modifiable Interface	Easy to maintain	Reliable	Durable	Totals
Easy to Clean		0	0.5	0	0.5	1
Modifiable Interface	1		0.5	0.5	0.5	2.5
Easy to Maintain	0.5	0.5		0	0.5	1.5
Reliable	1	0.5	1		0.5	3
Durable	0.5	0.5	0.5	0.5		2

Pairwise of Automation

	Automated Extrusion	Automated Stretch	Automated Removal	Automated Drying	Totals
~~Automated Extrusion~~					
Automated Stretch			0.5	1	1.5
Automated Removal		0.5		1	1.5
Automated Drying		0	0		0

Pairwise of Effectiveness

	Accuracy	Precision	Reproducibility	Minimize Thread Failure	Totals
Accuracy		0.5	1	0	1.5
Precision	0.5		0.5	0.5	1.5
Reproducibility	0	0.5		0.5	1
Minimize thread failure	1	0.5	0.5		2

Pairwise of Versatility

	Sterilization	Portability	Crosslinking	Modifiable Parameters	Totals
Sterilization		0.5	0	0	0.5
Portability	0.5		0	0	0.5
Crosslinking	1	1		0.5	2.5
Modifiable Parameters	1	1	0.5		2.5

Pairwise of Modifiable Parameters

	Strain rate	Stretch percentage	Extrusion head rate	Cycle time	Thread length	Totals
Strain Rate		0.5	1	1	1	3.5
Stretch Percentage	0.5		1	1	1	3.5
Extrusion head rate	0	0		1	1	2
Cycle time	0	0	0		0	0
Thread length	0	0	0	1		1

10.2 Appendix B: Weighted Comparison

Table B - 1: Weighted comparison chart

Level 1 Objectives	Design team	Client	User	Total	Modifing %
User friendly	0.5	0	0.5	0.3	0.05
Automated	2	3	2	2.4	0.4
Effective	3	2	2.5	2.4	0.4
Versatility	0.5	1	1	0.9	0.15
Level 2 User friendly	Design team	Client	User	Total	Modified total
Easy to clean	0.5	0.5	1	0.7	0.035
Modifiable interface	3	2	2.5	2.4	0.12
Easy to maintain	1.5	0.5	1.5	1.1	0.055
Reliable	3.5	3.5	3	3.3	0.165
Durable	1.5	3.5	2	2.5	0.125
Level 2 Automated	Design team	Client	User	Total	Modified total
Stretch	2	2	1.5	1.8	0.72
Removal	1	0.5	1.5	1	0.4
Drying	0	0.5	0	0.2	0.08
Level 2 Effectiveness	Design team	Client	User	Total	Modified total
Accuracy	2.5	0	1.5	1.1	0.44
Precision	2.5	2.5	1.5	2.1	0.84
Reproducibility	1	2.5	1	1.6	0.64
Minimize thread failure	0	1	2	1.2	0.48
Level 2 Versatility	Design team	Client	User	Total	Modified total
Sterilization	2	0.5	0.5	0.8	0.12
Portability	0	2	0.5	1	0.15
Crosslinking	1	0.5	2.5	1.4	0.21
Modifiable parameters	3	3	2.5	2.8	0.42
Level 3 Modifiable parameters	Design team	Client	User	Total	Modified total
Stretch speed	2.5	1	2.5	1.9	0.13
Stretch percentage	2.5	2.5	2.5	2.5	0.18
Cycle time	1	0.5	0	0.4	0.03
Thread length	0	2	1	1.2	0.08

10.3 Appendix C: Idea Comparison

For the following tables, a blue box denotes a design that was to be tested in preliminary testing due to its high score in this comparison chart based on the parameters listed in the table. A design that has been blacked out indicates that it could not meet all of our constraints, and therefore failed before the objective analysis.

Table C - 1: Frame idea comparison

Frame Idea Comparisons			Metal pegs	Weighted	Single roller	Weighted	Flat clamps	Weighted	Rotational clamp	Weighted	Roughened surface	Weighted	Seal	Weighted	Velcro	Weighted	Slanted gears	Weighted
C		Time limit (A-D term)	Y		Y		Y		Y		Y		Y		Y		Y	
C		Size (4x6x4 ft)	Y		Y		Y		Y		Y		Y		Y		Y	
C		Interface with existing system	Y		Y		Y		Y		Y		Y		Y		Y	
C		Limited budget ($524)	Y		Y		Y		Y		Y		Y		Y		Y	
O	0.3	**User Friendly**																
O	0.04	Easy to clean	3	0.11	2	0.07	4	0.14	5	0.18	1	0.04	2	0.07	1	0.035	2	0.07
O	0.12	Modifiable interface	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O	0.06	Easy to maintain	5	0.28	3	0.17	4	0.22	4	0.22	3	0.17	1	0.055	5	0.275	3	0.17
O	0.17	Reliable	5	0.83	3	0.5	4	0.66	4	0.66	2	0.33	4	0.66	4	0.66	3	0.5
O	0.13	Durable	5	0.63	3	0.38	5	0.63	4	0.5	3	0.38	3	0.375	5	0.625	5	0.63
O	2.4	**Automated**																
O	0.72	Stretch	3	2.16	3	2.16	5	3.6	5	3.6	4	2.88	4	2.88	3	2.16	2	1.44
O	0.4	Removal	3	1.2	2	0.8	3	1.2	3	1.2	3	1.2	4	1.6	3	1.2	2	0.8
O	0.08	Drying	3	0.24	3	0.24	5	0.4	5	0.4	4	0.32	4	0.32	3	0.24	2	0.16
O	2.4	**Effective**																
O	0.44	Accuracy	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O	0.84	Precision	2	1.68	1	0.84	4	3.36	4	3.36	4	3.36	4	3.36	3	2.52	2	1.68
O	0.64	Reproducibility	2	1.28	1	0.64	4	2.56	4	2.56	4	2.56	4	2.56	3	1.92	2	1.28
O	0.48	Minimize thread failure	1	0.48	1	0.48	3	1.44	3	1.44	2	0.96	5	2.4	1	0.48	1	0.48
O	0.9	**Versatility**																
O	0.12	Sterilization	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O	0.15	Portability	4	0.6	2	0.3	3	0.45	4	0.6	5	0.75	3	0.45	3	0.45	2	0.3
O	0.21	Crosslinking	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O	0.42	Modifiable parameters	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O	0.42	**Modifiable Parameters**																
O	0.13	Strain rate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O	0.18	Stretch percentage	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O	0.03	Cycle time	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O	0.08	Thread length	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Total		**9.47**		**6.57**		**14.7**		**14.7**		**12.9**		**14.7**		**10.6**		**7.5**

Table C - 2: Stretch mechanism idea comparison

Stretch Idea Comparisons			Acordian	Weight	Motorized stretcher	Weight	Rollers	Weight	Angled hydraulic	Weight	Track/ tread	Weight
C		Time limit (A-D term)	Y		Y		Y		Y		Y	
C		Size (4x6x4 ft)	Y		Y		Y		Y		Y	
C		Interface with existing system	Y		Y		Y		Y		Y	
C		Limited budget ($524)	Y		Y		Y		N		Y	
O	0.3	**User Friendly**										
O	0.04	Easy to clean	2	0.1	4	0.1	1	0			1	0.035
O	0.12	Modifiable interface	3	0.4	5	0.6	3	0.4			2	0.24
O	0.06	Easy to maintain	3	0.2	3	0.2	2	0.1			2	0.11
O	0.17	Reliable	4	0.7	4	0.7	2	0.3			4	0.66
O	0.13	Durable	3	0.4	2	0.3	2	0.3			3	0.375
O	2.4	**Automated**										
O	0.72	Stretch	5	3.6	5	3.6	3	2.2			4	2.88
O	0.4	Removal	0	0	0	0	0	0			0	0
O	0.08	Drying	0	0	0	0	0	0			0	0
O	2.4	**Effective**										
O	0.44	Accuracy	3	1.3	5	2.2	2	0.9			4	1.76
O	0.84	Precision	5	4.2	5	4.2	4	3.4			5	4.2
O	0.64	Reproducibility	5	3.2	5	3.2	3	1.9			5	3.2
O	0.48	Minimize thread failure	3	1.4	3	1.4	3	1.4			3	1.44
O	0.9	**Versatility**										
O	0.12	Sterilization	0	0	0	0	0	0			0	0
O	0.15	Portability	3	0.5	5	0.8	4	0.6			2	0.3
O	0.21	Crosslinking	0	0	0	0	0	0			0	0
O	0.42	Modifiable parameters	3	1.3	5	2.1	2	0.8			2	0.84
O	0.42	**Modifiable Parameters**										
O	0.13	Strain rate	3	0.4	4	0.5	3	0.4			4	0.532
O	0.18	Stretch percentage	3	0.5	3	0.5	2	0.4			3	0.525
O	0.03	Cycle time	0	0	0	0	0	0			0	0
O	0.08	Thread length	5	0.4	5	0.4	2	0.2			1	0.084
		Total		**18**		**21**		**13**				**17.2**

Table C - 3: Bath idea comparison

Bath Idea Comparisons			Angled	Weight	Bottom drain	Weight	Foldable walls	Weight	Humidifier	Weight	Compartment humidifier	Weight
C		Time limit (A-D term)	Y		Y		Y		Y		Y	
C		Size (4x6x4 ft)	Y		Y		Y		Y		Y	
C		Interface with existing system	Y		Y		Y		Y		Y	
C		Limited budget ($524)	Y		Y		N		Y		N	
O	0.3	**User Friendly**										
O	0.04	Easy to clean	5	0.2	5	0.2			5	0.2		
O	0.12	Modifiable interface	0	0	0	0			0	0		
O	0.06	Easy to maintain	5	0.3	5	0.3			3	0.2		
O	0.17	Reliable	5	0.8	4	0.7			5	0.8		
O	0.13	Durable	5	0.6	5	0.6			5	0.6		
O	2.4	**Automated**										
O	0.72	Stretch	0	0	0	0			0	0		
O	0.4	Removal	0	0	0	0			0	0		
O	0.08	Drying	0	0	0	0			0	0		
O	2.4	**Effective**										
O	0.44	Accuracy	0	0	0	0			0	0		
O	0.84	Precision	0	0	0	0			0	0		
O	0.64	Reproducibility	0	0	0	0			0	0		
O	0.48	Minimize thread failure	0	0	0	0			0	0		
O	0.9	**Versatility**										
O	0.12	Sterilization	0	0	0	0			0	0		
O	0.15	Portability	4	0.6	5	0.8			2	0.3		
O	0.21	Crosslinking	0	0	0	0			0	0		
O	0.42	Modifiable parameters	0	0	0	0			0	0		
O	0.42	**Modifiable Parameters**										
O	0.13	Strain rate	0	0	0	0			0	0		
O	0.18	Stretch percentage	0	0	0	0			0	0		
O	0.03	Cycle time	0	0	0	0			0	0		
O	0.08	Thread length	0	0	0	0			0	0		
		Total		2.5		2.5				2.1		

Table C - 4: Removal\drying idea comparison

Removal - Drying Idea Comparisons			90 Turn	Weight	Windshield	Weight	Stretch to remove	Weight	Track	Weight	Drop down angled	Weight	Drop down pan	Weight	Lift away	Weight
C		Time limit (A-D term)	Y		Y		Y		Y		Y		Y		Y	
C		Size (4x6x4 ft)	Y		Y		Y		Y		Y		Y		Y	
C		Interface with existing system	Y		Y		Y		Y		Y		Y		Y	
C		Limited budget ($524)	Y		Y		Y		Y		Y		Y		Y	
O	0.3	**User Friendly**														
O	0.04	Easy to clean	4	0.14	2	0.07	5	0.18	4	0.14	4	0.14	4	0.14	3	0.11
O	0.12	Modifiable interface	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O	0.06	Easy to maintain	3	0.17	3	0.17	5	0.28	3	0.165	4	0.22	4	0.22	3	0.17
O	0.17	Reliable	4	0.66	4	0.66	5	0.83	4	0.66	5	0.83	5	0.83	4	0.66
O	0.13	Durable	4	0.5	4	0.5	5	0.63	4	0.5	4	0.5	4	0.5	4	0.5
O	2.4	**Automated**														
O	0.72	Stretch	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O	0.4	Removal	4	1.6	3	1.2	2	0.8	4	1.6	3	1.2	3	1.2	3	1.2
O	0.08	Drying	1	0.08	5	0.4	1	0.08	5	0.4	1	0.08	1	0.08	4	0.32
O	2.4	**Effective**														
O	0.44	Accuracy	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O	0.84	Precision	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O	0.64	Reproducibility	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O	0.48	Minimize thread failure	3	1.44	3	1.44	3	1.44	3	1.44	3	1.44	3	1.44	3	1.44
O	0.9	**Versatility**														
O	0.12	Sterilization	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O	0.15	Portability	2	0.3	3	0.45	5	0.75	2	0.3	3	0.45	3	0.45	2	0.3
O	0.21	Crosslinking	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O	0.42	Modifiable parameters	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O	0.42	**Modifiable Parameters**														
O	0.13	Strain rate	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O	0.18	Stretch percentage	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O	0.03	Cycle time	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O	0.08	Thread length	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Total		**4.89**		**4.89**		**4.97**		**5.21**		**4.86**		**4.9**		**4.7**

10.4 Appendix D: Budget and Cost Analysis

Table D - 1: Budget vs. cost analysis

Prototype Part	Item	Quantity (ea)	Cost	Total Cost
Motorized stretcher	1/4-20 Stainless steel rod	1	$5.24	
	1/4-20 lock nut	2	$1.18	
	1/4-20 wing nut	4	$4.72	
	1/4-20 1-1/2" screw	4	$2.36	
	1/4 in stainless steel rods	2	$12.00	
	Potentiometers	1	$12.99	
	Gear kit	1	$12.99	
	High strength gear kit	1	$29.99	
	Jumpers	1	$2.49	
	Vex LEDs	1	$9.99	
	Motor	1	$30.00	
	Motor interface	1	$19.99	
	Power sources	1	$0.00	
	5lb Acrylic	0.5	$13.28	$157.22
Bath	5lb Acrylic	0.5	$13.28	
	Auto/Marine Sealant	1	$4.57	
	Alligator Clips	1	$1.96	
	Silicone Sealant	1	$4.57	
	Loctite Glue	1	$4.98	
	Acetone acrylic glue	1	$17.58	
	Coating	1	$5.00	
	Plunger	1	$0.00	
	Stuffing box	1	$25.49	
	1" Brass hooks	1	$1.18	$78.61
Stretch-to-remove	Teflon pan	1	$10.00	$10.00
Adhesion system	PDMS	-	$0.00	
	PDMS mold	2	$20.00	
	Window squeegee	1	$4.49	
	10-24 wing nuts	2	$2.36	
	10-24 screw	2	$1.18	
	Plastic hinges	1	$4.55	$32.58
Validation testing				
TOTAL				$278.41

10.5 Appendix E: Vex Code

Figure E - 1: EasyC Pro code for system

```
#include "Main.h"

void main ( void )
{
    int m1count = 0;
    int m1dir = 0;
    int threadcount = 1;
    int m2count = 0;
    int m2dir = 0;
    long encoder_count = 0;
    int max_countp1m3 = 0;
    int speedp2m3 = 0;
    int x = 0;
    unsigned char emer_stop1 = 1;
    unsigned char continue2 = 1;
    int countp1m3 = 0;
    int degreesp2m3 = 0;

    Wait ( 2000 ) ;
    countp1m3 = GetAnalogInput ( 6 ) ; // P1 gets user input for stretching distance
    degreesp2m3 = GetAnalogInput ( 7 ) ; // P2 gets user input for stretching speed
    PrintToScreen ( "Count%d\n" , (int)countp1m3 ) ;
    PrintToScreen ( "Degrees%d\n" , (int)degreesp2m3 ) ;
    PresetQuadEncoder ( 1 , 5 , 0) ;
    StartQuadEncoder ( 1 , 5 , 0 ) ;
    encoder_count = GetQuadEncoder ( 1 , 5 ) ; // Encoder collects count as M3 turns
    PrintToScreen ( "%d\n" , (int)encoder_count ) ;
    StartInterruptWatcher ( 2 , 1 ) ;
    PrintToScreen ( "Stop: %d\n" , (int)emer_stop1 ) ;
    while ( threadcount <= 14 && continue2 == 1 ) // Lengthwise extrusion (runs to 11 cm)
    {
        continue2 = GetDigitalInput ( 8 ) ;
        emer_stop1 = GetInterruptWatcher ( 2 ) ;
        PrintToScreen ( "Continue: %d\n" , (int)continue2 ) ;
        SetDigitalOutput ( 8 , 0 ) ; // EXTRUSION
        SetDigitalOutput ( 11 , 0 ) ;
        Wait ( 1.75 ) ;
        SetDigitalOutput ( 11 , 1 ) ;
        Wait ( 1.75 ) ;
        m1count += 1 ;
        if ( m1count  == 2000 ) // Widthwise extrusion - (runs to 1 cm)
        {
            continue2 = GetDigitalInput ( 8 ) ;
            if ( threadcount < 14 )
            {
                continue2 = GetDigitalInput ( 8 ) ;
                Wait ( 1500 ) ;
                while ( m2count <= 3500 )
                {
                    continue2 = GetDigitalInput ( 8 ) ;
                    SetDigitalOutput ( 13 , 0 ) ;
                    Wait ( 1 ) ;
                    SetDigitalOutput ( 13 , 1 ) ;
                    Wait ( 1 ) ;
                    m2count += 1 ;
                }
                Wait ( 1500 ) ;
            }
```

```
            if ( m1dir == 0 )
            {
                m1dir = 1 ;
                SetDigitalOutput ( 12 , 1 ) ;
            }
            else if ( m1dir == 1 )
            {
                SetDigitalOutput ( 12 , 0 ) ;
                m1dir = 0 ;
            }
            threadcount += 1 ;
            m1count = 0 ;
            m2count = 0 ;
        }
    }
    while ( continue2 == 0 ) // Polymerization time!!
    {
        emer_stop1 = GetInterruptWatcher ( 2 ) ;
        SetPWM ( 1 , 127 ) ;
        continue2 = GetDigitalInput ( 8 ) ;
        PrintToScreen ( "Button = %d\n" , (int)continue2 ) ;
    }
    if ( degreesp2m3 <= 63 ) // Speed 1 @ 2.0 mm/sec
    {
        speedp2m3 = 109 ;
    }
    else if ( degreesp2m3 <= 191 ) // Speed 2 @ 4.7 mm/sec
    {
        speedp2m3 = 107 ;
    }
    else if ( degreesp2m3 <= 319 ) // Speed 3 @ 6.9 mm/sec
    {
        speedp2m3 = 103 ;
    }
    else if ( degreesp2m3 <= 447 ) // Speed 4 @ 8.4 mm/sec
    {
        speedp2m3 =  100 ;
    }
    else if ( degreesp2m3 <= 575 ) // Speed 5 @ 10.3 mm/sec
    {
        speedp2m3 = 97 ;
    }
    else if ( degreesp2m3 <= 703 ) // Speed 6 @ 11.8 mm/sec
    {
        speedp2m3 = 94 ;
    }
    else if ( degreesp2m3 <= 831 ) // Speed 7 @ 13.2 mm/sec
    {
        speedp2m3 = 91 ;
    }
    else if ( degreesp2m3 <= 959 ) // Speed 8@ 14.0 mm/sec
    {
        speedp2m3 = 88 ;
    }
    else if ( degreesp2m3 <= 1023 ) // Speed 9 @ 15.4 mm/sec
    {
        speedp2m3 = 85 ;
    }
```

```
117     PrintToScreen ( "Encoders\n" ) ;
118     PrintToScreen ( "Count %d\n" , (int)countp1m3 ) ;
119     Wait ( 1000 ) ;
120     if ( countp1m3 <= 63 ) // 0%
121     {
122         PrintToScreen ( "Zero\n" ) ;
123         SetPWM ( 1 , 127 ) ;
124     }
125     else if ( countp1m3 <= 191 ) // 50%
126     {
127         max_countp1m3 = 2500 ;
128         SetPWM ( 1 , speedp2m3 ) ;
129         PrintToScreen ( "speed %d\n" , (int)speedp2m3 ) ;
130         while ( encoder_count < max_countp1m3 )
131         {
132             Wait ( 3000 ) ;
133             encoder_count = GetQuadEncoder ( 1 , 5 ) ; // Encoder collects count as M3 turns
134             PrintToScreen ( "%d\n" , (int)encoder_count ) ;
135         }
136         continue2 = 1 ;
137         SetPWM ( 1 , 127 ) ;
138     }
139     else if ( countp1m3 <= 319 ) // 100%
140     {
141         max_countp1m3 = 5500 ;
142         while ( encoder_count < max_countp1m3 )
143         {
144             encoder_count = GetQuadEncoder ( 1 , 5 ) ; // Encoder collects count as M3 turns
145             PrintToScreen ( "%d\n" , (int)encoder_count ) ;
146             SetPWM ( 1 , speedp2m3 ) ;
147         }
148         continue2 = 1 ;
149         SetPWM ( 1 , 127 ) ;
150     }
151     else if ( countp1m3 <= 447 ) // 150%
152     {
153         max_countp1m3 = 8000 ;
154         while ( encoder_count < max_countp1m3 )
155         {
156             encoder_count = GetQuadEncoder ( 1 , 5 ) ; // Encoder collects count as M3 turns
157             PrintToScreen ( "%d\n" , (int)encoder_count ) ;
158             SetPWM ( 1 , speedp2m3 ) ;
159         }
160         continue2 = 1 ;
161         SetPWM ( 1 , 127 ) ;
162     }
163     else if ( countp1m3 <= 575 ) // 200%
164     {
165         max_countp1m3 = 11000 ;
166         while ( encoder_count < max_countp1m3 )
167         {
168             encoder_count = GetQuadEncoder ( 1 , 5 ) ; // Encoder collects count as M3 turns
169             PrintToScreen ( "%d\n" , (int)encoder_count ) ;
170             SetPWM ( 1 , speedp2m3 ) ;
171         }
172         continue2 = 1 ;
173         SetPWM ( 1 , 127 ) ;
174     }
```

```
175     else if ( countp1m3 <= 703 ) // 250%
176     {
177         max_countp1m3 = 14000 ;
178         while ( encoder_count < max_countp1m3 )
179         {
180             encoder_count = GetQuadEncoder ( 1 , 5 ) ; // Encoder collects count as M3 turns
181             PrintToScreen ( "%d\n" , (int)encoder_count ) ;
182             SetPWM ( 1 , speedp2m3 ) ;
183         }
184         continue2 = 1 ;
185         SetPWM ( 1 , 127 ) ;
186     }
187     else if ( countp1m3 <= 831 ) // 300%
188     {
189         max_countp1m3 = 17000 ;
190         while ( encoder_count < max_countp1m3 )
191         {
192             encoder_count = GetQuadEncoder ( 1 , 5 ) ; // Encoder collects count as M3 turns
193             PrintToScreen ( "%d\n" , (long)encoder_count ) ;
194             SetPWM ( 1 , speedp2m3 ) ;
195         }
196         continue2 = 1 ;
197         SetPWM ( 1 , 127 ) ;
198     }
199     else if ( countp1m3 <= 959 ) // 350%
200     {
201         max_countp1m3 = 20000 ;
202         while ( encoder_count < max_countp1m3 )
203         {
204             encoder_count = GetQuadEncoder ( 1 , 5 ) ; // Encoder collects count as M3 turns
205             PrintToScreen ( "%d\n" , (int)encoder_count ) ;
206             SetPWM ( 1 , speedp2m3 ) ;
207         }
208         continue2 = 1 ;
209         SetPWM ( 1 , 127 ) ;
210     }
211     else if ( countp1m3 <= 1023 ) // 400%
212     {
213         max_countp1m3 = 23000 ;
214         while ( encoder_count < max_countp1m3 )
215         {
216             encoder_count = GetQuadEncoder ( 1 , 5 ) ; // Encoder collects count as M3 turns
217             PrintToScreen ( "%d\n" , (int)encoder_count ) ;
218             SetPWM ( 1 , speedp2m3 ) ;
219         }
220         continue2 = 1 ;
221         SetPWM ( 1 , 127 ) ;
222     }
223     while ( continue2 == 1 ) // Press continue button to return stretcher plate to home... SHUT MACHINE OFF when it is home
224     {
225         continue2 = GetDigitalInput ( 8 ) ;
226         PrintToScreen ( "Button = %d\n" , (int)continue2 ) ;
227         SetPWM ( 1 , 127 ) ;
228     }
229     while ( encoder_count > 0 )
230     {
231         SetPWM ( 1 , 200 ) ;
232     }
233 }
```

10.6 Appendix F: Protocols

Fibrin Microthread Extrusion Protocol
Aliquot Preparation
Materials:
Fibrinogen (F8630, Sigma)
Thrombin (T4648, Sigma) – 1 KU
Calcium Chloride ($CaCl_2$; MW: 110.99)
Sodium Chloride (NaCl; MW: 58.44)
HEPES (MW: 238.3)

Procedure:
HEPES buffered saline (HBS) preparation
1. Definition: HBS contains 20 mM HEPES and 0.9% (w/v) NaCl
2. Add the following reagents to 200 mL:
    a. 2.25g of NaCl
    b. 1.1915g of HEPES
3. pH solution to 7.4 using NaOH/HCl.
4. Bring final volume to 250 mL.
5. Store at room temperature.

Fibrinogen aliquots (70 mg/mL)
1. Measure 14.3 mL of HBS into a 50 mL conical tube.
2. Weigh 1.00 gram of fibrinogen and pour into conical tube.
3. Put conical tube on rocker plate, adjusting the position every 30-40 minutes until fibrinogen goes into solution.
    NEVER SHAKE/VORTEX FIBRINOGEN SOLUTION!!!! THIS WILL CAUSE FIBRINOGEN TO FALL OUT OF SOLUTION AND BIND TO ITSELF!!!!
4. Incubate conical tube at 37 C overnight to ensure fibrinogen is completely dissolved.
5. The next morning, measure 1 mL aliquots in eppendorfs and store at -20 °C.

Thrombin aliquots (40 U/mL)
1. Add 25 mL HBS to bottle of 1KU thrombin, mix well.
2. Aliquot 200 µL into eppendorfs and store at -20 °C (Final concentration: 8U / 200 µL).

Calcium chloride preparation (40 mM)
1. Add 0.1776 g of $CaCl_2$ to 40 mL of diH_2O.
2. Store at 4 °C.

HEPES buffer bath stock solution
1. Definition: Stock solution will be prepared at 10X of 10 mM HEPES buffer (100mM).
2. Add 23.83g of HEPES to 900 mL of diH_2O.
3. pH to 7.4 using NaOH/HCl
    WILL REQUIRE LARGE AMOUNTS OF ACID/BASE- USE HIGHER CONCENTRATIONS CAREFULLY.
4. Bring final volume to 1000 mL.

5. Store at room temperature.

Extrusion Procedure

Materials:

Fibrinogen aliquot (warmed to room temperature)
Thrombin aliquot (warmed to room temperature)
Calcium chloride solution (40mM, warmed to room temperature)
HEPES buffer bath stock solution (10X)
Metal non-stick pan
25 Gauge blunt end needle
0.86 mm I.D. polyethylene tubing (Intramedic PE90 427421)
2-1 mL syringes
Blending connector (SA-3670; Micromedics, MN)

Setup:

1. Place blunt end needle (25 gauge, BD) into 0.86 mm I.D. polyethylene tubing.
   CAN REUSE THESE MATERIALS IF PREVIOUS USER WASHED PROPERLY
2. Leur lock blunt end needle/tubing onto the front end of blending connector.
3. Turn syringe pump on.
   a. Press SELECT.
   b. Toggle to Table, press SELECT.
   c. Toggle to Bec. Dic. Plastic, press SELECT.
   d. Toggle to 1 cc 4.70 mm, press SELECT.
   e. Enter volume: 1.0 mL, press ENTER.
   f. Enter extrusion rate: 0.225 mL/min, press ENTER.
4. Place a metal non-stick pan next to the syringe pump.
5. Prepare 300 mL of 1X HEPES buffer solution (30 mL of stock solution and 270 mL diH_2O), pH to 7.4
6. Fill pan with 300 mL HEPES buffer solution
7. Add 150 µL of thrombin aliquot to 850 µL of calcium chloride solution, mix well.

Extrusion:

1. "Prime" 2 1 mL syringes by moving the plunger several times.
2. Collect all of the thrombin and fibrinogen solutions into 1 mL syringes.
   COLLECT THE FIBRINOGEN SOLUTION SLOWLY AND CAREFULLY, FAILURE TO DO SO MAY RESULT IN INSOLUBLE FIBRINOGEN FORMATION!!
3. Invert syringe, remove all bubbles, and ensure that both syringes have equal volumes.
4. Place each 1 mL syringe of fibrinogen and thrombin solutions into the back end of the blending applicator.
   ALWAYS PUT FIBRINOGEN SOLUTION IN THE BLENDING APPLICATOR OPENING WITH THE CIRCLE ON IT.
5. Secure syringe/blending applicator construct into syringe pump.
6. Press RUN on the syringe pump and wait for fibrin solution to flow out of the tip of the tubing.
7. Using a metal bar if necessary, draw threads into the buffer solution, taking 6-10 seconds to

draw each thread.
8. If the pump does not automatically stop when the syringes empty, press STOP.
9. Wash tubing/blending applicator with cold water and a 5 mL syringe, plugging the other opening with your thumb (at least 5 water rinses per blending applicator opening).
10. Flush water out of blending applicator/tubing repeating step 9 using an empty 5 mL syringe.
11. Fibers can be removed from the bath after 10-15 minutes and stretch threads to make 3 threads along the cardboard box (~7.5 inch threads).

Hand stretching with manual parameters:
1. Follow thread creation as listed previously in **Fibrin Microthread Extrusion Protocol**.
2. Wearing gloves and using a pair of forceps (one in each hand) pick up the edges of the threads gently, and rock them side to side, sliding them in and out of the water in a parabolic motion.
3. As a thread appear adequately stretched, gently lift it from the bath and secure one end to the cardboard box by gently pressing it.
4. Stretch the thread and choose a spot in the middle of the thread to place down on the box and secure.
5. Clip the thread after it is secured, and do the same with the other half of the thread.
6. Repeat these steps until batch is complete. Should yield about 35-40 threads.

Thread diameter measurement procedure:
1. Remove dried threads from threads are cut into 2cm pieces.
2. Using acetic glue, the 2 cm fibrin microthreads are mounted on to vellum paper.
3. Allow glue to dry overnight.
4. Using a Nikon Eclipse E600 microscope measure the diameter of the threads at each end and once in the middle.
5. Record the dry diameters and take the average.
6. Soak threads in PBS for 45 minutes.
7. One by one, remove threads from PBS and measure the hydrated thread diameters, again one from each end and one at the center.
8. Record and average the hydrated thread diameters.

Instron and Matlab procedure:

After dry diameter measurements, and while threads are hydrated for wet diameters:

Set up Instron machine as follows:
1. Attach 1N load cell (CAREFULLY) to the right end of the Instron on the station that does not translate and plug input into the back of the Instron.
2. Set the translation length of the left Instron piece to its maximum length to ensure that the Instron does not return and collide with the 1N load cell.
3. Calibrate load cell.
4. Attach custom grips to both the left-translational end and right-static load cell.
5. Balance load cell.
6. Open BlueHill and ElectroPlus software and open 110811JG Fibrin Threads.im_tens (stretch speed: 10 mm/min until a drop in load of over 90%).
7. Enter the maximum and minimum limits for the load cell (Max: 0.95N and Min: -0.95N) to ensure the safety of the load cell.

8. Enter average wet diameter in the appropriate input AND PRESS ENTER to ensure the program accepted the value.

To test threads:

1. Place the vellum frame on the custom grips so that the orientation of the thread is parallel to the straining axis, label to the left.
2. Attach securing grips to each end of the vellum frame.
   MAKE SURE THE LOAD ON THE LOAD CELL END DOES NOT EXCEED THE LIMITS
3. Cut the vellum frame on both the proximal and distal ends (without cutting the thread).
4. Run the program until complete failure of the thread and record its failure point. The thread will fail during the running of the program and tear at the left-glue (LG), right glue (RG), or in the middle (M) of the thread.

Matlab analysis of Instron Data:

1. After completion of all test groups for Instron mechanical testing, compile all data (.csv, .pdf) documents into one folder.
2. Open Matlab and run Fibrinmechanics03.m (seen in **[Appendix H].**
3. When prompted for an input file, select the folder of the correct test (ex. Fibrin test 1).
4. When prompted with a window with the stress-strain curve of each thread, click within the window along the thread line 4 times. The first two times are to capture the initial modulus of the thread and the second 2 clicks measure the ramped modulus of the thread. Click at the beginning and end of the initial modulus, attempting to make a line consistent with the data. The second two clicks are repeated in the same way for the ramped modulus before failure.
5. After completion of the program, find the output folded and accompanied csv and use this data to compile averages for: dry and wet diameters, swelling ratio (defined as (wet – dry) / (wet)), strain at failure, load, maximum tangent modulus (MTM or stiffness), and ultimate tensile strength.
6. Plot this data with accompanied standard deviations to visualize differences in test groups
7. Additional statistical analysis may be needed with performed one-way ANOVA with Holm-Sidak post hoc analysis ($p \leq 0.05$).

UV absorbance procedure:

1. Make diluted HEPES according to the procedure listed above.
2. Place HEPES in old bath, new bath, and leave some in the beaker for 1 hour.
3. Using quartz cuvettes, fill samples of each and place caps.
4. Turn on the computer and open BlueHill software
5. Place control HEPES cuvette in machine and press “Zero”.
6. After the zeroing analysis is complete it will instruct you to remove the control and place sample 1. You will place the old bath sample first, and rename your sample OldBath
7. Once that analysis is complete you can name a new sample NewBath, and place it in the machine. Allow it to analyze.
8. Press “Complete” to finish your analysis.

Machine parameter validation procedure

1. Ensure proper code and motor setting is typed in on EasyC Pro software, and the battery is fully charged and plugged into the machine
2. Download code to machine by plugging in the orange wire into the serial port, and your USB on your computer
3. Press “Build and Download”.
4. After it prompts you twice, and you press “Yes”, turn on the machine to allow the program to download then shut the machine off.
5. Take an initial distance measurement in mm from the back of the non-moving stretcher plate to the front of the moving stretcher plate.
6. Run the machine to your set parameters by turning it on and following the procedure listed in **[Appendix J**: User Manual**]**.
7. If running a stretch speed test, time the time it takes to stretch the threads.
8. Remeasure your stretcher plate distance from the back of the non-moving stretcher plate, to the front of the moving stretcher plate.
9. Press “Continue” to return stretcher plate to home position.
10. When it arrives at the starting position, flush against the extrusion pan, turn the machine off.
11. Repeat until measurements complete.

10.7 Appendix G: Motor Parameter Validation

Table G - 1: Stretch percentage validation – each stretch percentage was tested five times. A trial passed if it was within 10% of the set stretch percentage.

Trial	Set stretch %	Encoder setting	Initial location (mm)	Final location (mm)	Actual stretch %	Deviation from set	Pass (Y/N)
1	0	0	77	77	0	0	Yes
2	0	0	77	77	0	0	Yes
3	0	0	77	77	0	0	Yes
4	0	0	77	77	0	0	Yes
5	0	0	77	77	0	0	Yes
1	50	2500	77	115	49.35	0.64	Yes
2	50	2500	77	113	46.75	3.24	Yes
3	50	2500	77	113	46.75	3.24	Yes
4	50	2500	77	114	48.05	1.94	Yes
5	50	2500	77	113	46.75	3.24	Yes
1	100	5500	77	153	98.70	1.24	Yes
2	100	5500	77	154	100	0	Yes
3	100	5500	77	153	98.70	1.29	Yes
4	100	5500	77	154	100	0	Yes
5	100	5500	77	154	100	0	Yes
1	150	8000	77	195	153.24	-3.24	Yes
2	150	8000	77	193	150.64	-0.64	Yes
3	150	8000	77	194	151.94	-1.94	Yes
4	150	8000	77	193	150.64	-0.64	Yes
5	150	8000	77	193	150.64	-0.64	Yes
1	200	11000	77	236	206.49	-6.49	Yes
2	200	11000	77	234	203.89	-3.89	Yes
3	200	11000	77	236	206.49	-6.49	Yes
4	200	11000	77	235	205.19	-5.19	Yes
5	200	11000	77	234	203.89	-3.89	Yes
1	250	14000	77	273	254.54	-4.54	Yes
2	250	14000	77	275	257.14	-7.14	Yes
3	250	14000	77	269	249.35	0.64	Yes
4	250	14000	77	270	250.64	-0.64	Yes
5	250	14000	77	272	253.24	-3.24	Yes
1	300	17000	77	300	289.61	10.38	No
2	300	17000	77	303	293.50	6.49	Yes
3	300	17000	77	298	287.01	12.98	No
4	300	17000	77	304	294.80	5.19	Yes
5	300	17000	77	304	294.80	5.19	Yes

Table G - 2: Stretch speed validation testing – below is the full chart of stretch speed validation from motor power setting 109 through 82 for any setting we believed would be used for the machine. In yellow is our currently accepted stretch speed used for the threads we mechanically tested.

Setting on hardware interface (1-9)	Trial #	Motor power setting (0-127)	Initial location (mm)	Final location (mm)	Total run time (sec)	Actual stretch speed (mm/sec)	Average stretch speed (mm/sec)	% Error
1	1	109	84	160	367	2.07	2.07	-0.01
	2	109	84	160	365	2.08		
	1	108	84	135	143	3.56	3.57	-0.01
	2	108	84	160	212	3.58		
2	1	107	84	160	159	4.780	4.78	0.00
	2	107	84	160	159	4.78		
	1	106	84	160	141	5.39	5.39	0.00
	2	106	84	160	141	5.39		
3	1	103	85	161	109	6.97	6.91	0.12
	2	103	85	161	111	6.84		
4	1	100	84	160	90	8.44	8.39	0.09
	2	100	84	160	91	8.35		
5	1	97	85	161	73.4	10.34	10.31	0.08
	2	97	85	161	74	10.27		
6	1	94	84	160	64	11.87	11.78	0.18
	2	94	85	161	65	11.69		
7	1	91	85	161	58	13.10	13.28	-0.23
	2	91	84	160	57	13.33		
8	1	88	84	160	54	14.07	13.96	0.25
	2	88	84	160	55	13.81		
9	1	85	85	161	49	15.51	15.35	0.31
	2	85	84	160	50	15.20		
	1	82	84	160	48	15.83	15.83	0.00
	2	82	85	161	48	15.83		

10.8 Appendix H: Matlab Code for Mechanical Testing

```
%  FILE:         FibrinMechanics03.m
%  SCRIPT:       FibrinMechanics03
%  DESCRIPTION: This script opens folders with extension .is_tens_RawData
%               and files with extension .csv, importing data, plotting,
%               and applying a moving average filter, outputing results
%  UPDATE:       081412: Moved initialize output, apply moving average,
%               and maximum tensile stress to immediately follow data input
%               MTM calculations are now based on the moving average (Q)
%               012313: Modified parameters to match new data taken with a
%               new method
%
%  AUTHOR:       Jonathan Grasman & Michael Chrin
%
clear all;
clc;

%Create a window
set(gcf,'Units','normalized','MenuBar','none','NumberTitle','off','Position',
[.2 .2 .5 .5],'Name','Data Analyzer','Color',[.8 .8 .8]);
axis('off')

%Create waveform axes.
axes_list = axes('Position',[.1 .1 .8 .8]);

%Call data file
%Note that each file contains the following in the first five columns:
%Time(sec),Extension(mm),Load(N),Tensile strain(mm/mm),Tensile stress(MPa)

%Default File Location
FileName = uigetdir('C:\Users\Jon Grasman\Documents\Research\');

%User Selected Folder
DirCheck = dir(fullfile(FileName,'\*.is_tens_RawData'));
%If at in a sample directory, allow pass through FldrCnt once
if isempty(DirCheck)
    SampleCheck = dir(fullfile(FileName,'\*.csv'));
    if isempty(SampleCheck)
        msgbox('No Samples in this folder location','User Error','warn');
    else
        DirCheck = [];
        DirCheck.name = 'S';
    end
%Else at an outer directory so make a results folder
else
    mkdir(FileName,'Results')
end
%Iterate for each folder
for FldrCnt = 1:length(DirCheck)
    if DirCheck(FldrCnt).name == 'S';
        SampleList = dir(fullfile(FileName,'\*.csv'));
    else
        CurrentFolder = (DirCheck(FldrCnt).name);
        Folder = strcat(FileName,'\',CurrentFolder);
```

```
        SampleList = dir(fullfile(FileName,'\',CurrentFolder,'\*.csv'));
    end
    %Iterate for each sample
    SampleNum = length(SampleList);
    SampleLoop = 0;
    while(SampleLoop < SampleNum)
        SampleLoop = SampleLoop + 1;
        DataName = strcat('Specimen_RawData_',num2str(SampleLoop),'.csv');
        %Read Data
        if DirCheck(FldrCnt).name == 'S';
            DataLoc = strcat(FileName,'\',DataName);
        else
            DataLoc = strcat(Folder,'\',DataName);
        end
        X = csvread(DataLoc, 4, 0);
        %X(:,6:7)=[];
        % Initialize Output
        Q =[];
        % Apply Moving Average
            for m = 1:6
                Q(:,m) = filter(ones(1,10)/10,1,X(:,m));
            end
        %Maximum tensile stress
        [C,I]=max(Q(:,6));

        axes(axes_list);

        plot(Q(:,5),Q(:,6))
        hold on
        for AddL = 1:2
            [slopeX(:,AddL),slopeY(:,AddL)]=ginput(2);
            %Take the output of ginput, and find the closest data point
            for pts = 1:2
                [error,ind]=min(abs(Q(:,5)-slopeX(pts,AddL)));
                slopeX(pts,AddL)=Q(ind,5);
                slopeY(pts,AddL)=Q(ind,6);
            end
            %Plot the result on top of the data
            plot(slopeX(:,AddL),slopeY(:,AddL),'r')
            slope(AddL)=(slopeY(2,AddL)-slopeY(1,AddL))/(slopeX(2,AddL)-
slopeX(1,AddL));
        end
        pause(1)
        hold off
        %Maximum Tangent Modulus Calculation
        dim=size(X);
        window=round(dim(1,1)*0.2);
        TM=zeros(dim(1,1),1);
        w=1;
        p=1;
        while w<dim(1,1)-(window)
            x=Q(w:w+window,5);  % determines the strain
            y=Q(w:w+window,6);   %determines the stress
            LREG=polyfit(x,y,1);
            TM(p,1)=LREG(1,1);
            w=w+1;
```

```
            p=p+1;
        end
        Regrs=max(TM);

        %Stores Time, Extension, Load & Tensile strain at max Tensile stress
        Soln(SampleLoop,1:6)=Q(I,1:6);
        %Soln(SampleLoop,6:7)=[E;ECurve];
        Soln(SampleLoop,7)=[Regrs];
        Soln(SampleLoop,8:9)=slope;

    end
%Soln(:,:) - For Debugging
%FILE Output
    if DirCheck(FldrCnt).name == 'S';
        SaveFile = strcat(FileName,'.csv');
    else
        FileDelim = strfind(CurrentFolder,'.is_tens_RawData');
        SaveFile = CurrentFolder(1:FileDelim);
        SaveFile = strcat(FileName,'\Results\',SaveFile,'.xls');
    end
    xlswrite(SaveFile,Soln);
end
```

10.9 Appendix I: Thread Validation Results

Table I - 1: Thread validation results

Parameter	Change	Total number of threads	Failed before stretching	Percent failure (%)
Change in PE tubing length	54 cm	26	22	85
	74 cm	72	30	42
Extrusion pump rate	0.11	17	16	94
	0.225	13	9	69
Extrusion	Automated	86	76	88
	Manual	146	35	24
Drag/Drop	0 mm	8	8	100
	10 mm	8	5	63
	Drag manual	146	35	24
Teflon pan interface	2 mm	29	28	97
	0.1 mm	22	17	77
Removal method (failure determined during removal)	Frame	18	17	94
	Manual	16	14	88
	Transfer	146	40	27

10.10 Appendix J: User Manual

1. Introduction

Fibrin microthreads, a scaffold material that is morphologically similar to skeletal muscle, ligaments, and tendons, are comprised of natural materials and represent a promising artificial scaffold. Fibrin microthreads not only mimic the fiber-like structure of native tissue, but also degrade naturally during the wound healing cascade. Fibrin microthreads were originally created through an extrusion process; post-production modifications including stretching, sterilization, and crosslinking have been incorporated into the fibrin microthread production process (Cornwell, 2007). Fibrin microthreads have been used for the restoration of skeletal muscle injuries in mouse models (Page, 2011), the delivery of human mesenchymal stem cells (hMSC) on culture plates, and other applications. Although fibrin microthreads are arising in many applications, the way in which the threads are produced is limiting their large-scale use in research laboratories.

Fibrin microthreads are currently fabricated in batches through a hand-drawn extrusion process with manual post-production modifications including stretching, crosslinking, and sterilization. Manual extrusion and manipulation of the threads induces high batch-to-batch variability in terms of thread mechanical and structural properties. In order to develop more uniform threads with consistent properties, a need exists to automate the current process and eliminate manual handling of the threads.

Although previous attempts have been made to automate the production of microthreads, development of a system that encompasses automated extrusion and stretching of the threads does not exist. Based on unpublished data, stretching fibrin microthreads is a critical step during production because it aligns the polymer molecules present in the amorphous threads. The alignment of the polymer molecules within the threads leads to increases the moduli, strengths, and maximum strains of threads.

The Automated Fibrin Microthread Processing System detailed in this user manual is a system to encompass both automated extrusion and stretching of fibrin microthreads. Through its use, fibrin microthreads can be produced quickly and precisely in multiple laboratories, within several modifiable parameters. The following document details the use and maintenance for this system.

2. Scope

The scope of this project involved the creation of an automated post-production modification system for the threads, as well as the integration of this system with the previous extrusion system. This manual is to be used for the operation, maintenance, and modification of the system for laboratory use.

3. Reference Documents

- Fibrin Microthread Extrusion Protocol
- Report, Worcester Polytechnic Institute MQP – GXP 1201

4. Terms and Definitions

The following are terms and definitions that will be used throughout the manual to describe key components of the system.

Extrusion head

This is the automated extrusion system used to produce fibrin microthreads. It was originally created by MQP team GXP 1015 in 2010 for collagen threads and has been modified for this system.

Angled Bath

This is the term used to describe the hydration bath that is filled with HEPES to hydrate the threads. It has an angled corner to allow for fluid to drain more efficiently through the valve at the bottom. This bath houses the majority of the motorized stretcher system, and it is important to note that the threaded rod and the three bottom stretcher plates are attached to the bottom of the bath and should not be removed without further instruction.

Removable Frame

The removable frame is a portion of the motorized stretcher that lifts out of the bath to allow the threads to dry without human handling. It is composed of the top three stretcher plates, two guide bars, and two squeegee clamps.

Squeegee Clamps

These are the clamps used to secure the fibrin microthreads to the frame. There are a total of 2 per frame, and they consist of a PDMS squeegee, aluminum frame, and 4 alligator clips to secure the clamp to the frame.

PDMS Mold

This is the ABS plastic mold that is used to create the PDMS squeegee for the squeegee clamp system. The protocol to make the PDMS squeegee can be found in the GXP 1201 PDMS Mold Protocol.

Hardware System

The hardware system is the front panel user interface for the system located on the right side of the extruder head. It is composed of 2 dials, 6 LED light displays, and 2 buttons. The dials are used to set the modifiable parameters for the system and the LED lights indicate what step of the process is currently taking place during use. The EMERGENCY STOP button is located on the bottom right corner of the hardware system and the CONTINUE button is on the top right conor. The entire hardware system can be removed from the extrusion head machine for any maintenance or modifications.

VEX Controller

This is the control for the system. It is located underneath the extruder head and is where all of the wires plug in. The locations for the wires, along with its maintenance, is located in the set up and troubleshooting sections of this protocol

Stretch Percentage

This is the terminology used for how far the threads are stretched with the motorized stretcher. 0% is when the threads are left unstretched and the other percentages are the stretched length based on the initial length of the threads. For example, 100% stretch

means that the 6cm threads were stretched an additional 6cm, giving a final length of 12cm threads.

Strain Rate

This is the terminology used for the rate, in mm/s, that the threads are stretched at. The numbers 1-9 on the hardware system correlate to nine set strain rates. The specific rates are listed in the instructions portion of this manual as well as the appendix.

Extrusion Rate

This is the terminology used for the rate at which the extruder head moves lengthwise to extrude the threads. The extruder pump rate can be found in the setup section of this report.

Potentiometer

A potentiometer is a device that measures angular distance. They are used in this system as dials for the hardware system to control strain rate and stretch percentage. The dials are programed to allow for user error when selecting different settings, set the dial as close to the intended line as possible.

5. Materials

- Automated Fibrin Microthread Processing System
  - 7.2V Robot Battery NiMH 3000mAh, charged (VEX P/N: 276-1491)
  - Extrusion Head
  - Stretching System
  - Hardware Interface
  - Squeegee Clamps
  - Removable Frame
  - 3/32 in, 5/64 in allen wrenches
- Fibrinogen aliquots (70 mg/mL)
- Thrombin aliquots (40U/mL)
- HEPES buffered saline
- Calcium Chloride (40mM)
- 25 Gauge blunt end needle
- 0.86 mm I.D. polyethylene tubing (Intramedic PE90 427421)
- 2 - 1mL syringes
- Blending connector (SA-3670; Micromedics, MN)

6. Setup

Fibrinogen and Thrombin Setup

HEPES buffered saline (HBS) preparation

1. Definition: HBS contains 20 mM HEPES and 0.9% (w/v) NaCl
2. Add the following reagents to 200 mL:
   a. 2.25g of NaCl
   b. 1.1915g of HEPES
3. pH solution to 7.4 using NaOH/HCl.
4. Bring final volume to 250 mL.
5. Store at room temperature.

Fibrinogen aliquots (70 mg/mL)

1. Measure 14.3 mL of HBS into a 50 mL conical tube.
2. Weigh 1.00 gram of fibrinogen and pour into conical tube.
3. Put conical tube on rocker plate, adjusting the position every 30-40 minutes until fibrinogen goes into solution.
    a. **NEVER SHAKE/VORTEX FIBRINOGEN SOLUTION!!!! THIS WILL CAUSE FIBRINOGEN TO FALL OUT OF SOLUTION AND BIND TO ITSELF!!!!**
4. Incubate conical tube at 37 C overnight to ensure fibrinogen is completely dissolved.
5. The next morning, measure 1 mL aliquots in eppendorfs and store at -20 °C.

Thrombin aliquots (40 U/mL)

1. Add 25 mL HBS to bottle of 1KU thrombin, mix well.
2. Aliquot 200 µL into eppendorfs and store at -20 °C (Final concentration: 8U / 200 µL).

Calcium chloride preparation (40 mM)

1. Add 0.1776 g of $CaCl_2$ to 40 mL of diH2O.
2. Store at 4 °C.

HEPES buffer bath stock solution

1. Definition: Stock solution will be prepared at 10X of 10 mM HEPES buffer (100mM).
2. Add 23.83g of HEPES to 900 mL of diH2O.
3. pH to 7.4 using NaOH/HCl
    a. **WILL REQUIRE LARGE AMOUNTS OF ACID/BASE- USE HIGHER CONCENTRATIONS CAREFULLY.**
4. Bring final volume to 1000 mL.
5. Store at room temperature.

Extrusion Setup

1. Place blunt end needle (25 gauge, BD) into 0.86 mm I.D. polyethylene tubing.
    a. **CAN REUSE THESE MATERIALS IF PREVIOUS USER WASHED**
2. Leur lock blunt end needle/tubing onto the front end of blending connector.
3. Turn syringe pump on.
    a. Press SELECT.
    b. Toggle to Table, press SELECT.
    c. Toggle to Bec. Dic. Plastic, press SELECT.
    d. Toggle to 1 cc 4.70 mm, press SELECT.
    e. Enter volume: 1.0 mL, press ENTER.
    f. Enter extrusion rate: 0.225 mL/min, press ENTER.
4. Prepare 2700 mL of 1X HEPES buffer solution (270 mL of stock solution and 2430 mL diH2O), pH to 7.4
5. Fill bath with 2700 mL HEPES buffer solution
6. Add 850 µL of calcium chloride solution to150 aliquot µL of thrombin, mix well.
7. “Prime” 2-1 mL syringes by moving the plunger several times.
8. Collect all of the thrombin and fibrinogen solutions into 1 mL syringes.

a. **COLLECT THE FIBRINOGEN SOLUTION SLOWLY AND CAREFULLY, FAILURE TO DO SO MAY RESULT IN INSOLUBLE FIBRINOGEN FORMATION!!**

9. Invert syringe, remove all bubbles, and ensure that both syringes have equal volumes.
10. Place each 1 mL syringe of fibrinogen and thrombin solutions into the back end of the blending applicator.
    a. **ALWAYS PUT FIBRINOGEN SOLUTION IN THE BLENDING APPLICATOR OPENING WITH THE CIRCLE ON IT.**
11. Secure syringe/blending applicator construct into syringe pump.

Automated Microthread Processing System Setup

1. Ensure that the battery is completely charged. If the red light on the charger is blinking, the battery has enough power to run the system at least once.
2. Place the top portion of the stretching frame into the bath, ensuring the center plate lines up with the bottom center plate and the Teflon pan, Figure 71.

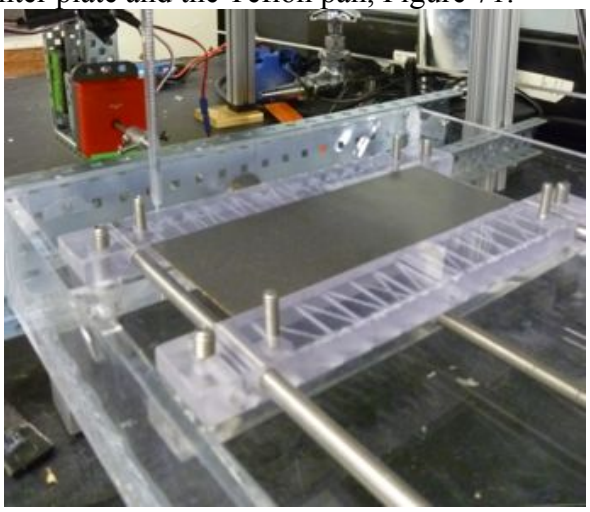

Figure 71: Frame in bath – the removable frame is placed in the bath and line up with the Teflon pan

3. Ensure the extruder head and hardware systems are properly plugged into the PIC microcontroller. Figure 72 below shows all of the ports for the VEX controller and where to plug in each wire.

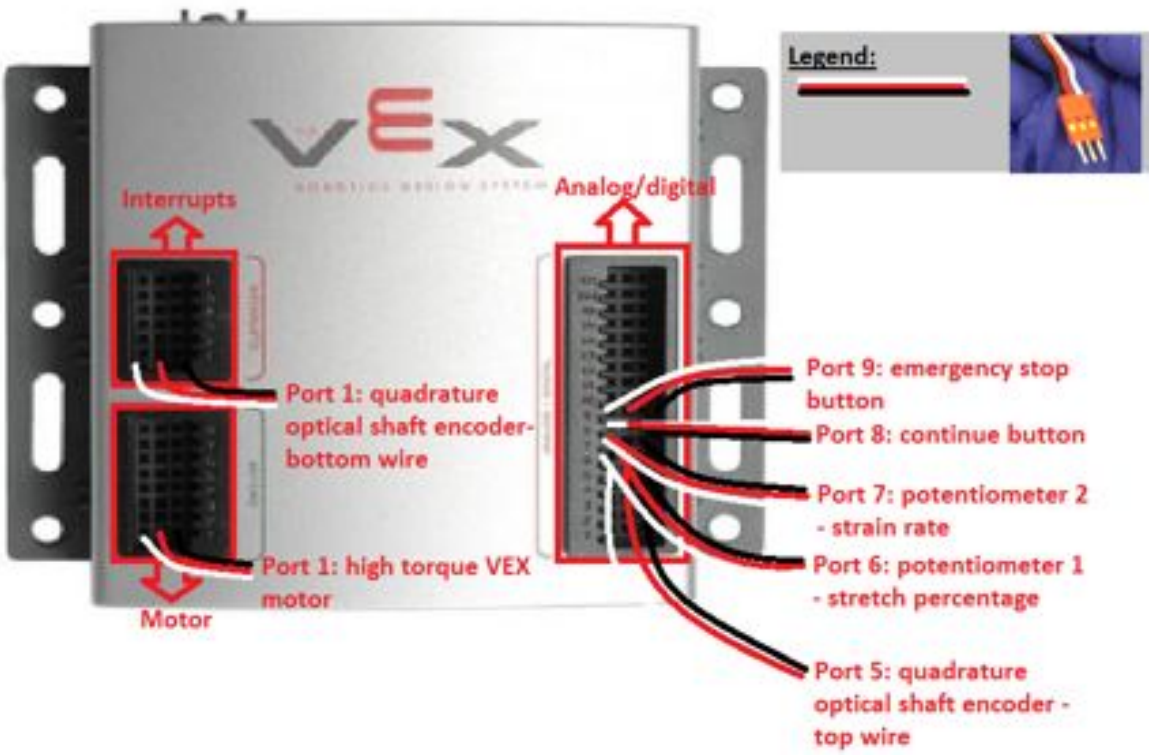

Figure 72: VEX controller ports - illustrates how to plug all of the wires for the stretching system into the correct ports

* Ensure when plugging in a triple wire that the white wire is closest to the groove on the PIC microcontroller, and when plugging in a double wire, do not use the groove but instead the black wire (ground) should be all the way to the right, away from the groove (Figure 73).

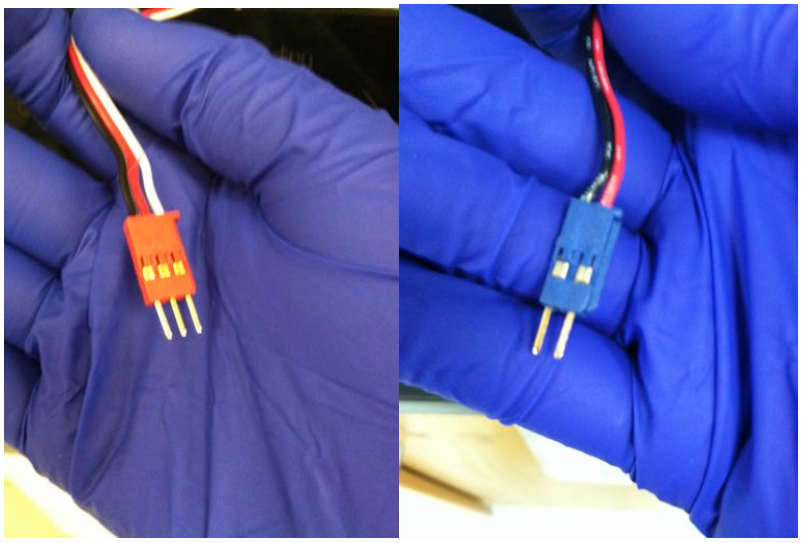

Figure 73: VEX port wires - (Left) diagram of the 3-prong plug for triple wire (Right) diagram of the 2-prong plug for the double wire

4. If necessary, flip the extruder head on its side, and plug in wires. Then, carefully flip the extruder head back over and use the Velcro to secure the hardware system to the sides of

the extruder head. The hardware system should be angled out and touch the lab bench (Figure 74).

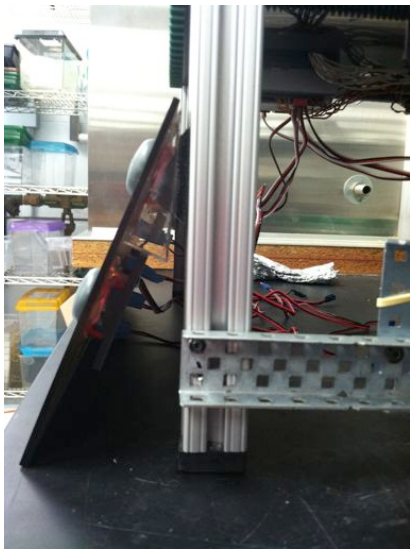

Figure 74: Side view of front panel - displays the front panel attached to the extruder head at the proper angle with the bottom side rested on the lab bench

5. Use the bath brace on the front of the extruder head to guide the bath into its location. The bath should press up against the brace and the threaded rod should fit securely into the coupling that connects it to the motor (Figure 75).

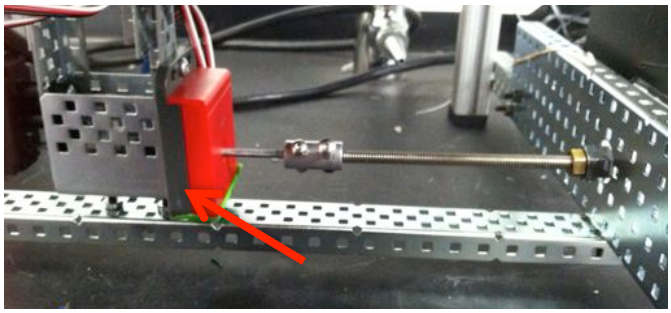

Figure 75: Motor coupling - shows the set up for the motor coupling between the threaded rod and the shaft of the stretcher motor

6. Use a 3/32 in Allen wrench to tighten the coupling so that the coupling and the threaded rod move as one unit. Test this by using the screws in the coupling to turn the rod, and watch for the turning threads.

   ***Note – make sure you tighten both setscrews evenly in order to ensure that the threaded rod spins evenly.**

7. Slowly, turn the coupling so that the dynamic stretcher plate touches up against the side of the pan. Ensure that there are no gaps or lips between the pan and the stretching plate (see troubleshooting if this is a problem).

8. Using the handle on the extruder head (Figure 76), move the pipet tip laterally until it is in the position shown in Figure 77. If the extruder head is not positioned lengthwise up against the back end of the pan, use your hand to push the extruder head backwards.

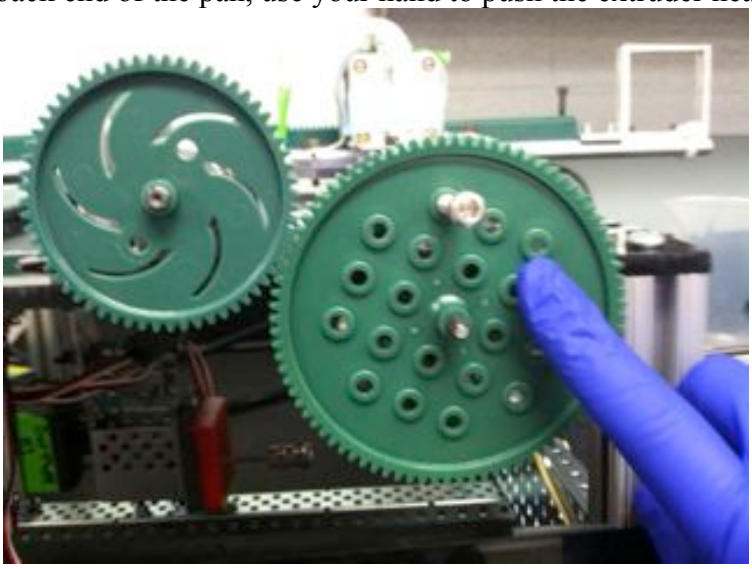

Figure 76: Handle on the extruder head - this illustrates the handle extruder head used to move the extruder head laterally

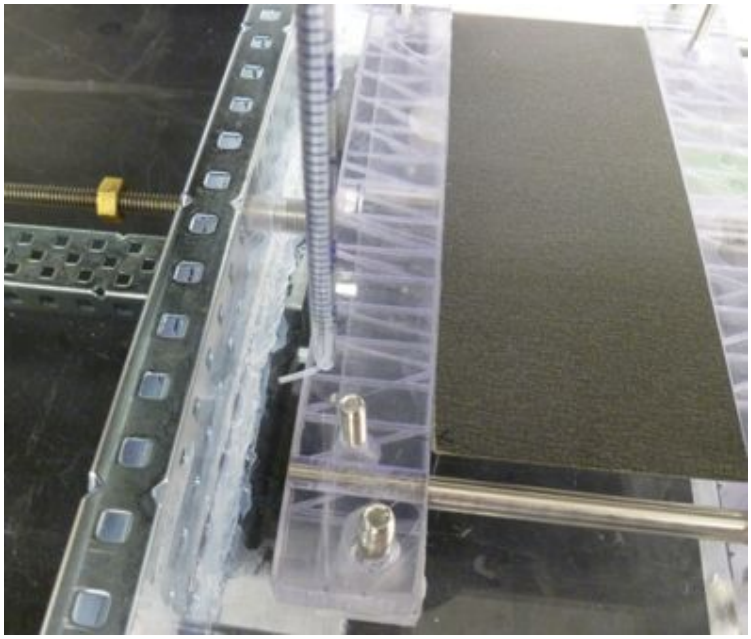

Figure 77: Starting position of the pipet – the pipet is moved to the rear of the stretching frame, closest to the control panel

9. Feed the polyethylene tubing through the glass pipet until the tape marker is at the top of the glass pipet, the tubing should stick out 10 mm. Figure 78 shows the glass pipet with the polyethylene tubing protruding from the glass pipet.

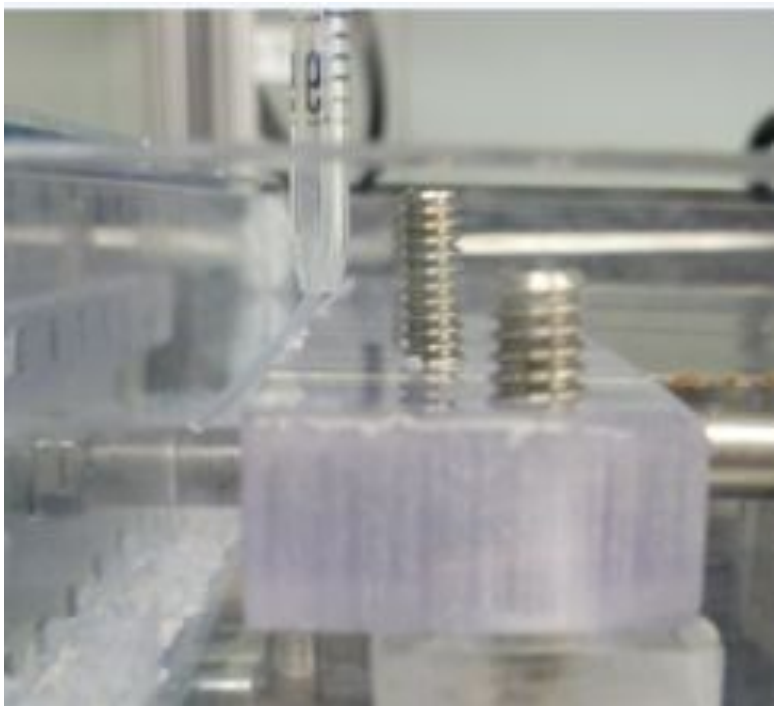

Figure 78: Position of polyethylene tubing - the polyethylene tubing sticking out of glass pipet should be 10 mm

7. Instructions

1. Set the desired stretch percentage and stretch speed with the dials on the hardware interface.
2. Hit run on the extrusion pump and watch the solution pump through the tubing. When the clot forms and reaches the end of the tubing (at the pan), switch on the system using the VEX controller (Figure 6).
3. If threads are hand-drawn, then follow manual extrusion protocol and transfer the threads to the stretching frame. To start the program from the stretching step, hold the continue button down for 5 seconds after turning on the machine.

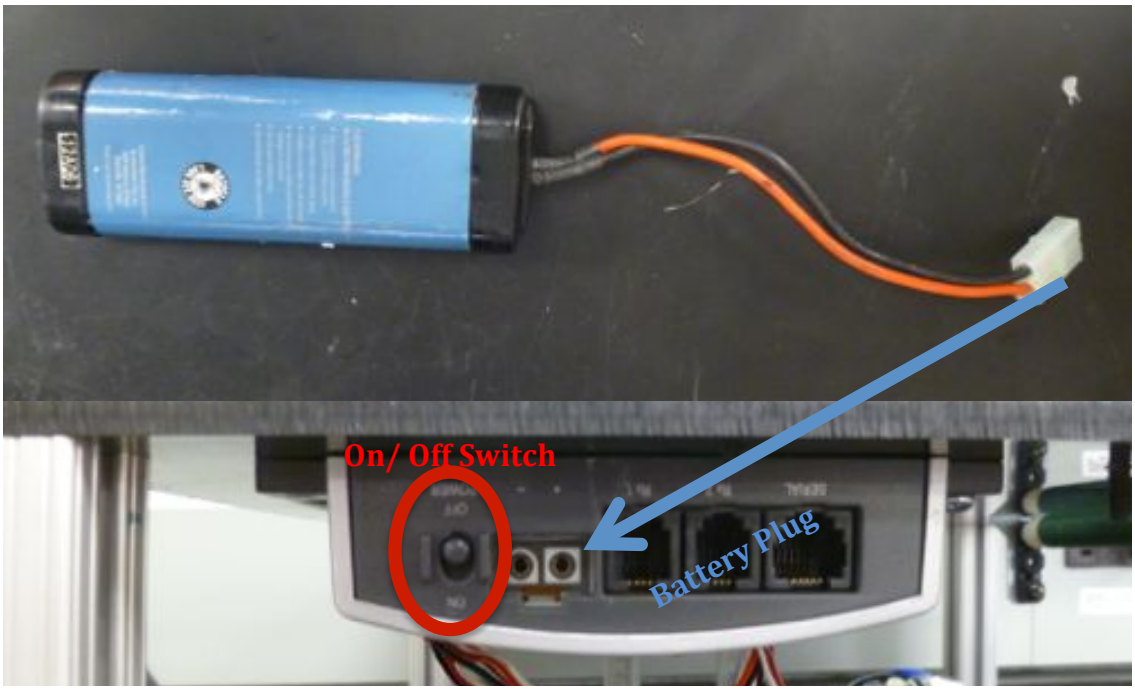

Figure 79: Battery Insertion - above is the battery and where to plug it into the microcontroller, as well as the on switch

4. The stretching LED will turn on and the extruder head will start to run. Watch the extruder head run, and guide the rails with your hand to ensure that the motors do not stall.

   *If it stalls lengthwise, put your hand on the end of the extruder head arm to coax it forward (Figure 7). If it stalls laterally, use the handle on the gears to continue its progression (Figure 5).

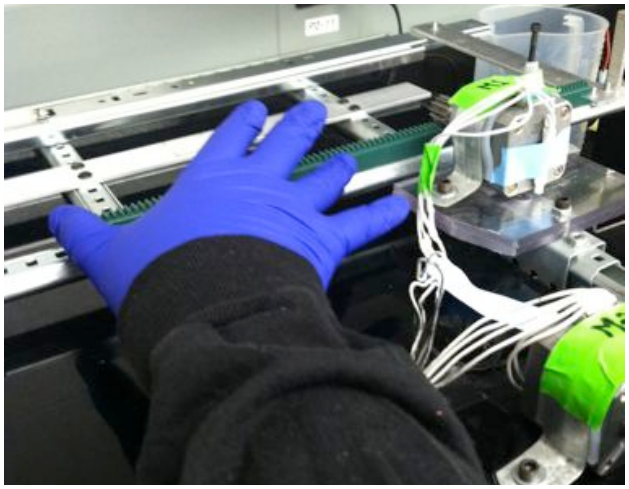

Figure 80: Lengthwise adjustment - illustrates how to push the extrusion head if it stalls during thread extrusion

5. When the extruder head stops and the polymerizing LED turns on, unplug the battery, shut off the system, and wait for 10 minute polymerization cycle to complete.

6. After about 11 minutes, use two forceps to remove threads (Use each forcep to clamp on each end of the thread and pull in opposite directions). This motion will remove the thread from the pan (Figure 8). Place each end of the thread on the rough ends of the acrylic plates.

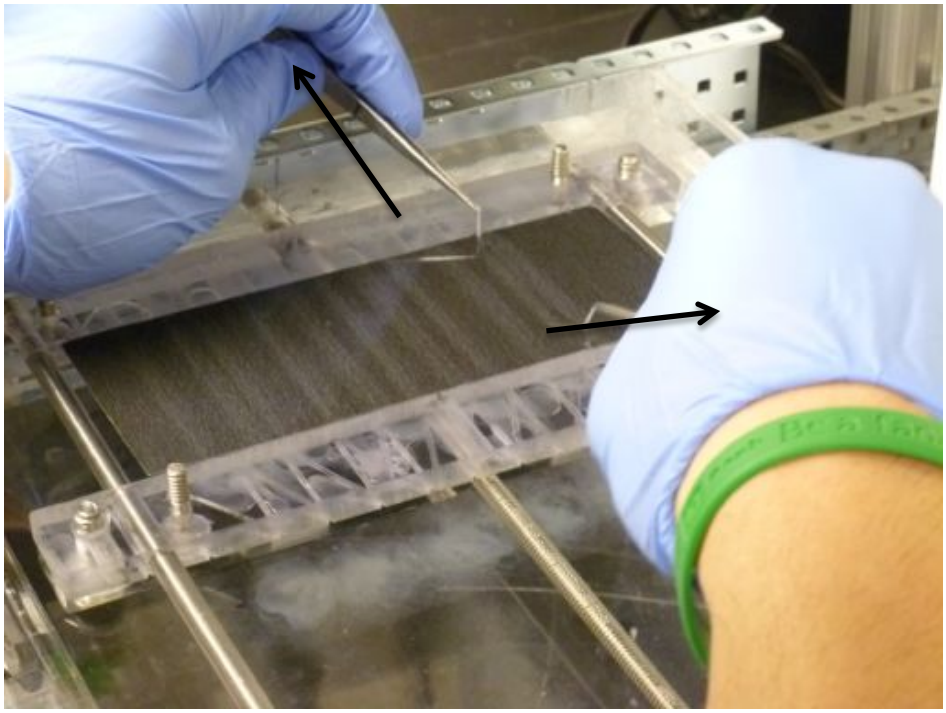

Figure 81: Lifting threads from pan - Ensure you are lifting outwards at an angle as to not shear the threads

7. Once all threads are free from the pan, place the aluminum squeegee clamps on each side. When placing clamps, ensure they are even and firmly clamped, then place alligator clips on top of the screws to secure them (Figure 9).

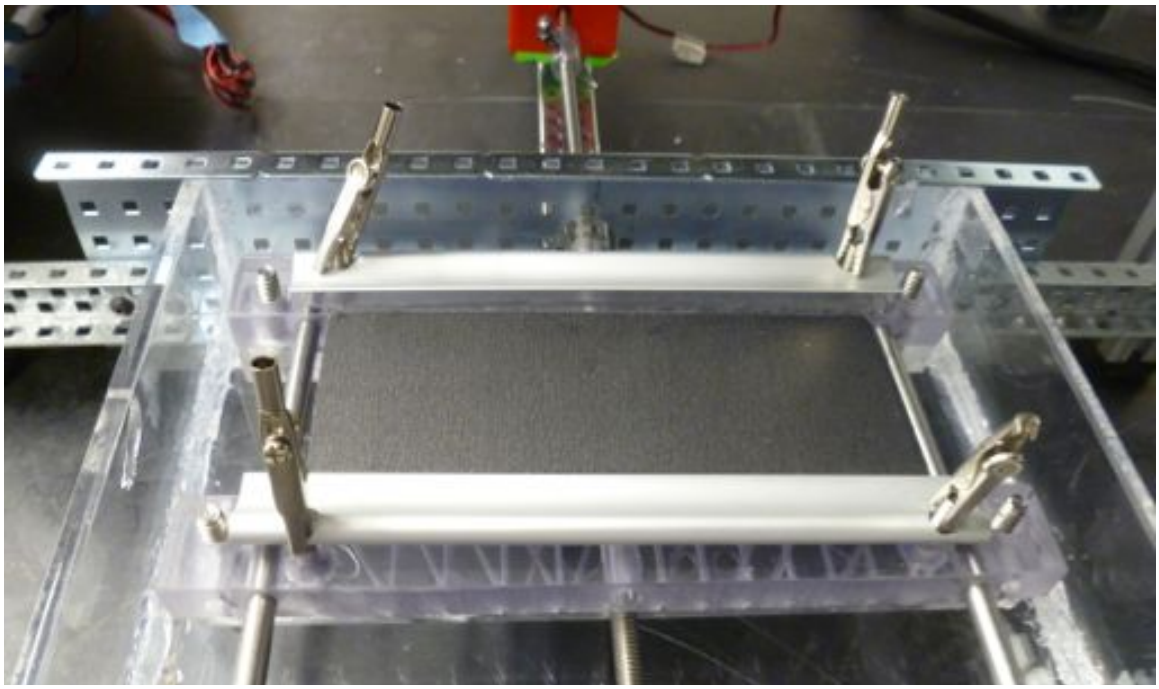

Figure 82: Alligator clips on clamps – clamps secure threads between the PDMS and stretching frame

8. When clamps are in place and threads are secure, replug in a battery into the Vex microcontroller. Press and hold "Continue" and wait for the robot to switch to the stretching motor (this could take up to 20 seconds). Wait for stretching to complete.
9. Once stretching is complete the "remove threads" LED will turn on, place clamps on each side of the middle acrylic plate to eliminate movement.
10. Pick up the frame at an angle by first removing the side without threads slowly, then picking up the side with threads. Be sure to do this very carefully as the threads are delicate at this stage and may be statically attracted to the end plate (Figure 10).

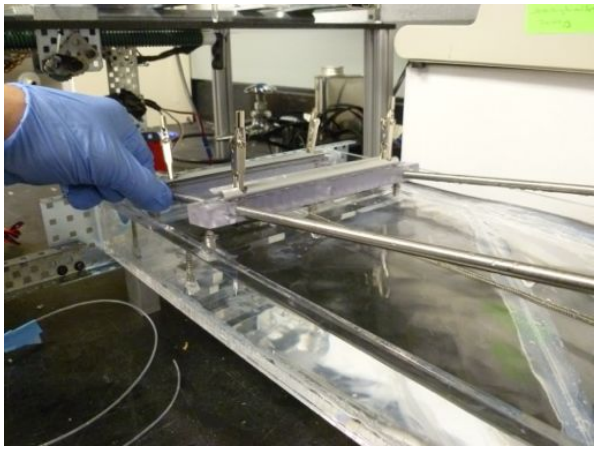

Figure 83 Frame Removal - peeling the small end plate up first allows the user to reduce the force of the surface tension on the threads

11. Once the frame is removed, press the "Continue" button once more. This will turn on the motor and return the bottom stretcher plates back to their original starting position.
12. When the "Done" LED is lit, the system has completed it cycle and can be turned off.
13. If desired, unlock the valve to allow the bath to drain (Figure 11).

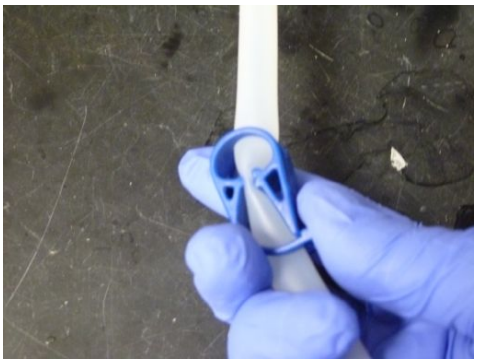

Figure 84: Valve for drainage – open valve to allow HEPES to flow out of the angled bath

14. Clean up your lab bench and materials.

8. Troubleshooting

The following is a list of common problems found when running the machine, and quick solutions to the problems. Problems with the procedure itself, or the way the machine is programmed should be fixed in the EasyC Pro program.

Frame Instability

Frame instability is caused by the fact that the bottom stretcher plates do not lay flat on the bottom of the angled bath. Instead, they float a couple of millimeters off the bottom of the pan and supported by screws. To adjust the stability of the pan, the post screws on the ends of the stretcher plates can be screwed in or out to adjust the height, Figure 85.

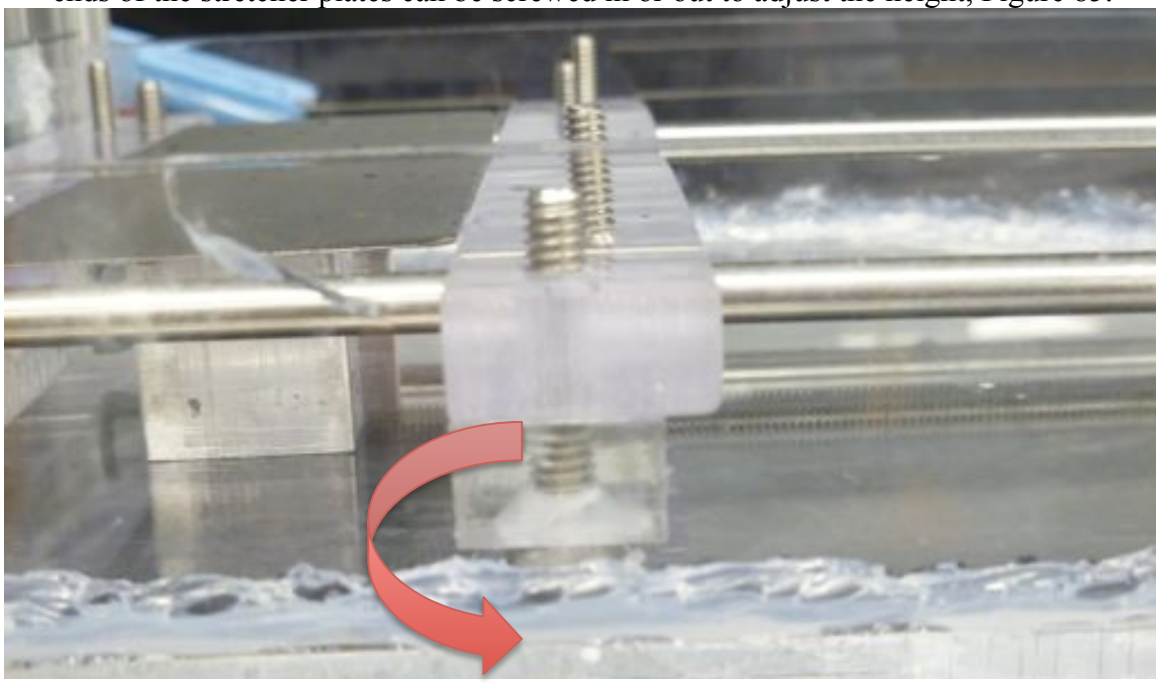

Figure 85 Stability Screws - Turn screw to ensure proper height and leverage from bottom of bath

Extrusion Head

The extrusion head has a tendency to stick because the motors do not have enough torque to move the gears across the track at the speed required to produce viable fibrin

microthreads easily. If this occurs, the user must push the track by hand and follow it to ensure it does not stall because if it stalls it will form inconsistent threads.

Programming Code

The code is included in Appendix E of the MQP final report. If there are issues regarding how the program is set, and how everything runs, please refer to the commentary in the code for which value to change, and how to change it.

Hardware System

Sometimes a button or a switch may not work on the hardware system. If this is the case follow the following steps to ensure they are correctly set up:

1. Check the wiring according to Figure 72 and ensure everything is plugged into the correct port.
2. Ensure that all the parts are securely glued into the hardware system, and that nothing has become undone because settings are particular to the angle.
3. If the top 2 are fine, plug the PIC microcontroller into a computer using the long orange Serial port to USB cord. Plug the battery into the machine, and turn it on. Pull up the EasyC Pro program. Find the file name, FinalGXP1201Code under the Projects folder, then click "Build and Download" and click "Build and Download" again. This will trigger a series of prompt boxes which will ask you to ensure the machine is turned on, click "yes", then it will ask you to begin download, click "begin". This will allow the program to download. A textbox will come up with some values. Ensure that the values of the Continue button and emergency stop button match those programmed, and ensure that the initial encoder count is 0. If this is true, download the program again and try to reset all parameters.

Bath Leaking

The bath may begin leaking over time. Use ONLY pure silicone, aquarium safe sealant and seal the bath from the inside around the edges. Across the middle angled part of the bath, seal it on the outside to ensure the friction from the sealant will not stop the stretching frame from sticking.

9. References

Cornwell, K. (2007). Collagen and Fibrin Biopolymer Microthreads for Bioengineered Ligament Regeneration. PhD dissertation.

Page, R. (2011). Restoration of Skeletal Muscle Defects with Adult Human Cells Delivered on Fibrin Microthreads. *Tissue Engineering Part A, 17*(21-22), 2629-640.