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Design of a Multifunctional Semi-Automated Production System for Biopolymer Microthreads

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Table of Contents

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1.0 Abstract

Biopolymer microthreads are a type of scaffold used in tissue engineering due to their high mechanical strength, structural similarity to native tissue, ability to encourage cell alignment, and capacity to form hierarchically-ordered scaffolds with complex architectures. The current production method for biopolymer microthreads is inefficient, time consuming, and produces threads with inconsistent properties. The goal of this project was to design and construct a reliable and precise automated system that produces biopolymer microthreads for uniaxial load bearing regenerative therapies and allows for post-production modifications. To accomplish this, the design team used the design process to develop a final device consisting of a motor controlled bi-directional extrusion system, heated outer bath, angled inner extrusion bath, removable anchor system, hopper fill system, and aspirator drain system. System testing with type I collagen demonstrated the device's ability to extrude straight, anchored threads at three different speeds (0.496, 0.617, and 0.816 cm/s) and at a constant temperature. Qualitative microscopic observations confirmed straighter and more uniform collagen microthreads than the current hand-drawn method. Diameter measurements demonstrated the device's ability to create microthreads with average diameters ranging from 60.3 – 78.4 µm and diameter variations that were statistically smaller than hand-drawn threads. Additionally, machine extruded threads were statistically stronger than hand-drawn threads with UTSs ranging from 1.22 ± 0.36 to 1.93 ± 097 MPa. The hand drawn threads had an average UTS of only 0.93 ± 0.37 MPa. These findings suggest that this automated extrusion device could vastly improve the quality of collagen microthread production and save the client time and effort.

Table of Figures

Table of Tables

2.0 Introduction

With over 33 million musculoskeletal injuries occurring in the United States, of which 50% are injuries to soft tissues, tendon and ligament damage is becoming more prevalent, especially among athletes [Calve, 2004]. Each year over 50,000 reconstructive surgeries are performed on torn tendons and over 150,000 reconstructive surgeries are performed on severely torn or ruptured anterior cruciate ligaments (ACL) at a cost of $3-6 billion [Maffulli, 2003, U.S. News and World Report, 2009]. The current treatments include the use of autografts, allografts, and synthetics which require surgical procedures that can take up to 6 months to recover from. While each treatment has advantages, there are also drawbacks.

Autografts can successfully be used to repair a torn ligament or tendon; however, they require the use of a patient's own patellar tendon or hamstring as a replacement for the damaged tissue. This weakens one area of the patient's body to heal another. In the case of anterior cruciate ligament surgery for example, this could mean weakness and buckling of the knee as well as chronic knee pain [Prodromos, 2007, Fauno, 2005, Johnson, 2004]. Allografts use harvested tissue from a cadaver or donor rather than from the patient's own body. While they require only one surgical procedure, as opposed the two necessary for autografts, and eliminate the need for a donor site, they are inherently weaker than autografts and have increased rates of failure and disease transmission [Chang, 2003, Luber, 2008]. Synthetic treatments were originally pioneered in the 1980's and use grafts primarily composed of polyethylene derivatives as replacements for damaged ligaments and tissues. They have a much higher stiffness and elastic modulus than ligaments and tendons, which can lead to stress shielding, and have high rates of fatigue related failure [Ventura, 2009, Davidson, 1995, Rubenstein, 1998].

The main limitation of the current treatments is poor integration of the implant with the surrounding tissue, primarily due to the avascular makeup of ligaments and tendons. Because of the small amounts of blood flow to these areas, there is little ability for self-healing and low potential for

new cell growth in, on, or around the implants. This leads to an implant which is weaker than the original ligament or tendon and can cause the implant to fail, requiring another surgery. As a result of the various limitations associated with current treatments, there is a need for new, innovative techniques for the repair of damaged ligaments and tendons.

One promising solution to this problem is the use of tissue engineering. Primarily composed of three components, cells, regulatory factors, and the extracellular matrix, tissue engineering seeks to overcome the problems associated with current procedures through the use of novel materials, signaling agents, and living components [Harbers, 2006]. In healthy tissue, the extracellular matrix (ECM) helps to deliver regulatory signals and provide structural support for cells. When the ECM damaged, it must be replaced by something that can mimic its mechanical and chemical properties. In tissue engineering, this is accomplished by using biomaterial scaffolds. Scaffolds are used in various morphologies and can support cellular in-growth and proliferation through the use of a 3D architecture designed to encourage cell migration and survival. The most relevant morphology for the repair of ligaments and tendons is the microthread.

Collagen microthreads are used due to their high mechanical strength [Gentleman, 2003], structural similarity to native ligaments and tendons, ability to encourage cell alignment, and capacity to form large, complex networks [Cornwell, 2007a]. Additionally, their physical and chemical properties can be altered through the use of post-production processing techniques such as crosslinking [Figueiró, 2004], bundling [Cavallaro, 1998], twisting [Ferretti, 2003], braiding [Ayranci, 2008], and surface modification [Andrade, 1985]. Tissue engineered scaffolds composed of microthreads offer advantages such as biocompatibility and the ability to interact with the surrounding environment. Cell proliferation and tissue in-growth can be supported through the release of growth factors or stem cells which have been seeded onto the implants. The growth of new cells will allow for faster healing and better recovery at the injury site. By pre-seeding the implants with stem cells, upon implantation specific cell types can

be made to proliferate on the implant, which can lead to better integration with the surrounding environment, and a stronger implant.

The current systems for producing collagen microthreads include manual hand-drawn production, an automated 2D plotting system developed by a previous Major Qualifying Project (MQP) [Bishop, 2005], a patent by Organogenesis [Kemp, 1995b], and a patent by Salo [Salo, 1952]. All devices involve extrusion of a material solution in a series of buffer solutions. The client has developed technology to use both the hand drawn method and the device from the previous MQP; however, his laboratory only uses the hand drawn method because of various limitations with the MQP device. These include the incompatibility of the device with the current water bath system used in the laboratory and the high level of user knowledge required to operate the device. The hand drawn method is subject to human extrusion error and has relatively low production rates as well as high variability in the dimensional and mechanical properties of the produced fibers. Both patented systems involve the use of various baths and buffer solutions, and both are limited in that they have the ability to produce only one continuous thread at a time. If a break occurs, the entire process must be reset.

Because of the problems with the current procedures for the production of microthreads, it is necessary to design a device which can overcome these limitations. This device aimed to minimize human contribution as well as increase precision and mobility of produced threads. Functions of this device included the ability to produce threads with varying physical properties and the ability to easily move the threads through post-production processes. The design team used the engineering design process to generate and evaluate design goals, develop design solutions, and make informed, unbiased decisions.

They were able to successfully design, built, and test a final device with four main components: an anchor system, a bath system, an extrusion system, and a drain and fill system. System testing with type I collagen demonstrated the device's ability to extrude straight, anchored threads at three different

speeds (0.496, 0.617, and 0.816 cm/s) and at a constant temperature. Qualitative microscopic observations confirmed straighter and more uniform collagen microthreads than the current hand-drawn method. Diameter measurements demonstrated the device's ability to create microthreads with average diameters ranging from 60.3 – 78.4 μm and diameter variations that were statistically smaller than hand-drawn threads. Additionally, machine extruded threads were statistically stronger than hand-drawn threads with UTSs ranging from 1.22 ± 0.36 to 1.93 ± 097 MPa. The hand drawn threads had an average UTS of only 0.93 ± 0.37 MPa. These findings suggest that this automated extrusion device could vastly improve the quality of collagen microthread production and save the client time and effort. Future work on this device could include a completely automated drain and fill system so that a laboratory worker would not have to be present during this portion of the production process. In addition, a stretching mechanism could be build that would easily integrate with the anchor system which has removable middle portions to simplify this process. This device could also be scaled up in order to produce more threads at a faster rate. Finally, this device could be used with other implantable biomaterials, in therapeutic biologics, or in other clinical areas such as cardiac and stem cell research.

3.0 Literature Review

3.1 Clinical Motivation

The purpose of this project is to design and develop a multifunctional production system that will facilitate the reproducible production, processing, and scale-up of biopolymer microthreads for regenerative therapies. As was indicated by the client, the primary use for these threads will be for uniaxial load bearing applications such as tissue engineered ligament or tendon. In order to accomplish this goal it is necessary to understand the need for such a project.

3.1.1 The Problem

Each year over 33 million musculoskeletal injuries occur in the United States, of which 50% are injuries to soft tissues such as ligaments or tendons [Calve, 2004]. The most common forms of injury are to ligaments (Figure 1), and occur when the tissue is subjected to rotational or hyperextensive forces which it cannot withstand. These forces can cause the ligament to tear and most often occurs during physical exercise or while playing sports [Mithoefer, 2009]. While a minor tear, which is referred to as a sprain, heals over time and generally does not have long lasting side effects, a complete tear of a ligament is a severe injury which requires reconstructive surgery. Each year over 150,000 reconstructive surgeries are performed on severely torn or ruptured anterior cruciate ligaments (ACL) at a cost of $3-6 billion [Maffulli, 2003, U.S. News and World Report, 2009].

3.1.2 Current Solutions

Current treatments for a severely torn ligament, such as the ACL, include surgical procedures involving the removal of the damaged ligament and the implantation of a graft which is then fixed to the surrounding bone. In this procedure, there are three types of implants that can be used: autografts, allografts, and synthetic grafts.

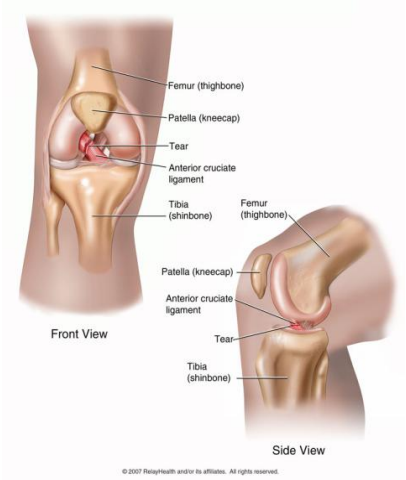


Figure 1: Ruptured ACL: (http://www.med.umich.edu/1libr/sma/ancrulig.jpg)

3.1.2.1 Autografts

Autografts are the most common form of ligament reconstructive surgery and is most often performed for ACL reconstruction. In ligament reconstruction surgery, most autografts are taken either from the patient's patellar tendon or hamstring. The former involves the removal of the middle portion of the patient's patellar tendon through arthroscopic surgery. This is conducted by creating two small incisions below the patellar tendon. Once the middle portion of the patellar tendon is removed, small holes between 8mm-11mm in diameter are drilled into the patient's femur and tibia, and the extracted portion of the patellar tendon is threaded through these holes and attached through the use of bone screws. Typically these bone screws are constructed of Ti-6Al-4V, 316L Stainless Steel, or bio-absorbable materials such as PLA, PGA, or PLC [Caborn, 1997, Lind, 2009].

The second option for autograft ACL reconstruction surgery involves the use of two hamstring tendons, the semimembranosus tendon and the gracilis tendon, and a similar surgical procedure to that of the patellar tendon autograft. Each procedure offers advantages and disadvantages. The physical makeup of the patellar tendon more closely resembles that of the ACL and patellar tendon autografts have been associated with higher initial stiffness and higher initial ultimate loads than hamstring autografts [Park, 2001]. Additionally, patellar tendon autografts allow for bone to bone healing through bone plug incorporation which reduces patient recovery time and creates a strong bond between the bone and the graft [Johnson, 2004]. Disadvantages include increased rates of chronic pain, patellar fracture, and overall weakening of the knee.

Use of the hamstring tendons as autografts has been associated with less post-operative pain, increased knee stability, and lower harvest site morbidity than patellar tendon autografts. Also, hamstring autografts have significantly higher ultimate tensile strength [Prodromos, 2007, Johnson, 2004]. However, the overall stability of the hamstring autograft depends largely on the proper fixation of the implant to the tibia and the femur. While patellar tendons utilize bone to bone healing, hamstring

autografts fix soft tissue to bone which takes longer to heal [Johnson, 2004]. This can be problematic because attachment at the bone sites is more difficult than patellar tendon autografts and, if performed incorrectly, can lead to increased rates of tunnel widening, premature failure, and bone resorption [Fauno, 2005]. Hamstring autografts also tend to be thinner than patellar tendon autografts and can loosen over time. Although not as common as for ligament repair, autografts can be used to repair tendons. Chiou et al. documented the successful repair of a severed patellar tendon through the use of an Achilles tendon autograft [Chiou, 1997].

A fundamental problem with autografts is that they restore function to a damaged area of the body by weakening another area. Another disadvantage is that due to poor vascularization of ligaments and tendons, the autograft has little potential to encourage cell proliferation. New tissue does not easily form around the implant, and there is little integration between the implant and the surrounding environment. This leaves the implant weak and often causes problems for patients. Approximately 25% of athletes are unable to return to the same levels of physical activity after ACL autograft reconstruction surgery [Chang, 2003]. The inability of the implant to integrate with its surroundings can lead to premature weakening and failure of the implant. Failure rates of autografts are relatively low. Kang et al. found a 6% failure of patellar tendon autografts after 3 years, and a study by Salmon et al. showed hamstring autografts had a 10% failure rate after 7 years. [Sun, 2009, Salmon, 2006]. If failure of the implant occurs and there is the need for a second surgery, an autograft from a different location or an allograft must be performed because of weakening of the original donor site.

3.1.2.2 Allografts

Another method for ligament and tendon repair is the use of cadavers as a source of the necessary tissue, called an allograft. Upon extraction from the cadaver, the allograft is freeze-dried in order to minimize the risk of an immune system response in the patient's body, remove any living cells

on the implant, and prepare the allograft for storage [Prokopis, 1999]. The procedure for implantation of an allograft is similar to that for an autograft.

Allografts for ligament and tendon repair are viable options for reconstruction surgery which have been in use for over 35 years. They offer both advantages and disadvantages compared to autografts. Only one surgical procedure must be performed instead of two, as is necessary in autograft reconstruction surgery. This allows for a shorter procedure and eliminates the need for a donor site from the patient. Additionally, large grafts can be extracted from cadavers and modified to fit a patient's specific needs [Prokopis, 1999]. Allograft procedures are also associated with less post-operative knee stiffness than with autografts, and if failure of the implant occurs, the patient can be given another allograft or an autograft procedure [Johnson, 2004].

Some disadvantages with allografts are that they are not as mechanically stable as autografts and have a higher chance for traumatic failure, especially in younger, more active patients [Chang, 2003]. A study by Luber et al. showed that in patients less than 40 years of age, there was a 24% failure rate of allografts after 2 years which suggests that there is a decrease in reliability when comparing autografts to allografts [Luber, 2008]. Because of this, allografts are more advantageous to patients who live a sedentary lifestyle and perform less physical activity. Chang, et al. showed that on average only 65% of allograft procedure patients are able to return to their previous levels of activity compared to approximately 75% of autograft patients [Chang, 2003]. Furthermore, allografts carry a higher risk of disease transmission than autografts and must be sterilized and extensively screened for contamination before implantation. Even with proper screening, there is the possibility of an immune response and rejection of the implant. This occurs if cells have been left intact on the implant and cause the immune system to target the implant [Prokopis, 1999]. This leads to rapid failure of the implant and necessitates another surgical procedure.

3.1.2.3 Synthetic Materials

Synthetic grafts were primarily used for ligament reconstruction surgery in the 1980's and 1990's and were sold under names such as Dacron™ (polyethylene terephthalate), Gore-Tex™ (polytetrafluoroethylene), and the Kennedy Ligament Augmentation Device™ (LAD). They were composed of large arrangements of fibers and offered advantages such as high mechanical strength, ease of implantation, long shelf life, and short recovery times [Davidson, 1995, Rubenstein, 1998]. While synthetic grafts had acceptable short term properties, their long term performance was less than desirable. One study observed the failure rate of Gore-Tex™ grafts in patients averaged 10% after two years and 33% after four years [Davidson, 1995]. Another study showed that after 4 years, 37.1% of Dacron implants used for ACL reconstruction had failed, and Ventura et al. concluded that in addition to a high failure rate, patients who had received Dacron implants were prone to developing degenerative osteoarthritis [Ventura, 2009, Richmond, 1992]. The elastic properties of synthetic grafts were poor, with stiffness values between 322-420 N/mm, as compared to the undamaged ACL with a stiffness = 242 N/mm, and because they were not composed of biological materials there was little ability for tissue in-growth, no ability for remodeling, and the strength of the implants decreased over time [Weitzel, 2002, Woo, 1991].

The LAD was one of the first devices which tried to integrate tissue in-growth into the design by using a porous fibrous scaffold composed of polypropylene. Although levels of cell growth increased on the implant, the cells grew in an unorganized manner [Weitzel, 2002]. A study by Barceló also reported biocompatibility issues and synovitis in 63% of patients [Barceló, 1994]. While not as common, ruptured tendons can be repaired through the use of synthetic grafts. Fukuta et al. used the Leeds-Keio™ prosthetic ligament, composed of polyester, to repair a ruptured patellar tendon [Fukuta, 2003]. General disadvantages of synthetic grafts include both loosening of the implant as well as the buildup of wear particles caused by degradation. Those particles were shown to cause osteolytic reactions,

inflammation of the surrounding tissue, chronic pain, osteoarthritis, and bone resorption [Ventura, 2009, Miller, 2006]. The ultimate tensile strength and stiffness of various materials are shown in Table 1. Based on the disadvantages associated with synthetics, and the previously mentioned disadvantages associated with autografts and allografts, there is a clinical need for new and better treatment options.

Table 1: Ultimate Tensile Strength and Stiffness of Various Materials Used for Ligament and Tendon Repair

Graft Type	UTS (N)	Stiffness (N/mm)
Gore-Tex [1]	5300	322
Dacron [1]	3631	420
Undamaged ACL [2]	2160	242
Patellar Tendon [3]	1784	210
Hamstring Tendons [4]	4090	776

[1] Core-Tex and Dacron Data (Weitzel, 2002)
[2] ACL Data (Woo, 1991)
[3] Patellar Tendon Data (Wilson, 1999)
[4] Hamstring Data (Hamner, 1999)

3.2 Tissue Engineered Ligaments and Tendons

Because of the inability of the current methods to provide adequate tissue regeneration and return to normal tissue function after ligament injuries, there is a clinical need for a new treatment which better mimics the original structure and functions of ligaments and is composed of biological materials designed to accelerate healing and providing ideal long term results. These needs can be met through the use of tissue engineering, an interdisciplinary approach to the treatment of injury, disease, or other tissue damage that uses biological substitutes which better mimic the original tissue structure and allow for significantly increased healing rates and structured integration into the surrounding environment [Kim, 2005a]. Tissue engineering seeks to overcome the classic challenges associated with engaging host cells (and eventually tissue) to re-grow in areas where tissue is absent, damaged, or otherwise compromised [Harbers, 2006].

3.2.1 Components of Tissue Engineering

Tissue engineering is accomplished by developing strategies which combine novel materials, living components, and signaling agents into implantable delivery systems [Harbers, 2006]. As can be seen in Figure 2, tissue engineering has three main components: cells, regulatory signals and the extracellular matrix (ECM) [Li, 2003]. In order for an implant to substantially improve upon the current treatments it must incorporate aspects from each of the three components to encourage cell proliferation, tissue in-growth, and degradation of the implant by the body.

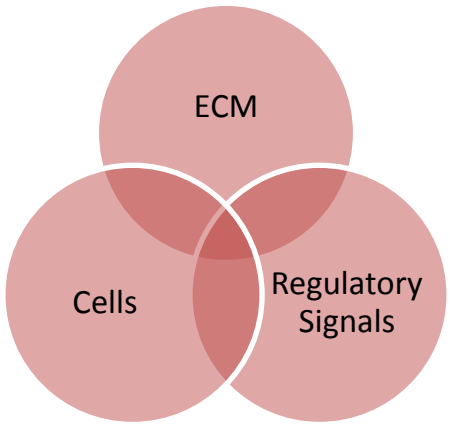


Figure 2: Primary Components of Tissue Engineering [Li, 2003]

3.2.1.1 Cells

Cells are used as "tissue precursors" [Harbers, 2006] to promote tissue regeneration in areas that have a low cellular density or low propensity for self repair. The midsubstance of tendons and ligaments for example has not only a low cell density but poor vascularization as well. Therefore, there is little potential for self-repair [Guo, 2006]. In an area such as this, cell seeding has been shown to accelerate regeneration and tissue repair [Lo, 1998a, Lo, 1998b, Murray, 2001].

Cells can be introduced into the body through general injection or infusion methods (especially when dealing with cells in the bloodstream), but often it may be useful to implant cells directly into a specific tissue area. For skeletal tissues (such as ligament and tendon), pluripotent bone marrow stromal cells (BMSCs) have been investigated. Young et al. found that seeding BMSCs on Achilles tendons

scaffolds increased healing rate and improved tendon repair [Young, 1997]. Not only are these cells capable of self renewing, but they can different into a host of different phenotypes including fibroblast lineages for ACL grafts. Additionally, they offer the advantage of being readily available in adult human bone marrow and easy to collect and isolate. In fact, a 25mL sample of bone barrow contains enough BMSCs for thirty ligament implants [Harbers, 2006].

However, native tissue consists of multiple cell types which work together and remain in constant communication through tactile and biochemical signals [Harbers, 2006]. Functional tissue generation requires more than simply introducing cells to a specific area. The surrounding microenvironment [Guo, 2006] and specialized architecture within tissue plays a critical role in functionality [Saltzman, 2004].

3.2.1.2 Regulatory Signals

Regulatory signals can include various bioactive molecules such as signaling peptides, recombinant growth factors, or synthetic drugs. These molecules are used to stimulate specific physiological responses or prompt desired interactions to occur between implanted materials and the surrounding biological environment [Harbers, 2006]. For example, Anaguchi et al. found that administering transforming growth factor-beta1 to rabbits with partially resected patellar tendon helped to increase the mechanical properties of regenerated tissue. Both the tangent modulus and tensile strength was significantly greater in rabbits that had received the treatment [Anaguchi, 2005].

3.2.1.3 The Extracellular Matrix

The extracellular matrix (ECM) acts as a delivery vehicle for regulatory signals and also offers mechanical support and guidance to cells [Li, 2003], both of which are necessary for healthy tissue development, function, and repair [Carey, 2009a]. This network of macromolecules interconnects the cells within tissue and facilitates the biochemical and mechanical interplay that directs cellular processes

such as migration, adhesion, differentiation, and apoptosis [Palsson, 2004]. When this component is damaged or missing, it must be replaced or augmented with something that will mimic the natural ECM and eventually function as a viable tissue-like living mass [Harbers, 2006]. In tissue engineering, this is done through the use of biomaterial scaffolds.

3.2.1.4 Scaffolds

In the absence of a healthy ECM, scaffolds must support cellular activity [Gentleman, 2003] and act as a delivery vehicle for cells, genetic materials, or growth factors [Harbers, 2006]. These cellular activities can include infiltration, adhesion, differentiation, and proliferation of cells from the surrounding tissue or the actions of cells seeded in or on the scaffold before implantation. The scaffold architecture guides the 3D geometry of cell organization and formation which makes it possible to not only control cell growth temporally and quantitatively, but spatially as well [Harbers, 2006, Ma, 2008].

Using scaffolds as delivery vehicles provides an effective method for getting necessary components to injured tissues [Carey, 2009a] and better controlling their concentration and distribution. This is important because successful cell therapies require the delivery of cells in adequate numbers. Current cell therapy protocols require tens of millions to a few billion cells while tissue-like cultures often require densities of about 10 million cells per milliliter [Harbers, 2006]. Furthermore, uniform distribution of these cells within a construct is necessary to promote uniform formation and distribution of new ECM and ultimately new tissue [Holtorf, 2006]. For regulatory signals, delivery via biomaterial scaffolds means the ability to release bioactive molecules in a specific local area in a defined time [Harbers, 2006]

3.2.1.4.1 Properties of Scaffolds

The design of a scaffold will largely depend on the application which it is being used for. Suitable properties must be established before implantation [Altman 2006] to ensure the scaffold effectively

mimics the ECM of natural tissue and provides a natural, controlled restoration of cell processes and tissue function [Harbers, 2006]. There are four basic properties that govern the performance of a scaffold: material selection, surface properties, bulk properties, and 3D structure and shape. For ACL repair and regeneration, the following principles are generally necessary to ensure success.

The material chosen for a scaffold can be natural or synthetic, but it must be a low density substance that is biocompatible and avoid the inflammatory or foreign body responses which lead to chronic healing problems [Leong, 2003, Weitzel, 2002]. Additionally, the material must be easy to work with in a manufacturing and processing setting [Leong, 2003]. Most important for ACL repair and regeneration is the elimination of the need to obtain scaffold materials from the patient themselves. As previously discussed, this causes complications with availability, the need for a second surgical site, and can increase the duration and intensity of rehabilitation. Materials should be obtained from sources that do not increase patient morbidity while also minimizing the risk of infection or disease transmission [Altman, 2006]. The material chosen must also satisfy the other necessary scaffold properties such as mechanical strength or degradability.

The biological response to a biomaterial implant is highly dependent on the chemical and structural properties of the material surface [Garcia, 2008]. The surface properties of a scaffold must be designed to address both mass transfer needs [Altman, 2006] and physiological interactions of the bulk scaffold [Leong, 2003]. Interconnectivity within the graft as well as a 3D highly porous surface are both necessary to facilitate nutrient influx as well as waste removal [Altman, 2006]. Surface morphology, as well as surface chemical properties, should promote intracellular signaling and aid in the recruitment of native tissue as well as the maintenance of correct phenotype expression [Leong, 2003].

Bulk properties include both mechanical and degradation behaviors of the scaffold. Mechanically, an ACL scaffold must match typical native tissue mechanical properties such as ultimate tensile strength, stiffness, elongation, creep, and stress relaxation. Additionally, stress-shielding caused

by high stiffness limits the longevity of a scaffold and should be avoided by good material match [Woo, 1990]. To better mimic natural physiology, scaffolds can perform cell signaling through the timed release of bioactive molecules [Harbers, 2006]. In order to accommodate this, scaffolds should undergo degradation at a predicable rate without releasing harmful agents [Leong, 2003]. Furthermore, the degradation rate should match tissue in-growth in order to maintain overall mechanical integrity and stability during cell migration, cell proliferation, and new matrix production [Weitzel, 2002]. A porous, fibrillar, or webbed macrostructure will aid in promoting these cell processes [Leong, 2003].

The design of scaffolds allows for finely tuned control of the morphology and shape of an implant [Harbers, 2006]. Scaffolds must not only correctly fit within the confines of the wound geometry, but their shape must provide immediate stabilization and facilitate reliable fixation [Weitzel, 2002]. Additionally, better geometrical approximation of native structure supports cell alignment and functional tissue formation [Altman, 2006]. In the ACL, this becomes especially important as the distribution and transmission of mechanical stimuli is essential for proper directionality and organization in regenerated tissue [Chu, 1995, Weitzel, 2002]. One way to accomplish this is through the use of yarn-like constructs, such as microthreads [Altman, 2006].

3.2.2 Microthread Scaffolds

Fiber-like structures have been studied previously for a variety of applications such as sutures and wound dressings. Fibrin microthreads have demonstrated the ability to serve as a suture-like delivery vehicle to support hMSC proliferation, survival, and differentiation ability as a part of therapies to regenerate cardiac tissue [Murphy, 2008]. One additional microthread cell technology involved the design of a co-culture system which use collagen microthreads to support the growth of human umbilical vein endothelial cells and human dermal fibroblasts for therapies that may potentially support angiogenesis [Carey, 2009a].However, one major field which studies and utilizes microthread technology is in uniaxial load bearing applications for ligament repair. The majority of ligament prosthetics in use

today take advantage of this fibrous structure [Harbers, 2006] because it offers several advantages including mechanical strength [Gentleman, 2003], structural similarity to native tissue, contact guidance, and the ability to be formed into more complex structures [Cornwell, 2007a].

Matrices formed from gels or sponges tend to lack the mechanical and structural integrity necessary for unaxial load bearing applications [Harbers, 2006, Cornwell, 2007a]. However, Cornwell and Pins showed that UV crosslinked fibrin microthreads could achieve mechanical strengths that were orders of magnitude higher than fibrin hydrogels [Cornwell, 2007a]. Subsequent studies with thread-like morphologies have proven that they not only possess good mechanical properties, but can be engineered to mechanically perform similarly to native tissue. Kato et al. found that fibers extruded from type I collagen demonstrated mechanical properties similar to that of native tendon [Kato, 1989], and Gentleman et al. were able to extrude 125µm-diameter collagen fibers that showed tangent modulus and peak stress values similar to human ligaments. Furthermore, Gentlemen et al. were able to implant these collagen fibers into collagen gels to create constructs with the flexibility of a gel and the mechanical strength of fibers [Gentleman, 2003].

Native tendons and ligaments contain a hierarchical arrangement of collagen fibers. This structural organization creates microenvironments for the surrounding cells and dictates the mechanical properties of the tissue as a whole [Gentleman, 2003]. Microthread scaffold technologies are able to mimic this natural formation and therefore would be advantageous for use in uniaxial load bearing applications [Cornwell, 2007a].

“Contact guidance” refers to the natural alignment and orientation of cells on thread-based scaffolds [Cornwell, 2007a]. The ability to control the morphology of cells is important in the eventual development of organized, aligned tissues. Rovensky and Samoilov have shown that cylindrical surfaces with a high degree of curvature can affect cell size, shape, and alignment. Their study found increased orientation and elongation in mouse embryo fibroblasts and certain kinds of epitheliocytes that were

cultured on quartz fibers [Rovensky, 1994]. Cornwell found that fibroblasts cultured on fibrin microthreads will align along the long axis of the microthreads and maintain this arrangement during culture. Based on these studies microthreads have demonstrated an ability to control cell orientation and morphology and potentially direct tissue formation.

Finally, microfibers are initially formed as singular entities, but they can be adapted into more complex structures though the use of braiding, twisting, bundling, or other physical modifications. Not only does this offer the opportunity to create constructs with increased structural properties, but it offers finer control over the porosity as well [Cornwell, 2007a].

3.2.2.1 Thread Materials

While many kinds of biomaterials have been studied in thread-like morphologies, natural sourced materials are generally thought to be more promising than synthetics because of their biocompatibility, non-toxicity, and potential for bioactivity [Knill, 2004]. These natural materials include silk, alginate, chitin/chitosan, collagen, and fibrin; however, only silk, collagen, and fibrin have been specifically studied for use in uniaxial load bearing applications.

3.2.2.1.1 Silk

Silk is a fibrous protein formed in specialized cells of some species of spiders and worms. It has a highly organized fibrous structure that has been used for centuries as a suture material, but has recently experienced an expansion into tissue engineering applications [Vepari, 2007]. There are several advantages to using silk as a biomaterial. Mainly, it has extremely high tensile strength (Table 2) and offers a wide range of morphological options. Silk cocoons can simply be unwound to produce fibers that can then be woven or twisted into ropes, or silk can be dissolved into an aqueous solution that can be electrospun, freeze dried, or formed into gels. Altman et al. used a wire-rope design to create a multi-fiber silk matrix that matched the mechanical and fatigue requirements of human ACL.

Furthermore, their scaffold supported the attachment, expansion, and differentiation of human progenitor bone marrow stromal cells toward ligament lineages [Altman, 2002]. Silk can also be used in conjunction with other materials to manipulate the overall performance of a scaffold. Panas et al. demonstrated that increasing the concentration of silk within a silk/collagen hybrid scaffold caused the UTS to increase. Additionally, greater cell proliferation was seen with 75% silk scaffolds [Panas, 2009].

However, one of the major drawbacks to silk fibers is their questionable *in vivo* biocompatibility. Silk fibers are held together by sericin, an adhesive protein [Winkler, 2000] which can cause hypersensitivity issues [Vepari, 2007]. This component must be removed before implantation in order to avoid complications and minimize the risk of allergic responses [Cornwell, 2007b].

3.2.2.1.2 Collagen

Collagen is the most abundant structural protein found in the human body [Gentleman, 2003] and a major component of the ECM [Li, 2003]. It makes up approximately 25% of mammalian protein mass [Harbers, 2006] and 86% and 70% of the dry weight of tendon and ligament respectively [Guo, 2006]. Because it is found naturally in the body, it is highly biocompatible and demonstrates mechanical properties similar to those of soft biological tissues (Table 2) [Gentleman, 2003]. Furthermore, these properties can be modified and adapted for use in a wide variety of applications through the use of crosslinking.

Collagen fibers have been extensively studied as scaffolding material for uniaxial load bearing applications. For example, Dunn et al. [Dunn, 1993] investigated the relationship between fiber diameter and the mechanical strength and degradation performance of collagen fiber scaffolds. Their study found that thinner fibers have higher mechanical strength and a more rapid degradation rate. This is advantageous because it offers a method for increasing the mechanical strength of the scaffold without increasing the amount of implanted material or decreasing the rate of degradation. Furthermore it demonstrates the versatility of using collagen fibers for ligament regeneration. There

have also been long term studies performed in animal models. Kato et al. observed the effects of carbodiimide and glutaraldehyde crosslinking on collagen fiber scaffolds after 52 weeks of implantation in the Achilles tendon of rabbits. Their study found that carbodiimide treated scaffolds achieve rapid repair because of their effective degradation and mechanical properties. Furthermore, there was no inflammatory response from these implants [Kato, 1991]. Similar results were show in a study done by Kemp et al. Carbodiimide (EDC) crosslinked collagen fiber scaffolds were used to replace the ACL in dogs and the behavior of these implants were observed after twelve weeks. While there were only two test subjects, the researchers found that the animals returned to nearly normal gait after twelve weeks, and there was significant replacement of scaffold with new tissue. Furthermore, there were no changes observed in the properties of the surrounding synovial fluid, suggesting good biocompatibility and integration [Kemp, 1995a].

Table 2: Properties of Microthreads Formed from Various Materials

Microthread Material	Source	Biocompatibility	Ultimate Tensile Strength (MPa)	Previous use with uniaxial load bearing applications
Silk	Spiders/Worms	Questionable	740[1]	Scaffold with ACL properties and cell differentiation [Altman, 2002]
Collagen	Structural protein in humans	High	1.5[2] 18-21[3] 25-50[4] 25-43[5]	Achilles tendon replacement in rabbit models [Kato, 1991] ACL replacement in dog models [Kemp, 1995a]
Fibrin	Plasma protein in wound healing cascade	High	4.5-7.8[6]	No
Tendon	-	-	53.0[7]	-

[1] Average from *B. mori* worm [Cunniff, 1994]
[2] Uncrosslinked [Cornwell, 2006]
[3] UV crosslinked, increasing levels [Cornwell, 2006]
[4] Single extruded fibers from 158-69µm in diameter, EDC crosslinked [Gentleman, 2003]
[5] DHT crosslinked, increasing levels [Cornwell, 2006]
[6] UV crosslinked, increasing levels [Cornwell, 2007a]
[7] Undamaged tendon [Gentleman, 2003]

3.2.2.1.3 Fibrin

Fibrin is a plasma derived protein which plays a major role in the natural wound healing cascade [Cornwell, 2007b]. Fibrin formation is initiated when thrombin cleaves fibrinogen. Double stranded twisting fibrils align and overlap, followed by lateral association and branching to further solidify the structure [Mosesson, 2006]. At the wound site, fibrin naturally forms into a scaffold material that directs tissue regeneration in the damaged area. Fibrin fibers were first experimented with in an effort to create a scaffold with the bioactivity of fibrin matrices and the structural properties of microthreads [Cornwell, 2007a].

While there has not been extensive study done on fibrin microthreads, initial investigations have demonstrated that this material is a viable option for tissue engineering applications. Cornwell found that uncrosslinked fibrin microthreads supported fibroblast attachment, alignment, and proliferation [Cornwell, 2007b], and mechanical properties of these microthreads could be doubled through the use of UV crosslinking [Cornwell, 2007a]. However, crosslinking did decrease fibroblast proliferation, and the ultimate tensile strength was much less than tendons or ligaments. None the less, fibrin threads still have potential in tissue engineering applications, especially with further research and development.

3.3 Components of a Microthread System

3.3.1 Production

As previously stated, this project focuses on the use of biopolymer microthreads, since their uniaxial load bearing capabilities make them suitable replacements for tendons or ligaments. The unique, fibrillar structure of microthreads is largely responsible for many of their advantageous characteristics. Therefore, in order to exhibit desired mechanical and physical properties, fabrication of a chosen biopolymer into a thread shape must be performed.

Fabrication of microthreads involves forming the material into a fibrillar morphology, usually through some form of extrusion, and allowing the formed thread to polymerize. At this point, the threads can be modified, dried, and stretched. Drying facilitates storage of the threads which can later

be rehydrated and used for various research or clinical purposes. Stretching aligns the material structure of the threads and can be performed during and after formation of the solid thread. Proper stretching under the threads own weight can lead to better mechanical properties [Pins, 1997].

3.3.1.1 The Current System

Currently, the client's laboratory uses a manual extrusion method to create collagen and fibrin microthreads used for research. The method was originally developed by Pins and Silver in 1995 [Pins, 1995] and has been adapted over the years into the system that is currently used today.

3.3.1.1.1 Collagen Fibers

Collagen is first extracted from rat tails using methods previously described by Elsdale and Bard [Elsdale, 1972]. A 10 mg/mL solution is made by combining dry collagen with 5mM HCl and allowing the mixture to shake overnight. A syringe pump extrudes this solution through small diameter FEP tubing into a fiber formation buffer while a laboratory worker draws the tubing along the buffer dish to create the fibers. The fibers are then left in formation buffer for approximately 24 hours before they are removed and placed in a fiber incubation buffer. The collagen fibers remain in the incubation buffer solution for an additional 24 hours, after which they are moved to a third bath of distilled water. After soaking in distilled water for 24 hours, the fibers are suspended on boxes and dried under their own weight. This entire process (Figure 3), up until drying, must be done in a heated water bath to keep solutions at a constant temperature of 37°C. [Bishop, 2005, Cornwell, 2006]

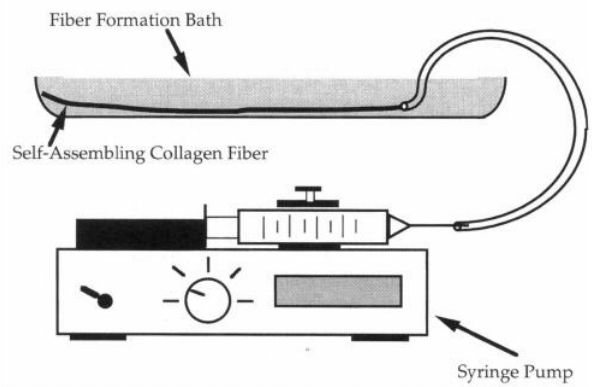


Figure 3: Schematic of Collagen Fiber Extrusion Process [Pins, 1995]

3.3.1.1.2 Fibrin Fibers

Production of fibrin fibers involves the co-extrusion of fibrinogen and thrombin solutions (Figure 4), which when combined initiate fibrin assembly [Mosesson, 2006]. A solution of fibrinogen in HEPES buffered saline (HBS) is placed in a 1mL syringe; another 1mL syringe is filled with a solution of thrombin diluted in HBS and $CaCl_2$. A syringe pump extrudes the two solutions through a blending applicator tip before the mixture travels through small diameter FEP tubing and into a 10mM HEPES bath. As with collagen fiber formation, a laboratory worker must draw the tubing along the buffer dish to create the threads. The threads must soak in solution for approximately 15 minutes before they are removed, stretched, and suspended on boxes to dry. This process can be done at room temperature, so it does not require the use of a heated water bath. [Cornwell, 2007a]

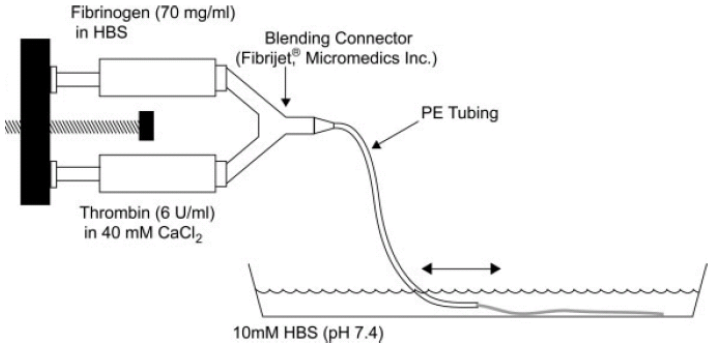


Figure 4: Schematic of Fibrin Fiber Extrusion Process [Cornwell, 2007a]

3.3.1.1.3 Disadvantages of the Current System

While the current method of thread formation is fairly simple and straightforward, there are many disadvantages to using a manual extrusion system. First and foremost is the problem of inconsistency. Though the solutions are extruded at a constant rate, because of the syringe pump, a given laboratory worker cannot move their hand as accurately or precisely. Natural variation in the speed of the worker's hand causes variation in the diameters of the formed fibers, which in turn affects the threads' mechanical properties. According to a study done by Bishop et al, fiber diameter can vary by as much as 14% when using this hand-drawn extrusion method [Bishop, 2005]. Second, the process is

highly inefficient. To make each batch of threads can take up to an hour and the process yields only about 12 threads, some of which may not be useable. Laboratory personnel are wasting time preparing threads instead of being able to use them for research. Third, a great amount of skill is required to make threads using manual extrusion. Laboratory workers must be trained in this process, and it takes some time before they develop the expertise necessary to create useable threads. Both time and materials are lost during this training period.

Fourth, production of these threads requires a great deal of handling; each time the threads are handled there is the risk of damage and material waste. Collagen threads are individually moved from bath to bath using forceps, which not only takes time but can result in threads being dropped, tangled, or otherwise damaged beyond use. Hanging threads to dry must also be done individually, which wastes more time. Often threads will slip out of the forceps and stick to the side of the box, rendering them unfit for use. Furthermore, if the threads are to go through any post-production modification processes, they will be exposed to additional handling. The loss of threads throughout these processes further contributes to the inefficiency of the overall process.

The design and use of an automated system would greatly increase the quality and production rate of collagen and fibrin threads by eliminating human error and the need for skilled workers and decreasing fabrication time, human handling, and material waste.

3.3.1.2 Patents

Disadvantages of manual extrusion have lead to the need for an automated system for thread formation. This section examines two patents developed to automatically extrude biopolymer microthreads in order to establish the major functions of a device which automatically fabricates biopolymer microthreads. A previous Major Qualifying Project (MQP) is also examined, which attempted to design a device with marked improvements over both manual extrusion and the patented devices.

3.3.1.2.1 Salo

Salo et al. (US Patent #2598608) patented a device for the development of extruded collagen fibers in 1946. The patent claimed to increase strength and enzymatic resistance due to orientation of the thread structure. Figure 5 shows the basic design of the Salo system. The process entails a single collagen thread extruded by a metering pump (8) and pulled downward to a nozzle (10) located above a dehydrating bath. The thread is stretched by gravity while traveling into the bath, which contains acetone. The fiber is then wrapped around a pulley (14) which adds a 15% stretch to the fibers. Constant tension is maintained in the fibers under their own weight. The fibers then travel to a bath of distilled water to wash away the acetone and are then dried in tension with a weight of 1.2 to 1.5 grams. Final dried fibers are wound onto a spool [Salo, 1952].

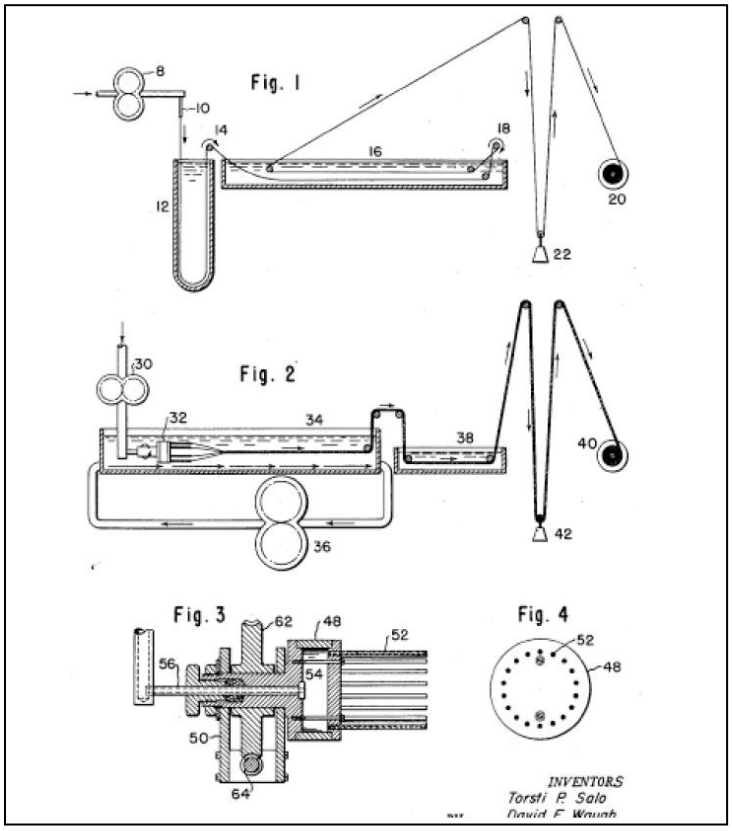


Figure 5: Preparation of Collagenous Materials [Salo,1952]

3.3.1.2.2 Organogenesis

The Organogenesis Inc. design was first described by Kato and Silver in 1989, and was purchased and modified by Organogenesis™ into its final design (US. Patent 5,378,469). Fibers produced from this system can have an ultimate tensile strength greater than 1MPa for non-crosslinked threads and 45MPa for crosslinked threads. Formed threads from this system can then be knitted, weaved, or formed into a tissue construct. Figure 6 shows the design of the device with components labeled. Collagen is extruded (1) from a syringe pump (2), consisting of a syringe (3), leader tubing (4), and a blunt needle (5), into a dehydrating bath (10), which includes a trough made of PVC and polycarbonate (11), a dehydrating agent (12), and a recirculation pump (13). The rinsing bath (20) is composed of a rinsing trough (21) and a rinse liquid (22). Drying the threads (30) is accomplished through the use of a drying cabinet (31), pulleys (43-47) and a heater/blower (32) [Kemp 1995b].

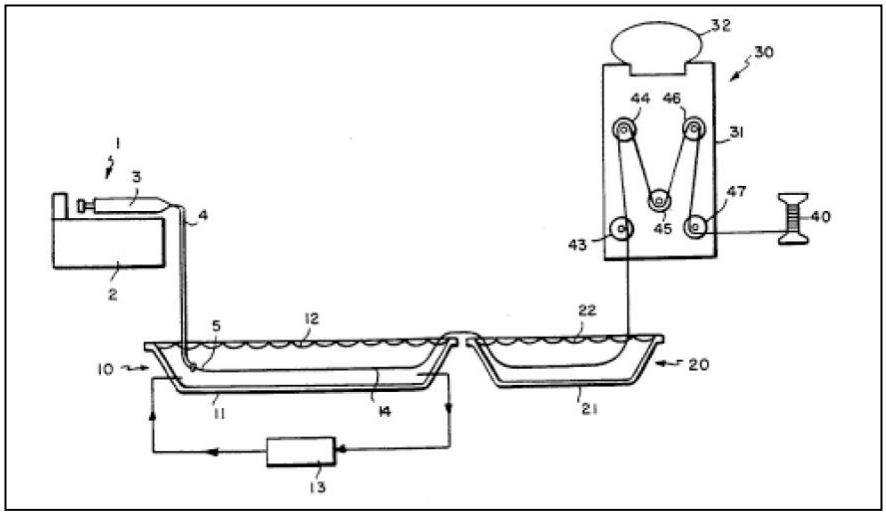


Figure 6: Collagen Threads [Kemp, 1995b]

3.3.1.3 Previous MQP

A previous MQP titled “Design of an Extrusion System to Optimize the Production of Self-Assembled Collagen Microthreads” developed an automated system for collagen thread extrusion that would improve upon manual extrusion. In the project, an automated system was designed which

allowed for the extrusion of collagen into a fiber formation buffer by means of a computer controlled bi-axial extrusion vehicle.

The first constructed component was a Lexan® (polycarbonate) bath system, with water flow channels to control the temperature of the medium that the threads would be soaking in. The inner bath was constructed out of black Lexan®, to make any formed threads more visible, and the outer out of clear Lexan®; both were fused together using acrylic cement. Two pieces of Lexan® were fused to the inside of the outer bath to form a path for water circulation. A silicone sealant was used to make the entire system watertight [Bishop, 2005].

Testing of the bath included experiments to see if a constant temperature could be achieved as well as a mass spectrometry analysis to check for possible reactivity between the thread material and the bath. The bath was filled with a room temperature buffer solution and a circulation pump was attached to the device and set for 37˚C. After forty minutes, the buffer reached a constant maximum temperature of 35.6˚C. The pump was adjusted in 1 degree increments until a desired constant buffer temperature of 37.6 (±0.4) degrees was attained. Another test included the use of mass spectrometry to determine if the Lexan® or silicone sealant could react with the thread material. Results of this experiment showed no reactivity for either component of the bath system [Bishop, 2005].

Construction on the second component began by first creating a physical three-dimensional model. A 3D model would bring to light any unseen errors before a final prototype was built, allowing for a cost effective method to test the extrusion vehicles functionality. This method also allowed for adjustments to be made regarding correct frame spacing and the positioning of moving parts without wasting final materials. The base was constructed of foam board with wooden dowels used as rollers. Through modeling, the design team made a number of important adjustments to the final design, including an expansion of the base to accommodate the motor, the addition of a counter weight to

reduce friction, and the adjustment of L-bracket positioning so as not to impede the moving parts on the device [Bishop, 2005].

For the final major component, an anchoring system, tests were performed to see which material would be most suitable. Candidates for possible materials included: pumice, artificial sponge, coral, Lexan®, and glass. Pumice and coral both showed signs of visible leaching and were removed from consideration in the final design. The remaining substances were subjected to mass spectrometry to test for any non-visible leaching. All of these materials were found to be inert and were subject to adhesion testing to see which could anchor the threads best. Hand etched glass and Lexan® functioned equally well; Lexan® was chosen because it is less brittle and more easily shaped [Bishop, 2005].

A major feature of the MQP device was the ability to automatically extrude numerous fibers into a fiber formation buffer, eliminating the issues associated with the extrusion of a single continuous strand. The device also implemented a system to control bath temperature and an anchoring method for the formed threads. The entire process was controlled through LabVIEW™, which allowed for some level of reprogramming for specific purposes [Bishop, 2005].

While this device did make better threads, it did not make the overall process easier. The lack of full automation of the fabrication process is most likely the reason it is not in use today. Specifically the devices main function was to automatically extrude threads evenly and without error; aside from this, the device did little to facilitate movement of the threads between buffers and it did not provide a platform for efficient thread stretching. The lack of efficient buffer changes and stretching platform left much of the thread making process the same as the hand drawing method.

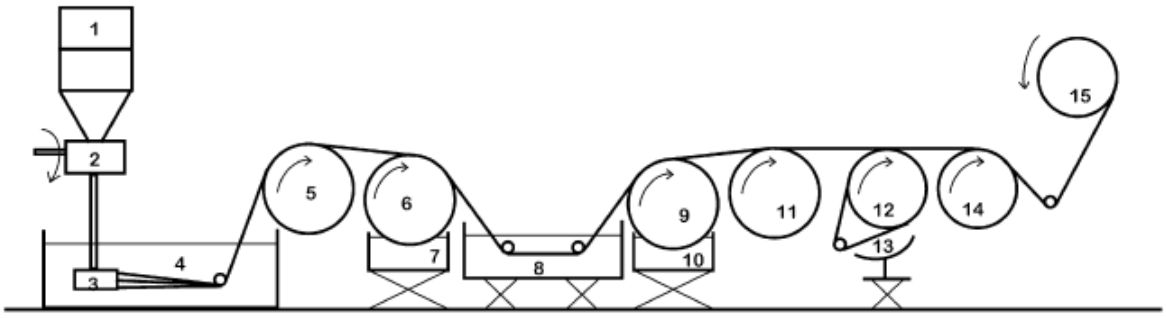
Some other improvements to the device should be considered. Currently the device has no ability to change buffer solutions or stretch the fibers during production, and automating both these processes would allow the device to have less user involvement. Automation in the buffer system can also facilitate the integration of post-production procedures that simply involve the thread soaking in a

bath, such as some crosslinking or sterilization methods. By implementing automated buffer changes and stretching, the design team hopes to create a device that would offer improvements and hopefully warranting implementation of the device in a lab setting by the user.

3.3.1.4 Additional Methods

There exist a number of methods to create synthetic polymer threads, as opposed to biopolymer threads. Dry spinning, centrifugal spinning, and polymer melt spinning all involve applying heat to a polymer and extruding it into a thread shape where it can cool into a solid form. The heat applied in these methods can denature biological materials and is therefore not suitable for biopolymers such as collagen or fibrin. The use of solvents or dehydration methods such as pressure assisted microsyringe extrusion, or wet spinning lend themselves better to thread formation involving biopolymers, because they do not require high temperatures [Samon 2001].

Wet spinning involves extruding the thread material through a spinneret into a buffer solution. The use of a spinneret allows for multiple threads to be extruded at once. The wet spinning method is similar to the syringe extrusion method in that both involve the material extruded by some means into a fiber formation buffer. Dry-jet wet spinning is a form of wet spinning that involves using a jet of air to stretch the material before it enters the fiber formation buffer [Fan 2006, Kim 2005b].



1. Dope tank; 2. Metering pump; 3. Spinneret; 4. Coagulation bath; 5,6. Take-up rollers;
7. Washing bath; 8. Orientation bath; 9,11. Stretching rollers; 10. Extraction bath;
12,14. Advancing roller; 13. Heater; 15. Winder

Figure 7: Typical Wet Spinning Process [Pillai, 2009]

By controlling a number of factors in the wet spinning process, mechanical properties can be tailored for specific use. A change to the diameter of the extrusion point or the rate of extrusion allows threads of varying diameters to be made, can effect on its tensile strength. Changes to the composition or time spent in the buffer solution can also affect the mechanical properties of threads formed. By fine tuning the variables involved in wet spinning, a variety of threads with distinct mechanical properties can be formed [Cornwell, 2007a].

The main advantage of the previous systems is their ability to perform the entire thread formation process in a continuous chain. The thread is formed, stretched, dried, and wound onto a spool in a uninterrupted process, creating a high output rate and volume. This is achieved through extruding a continuous thread, instead of a number of threads of a shorter length. This aspect of the systems also is the source of their main disadvantage.

The main disadvantage of the previous systems is their extrusion of a single continuous thread. If a break occurs on the thread, the entire system will no longer function properly and must be reset. This break can occur for a number of reasons, from an inconsistency in the extruded medium, to a disturbance of the system by some outside force. This issue can lead to considerable material loss if the automated process is not observed regularly for errors, which is usually the case.

3.3.2 Post-Production Modifications

Processing the threads is important because it alters both the mechanical properties and biological responses of the threads. For example, braiding makes threads mechanically stronger and more stable. This allows the threads to serve as a template, scaffold, or support system within the body [Attawia, 2005]. The process of surface modification biologically alters threads by adding elements such as drugs, growth factors, hormones, extracellular matrix components, genetic material, and antibacterial coatings to them [Kaplan, 2007]. These altered threads are then placed in the body to perform specific

functions based on the processing techniques used to create them. Therefore, processing threads is very important because specialized properties can be tailored for use in specific applications.

3.3.2.1 Procedure

Many different methods for processing threads currently exist. This paper will focus on sterilization, cell culture and seeding, crosslinking, bundling and twisting, braiding, and surface modification. This list encompasses methods that are currently used by the client and additional processes most relevant to uniaxial load bearing applications.

3.3.2.1.1 Sterilization

Post-production sterilization is very important because it eliminates the possibility of bacterial contamination. Although they do not necessarily need to be produced in a sterilized environment, purification is needed before cell seeding, biological implantation or the performance of studies where sterilization may affect results. This is an essential processing technique because if a thread is contaminated, it is not safe for use within the body due to the risk of infection. Sterilization of threads is done by either placing the threads in a chemical solution or exposing them to some sort of energy [Koob, 2001].

3.3.2.1.1.1 Chemical Methods

Ethanol is a common sterilization agent used for threads. Koob et al. showed it can be safely used with threads in a 70% solution. Some advantages of this method are that it is very reliable and does not attenuate mechanical properties when used at appropriate levels. It is also simple; it merely involves soaking the threads in a chemical bath, then removing the alcohol.

A second method of sterilization that has been used on threads is peracetic acid. This has been specifically tested and patented for use on collagen materials. It is a very strong oxidizing agent that is generally produced at approximately 35% concentration. It must be diluted to between 0.02% and 0.1%

when used to prevent damage the biological materials. For example, collagen threads dissolved in 0.1% peracetic acid, but remained intact in the other solutions. This method is beneficial because it is very fast; sterilization takes place in a matter of minutes [Kemp, 1995c].

A third method of sterilization used commonly in biological tissues is ethylene oxide (ETO), which is a colorless, flammable, explosive gas. ETO sterilization is generally effected by gas concentration, temperature, humidity, and exposure time. One advantage of ETO is that it can sterilize materials that are heat and moisture sensitive. In addition, this method is currently used by the client, and therefore it has been proven to be reliable in a laboratory setting. Disadvantages of ETO include the high cost, large time frame needed, and its hazards in high concentrations [Rutala, 1996]. According to Freiss, treatment with ETO is a reliable method for collagen, although slight denaturation can occur [Freiss, 1998].

In general, chemical methods are more likely to cause harm to the biological tissues than physical methods if used at incorrect amounts. This form of sterilization can also cause threads to be stiffer, harder to remodel, and are more likely to evoke an inflammatory reaction.

3.3.2.1.1.2 Energy Methods

Ultraviolet light (UV) sterilization involves shining light on the threads in order to kill pathogens. It is beneficial because it is relatively fast and is not a chemical method and therefore has no potential of emitting toxic byproducts [Cornwell, 2007b]. However, UV sterilization has the potential to change the mechanical properties and chemistry of the threads because it is also used as a crosslinking method. According to one study, UV sterilized fibers averaged lower tensile strength but higher elastic modulus compared to control fibers [Koob, 2001].

Many other sterilization methods for biological applications exist, but they cannot be used on threads. For example, heating methods such as microwaving, boiling, and autoclaving are generally not appropriate because they can coagulate soft tissue. In addition, manipulating collagen at any

temperature above 60°C will denature it [Kemp, 1995c]. Gamma irradiation has been shown to sterilize collagen very effectively in a controlled manner at 2.5 megagrads [Freiss, 1998]. However, one study showed that collagen is damaged by gamma radiation at 1 megagrad, and therefore it is not an effective method for application [Kemp, 1995c].

3.3.2.1.3 Crosslinking

Crosslinking involves joining molecules by covalent bonds and is a very important procedure which is used to strengthen and stabilize fibers. Both chemical and physical crosslinking methods exist, and each has different effects on the mechanical and physical properties of the fibers. The most relevant methods for crosslinking threads are nordihydroguaiaretic acid (NDGA) treatment, glutaraldehyde treatment, 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC) crosslinking, dehydrothermal (DHT) treatment, and ultraviolet (UV) treatment.

Individual threads that were NDGA crosslinked for 24 hours provide a viable chemical method; Koob et al. found that the material properties of these individual collagen fibers crosslinked with this chemical are very similar to those of natural tendon. In addition, it proved to be nontoxic to tendon fibroblasts. NDGA is simple in that it merely involves chemical baths. One disadvantage of this method is that it takes an extended amount of time. Koob et al. used two treatments of NDGA to crosslink collagen fibers, and each was left overnight. However, they also made the very important conclusion that NDGA-crosslinked fibers are not cytotoxic to tendon fibroblasts. Therefore, this method is feasible for this project's specific biological application.

The chemical glutaraldehyde is also a useful crosslinker that strengthens threads through a reaction between amide and aldehyde groups [Figueiró, 2004]. According to Freiss, optimal crosslinking occurs at a neutral pH. However, the more glutaraldehyde used, the less efficient the crosslinking can become. Finally, crosslinking with glutaraldehyde on has the ability to decrease the immunogenicity of collagen while making it more resistant to enzymatic degradation [Freiss, 1998]. Koob et al. explained

that some glutaraldehyde crosslinked biomaterials are cytotoxic, cause an inflammatory response in vivo, and may eventually become encapsulated as a result of the body's response to a foreign substance. Another drawback is that this is not a fast process; it takes approximately 24 hours. However, the chemical stabilizes collagen molecules and increases their thermal stability [Figueiró, 2004]. In addition, the mechanical properties of glutaraldehyde crosslinked collagen fibers are comparable to that of normal tendon [Koob, 2001, Yato, 1989, Dunn, 1993].

EDC crosslinking uses the 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride, and involves placing the threads in a chemical bath which conjugates carboxyls to primary amines [Thermofisher Scientific, 2009b]. The optimum pH for EDC crosslinking is about 5 [Freiss, 1998]. Most importantly, it is a zero length crosslinker, which means that it does not become a part of the final crosslink between molecules [Thermofisher Scientific, 2009b]. In addition, EDC crosslinked fibers were most likely to exhibit enzymatic stability when compared to other crosslinking methods [Koob, 2001]. Resistance to enzymatic degradation can be controlled by altering the degree of crosslinking via the reaction conditions [Freiss, 1998]. This protein crosslinking method increases mechanical properties to those close to native tendon by creating isopeptide bonds within the collagen fiber. However, these mechanical properties are not as strong as compared to those from UV of DHT crosslinking. Another benefit of this method is that threads have less of an inflammatory response and are more biocompatible than glutaraldehyde treated collagen microthreads. [Koob, 2001] One drawback of this method is that it takes time. For example, exposing threads to only one level of EDC crosslinking takes approximately 24 hours. However, Cornwell et al. explained that the tensile strength of fibers reaches a plateau after about 4 hours, so treatment for a full 24 hours may not be necessary. They also showed that cell migration was faster on chemically crosslinked EDC threads than on physically crosslinked threads. However, EDC is a chemical method, which can have toxic effects on the fibers. Also, according to Table 3 below, EDC crosslinked collagen fibers produce the lowest ultimate tensile strength and

lowest modulus when compared to other crosslinking methods [Cornwell, 2006]. Finally, research has shown that crosslinking EDC with N-hydroxysuccinimide (NHS) has also proved to be effective in crosslinking dermal sheep collagen [Olde Damink, 1996].

DHT crosslinking involves extreme dehydration of the threads, and is generally achieved by placing threads in a vacuum oven for a period of days. This is a physical crosslinking method, so there is no chance of chemical disruption. However, physical methods may unwind the triple helix of collagen fibers. In addition, cell migration rates decrease significantly when using this type of crosslinking. As seen from Table 3 below, it produces the best mechanical properties after three days of treatment, with an ultimate tensile strength of approximately 43MPa, a strain to failure of approximately 0.18, and an elastic modulus of approximately 249MPa [Cornwell, 2006].

Ultraviolet crosslinking is very simple; it involves placing the threads into a machine that emits light at a wavelength of approximately 250nm. The benefits of UV crosslinking are that it is very simple and fast; exposing collagen threads to light for fifteen minutes sufficiently crosslinks them. As can be seen from the Table 4 below, increasing the time spent under the UV light beyond 15 minutes does not significantly increase the mechanical properties of collagen fibers. Using fibrin fibers, however, the time does change the mechanical properties. The strength and modulus reach a peak and then decrease as can be seen Table 4 below. As mentioned previously, since it is a physical crosslinking method, it may unwind the structure of collagen fibers, but there is no danger of chemical byproducts [Cornwell, 2006].

Table 3: Effects of Crosslinking on the Mechanical Properties of Self Assembled Collagen Threads [Cornwell, 2007a]

	Sample Size	UTS (MPa)	Strain at Failure	Modulus (MPa)
UNX	20	1.5 ± 0.2	0.42 ± 0.12	4.0 ± 1.2
DHT-1	18	25 ± 10	0.17 ± 0.08	166 ± 74
DHT-3	16	43 ± 17	0.18 ± 0.05	249 ± 95
UV-15	16	21 ± 7	0.13 ± 0.08	192 ± 65
UV-30	18	18 ± 5	0.10 ± 0.03	188 ± 62
EDC	17	11 ± 4	0.17 ± 0.04	68 ± 31

UNX = uncrosslinked, DHT-(days) = dehydrothermal treatment, UV-(mins) = ultraviolet, EDC = carbodiimide.

Table 4: Mechanical Properties of Fibrin Microthreads with Increased UV Crosslinking [Cornwell, 2007a]

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

In conclusion, there have been many crosslinking methods used on threads. These have the ability to alter the mechanical properties of the threads, therefore making them more reliable for use *in vivo.* A summary table of the crosslinking methods can be seen below.

Table 5: Summary of Crosslinking Methods

	Pros	Cons
NDGA	-Simple[1] -Material properties similar to native tendon[1] -Not cytotoxic to tendon fibroblasts[1]	-Time consuming[1]
Glutaraldehyde	-Mechanical properties similar to native tendon[1,2,3]	-Can cause cytotoxicity, inflammation, encapsulation[4] -Time consuming[4]
EDC	-Zero-length crosslinker[5] -Exhibit most enzymatic stability[1]	-Time consuming[6] -Chemical method, can be toxic[6] -Low UTS and modulus[6]
DHT	-Physical method, no chemical disruption of threads[6]	-May unwind triple helix structure of collagen fibers[6] -Decrease cell migration rates[6]
UV	-Simple[6] -Fast[6] -No danger of chemical byproducts[6]	-May unwind triple helix structure of collagen[6]

[1][Koob, 2001]
[2] [Yato, 1989]
[3] [Dunn, 1993]
[4][Figueiró, 2004]
[5][Thermofisher Scientific, 2009b]
[6] [Cornwell, 2006]

3.3.2.1.4 Bundling/Twisting

Bundling is an important processing technique because bundles mimic the natural tissue arrangement of the body, especially in ligaments. One method to bundle threads is to create groups of fibers of various sizes such as 10, 50, or 200 ply by winding them around two pegs mounted onto a frame and securing them with tape to form a loop. Once the entire thread is wound, the loop ends are cut to form a bundle of fibers. Another method to form bundles involves obtaining threads that are approximately the same length and tying the ends to form a bundle [Cavallaro, 1998]. One final method is done by using a Phosphate Buffered Saline (PBS) Method, in which dried microthreads can be laid next to one other while droplets of PBS are dragged along the length of the threads until they become attached to one another [Murphy, 2008]. However, this can only be done for fibrin threads. Ferrettii et

al. showed that the maximum load for a group of parallel bundled cadaver tendons was 1709 ± 581.9N, which is comparable to the ACL which has a UTS of approximately 2160 N [Woo, 1991]. Although bundling significantly increases the mechanical properties, one drawback of the bundling method is that tensions of individual fibers may vary, which could result in an overall loss of strength [Ferretti, 2003].

Twisting is a similar processing technique which involves twisting a group of threads together to improve strength and stiffness. Ferretti et al. found that the maximum load for twisted tendons taken from cadaver knees was approximately 2428.3 ± 475.4N, compared to the previously mentioned maximum load of bundled tendons. Additionally, Cooper et al. reported that twisting fibers at a 90° angle significantly increased the tensile strength of the patellar tendon [Cooper, 1993]. Generally, twisting provides similar results, advantages, and disadvantages to the bundling method [Ferretti, 2003].

3.3.2.1.5 Braiding

Braiding microthreads has proven to be an effective technique for increasing the mechanical properties and resistance to failure of threads. Networks of fibers are interlaced in three or more strands, resulting in long, narrow structures which are adept at taking high loads of shear and torsional forces due to the interconnectedness of the structure [Ayranci, 2008]. Stiffness is also increased versus non-braided, parallel fibers, and a study by Sanders, et al. concluded that braided fibers can have higher resistance to radial shrinkage under tensile loads, which provides increased resilience [Sanders, 1977]. Braiding of fibers is usually conducted by machines which use multiple drivers that spin about a central point and intertwine the threads; however, it has been shown that braiding microthreads can be conducted by hand [Freeman, 2007]. Depending on the method for construction and the particular application, braids can be made into simple 3D shapes, or more complex 3D shapes. Simple 3D braids are either flat or round while complex 3D braids can be made into any number of shapes, such as a cube, a rectangular prism or a cylindrical tube [Ayranci, 2008].

It has been shown that the mechanical properties of braided networks of microthreads depend largely on the type of braid and factors such as the braid angle between the threads. These factors allow for the mechanical properties of the fibers to be fit for specific applications. Disadvantages of braiding microthreads include the high cost for proper setup and function of the machines as well as the potential to damage the final product due to slight variances in the machine. Braiding machines consist of large amounts of moving parts which must be precisely timed and extensively tested in order to perform correctly. Small changes in braid angle and thread tightness can negatively affect the mechanical properties of the threads by causing the uneven distribution of forces throughout the network and premature failure. [Ayranci, 2008, Cooper, 2005, Freeman, 2007]

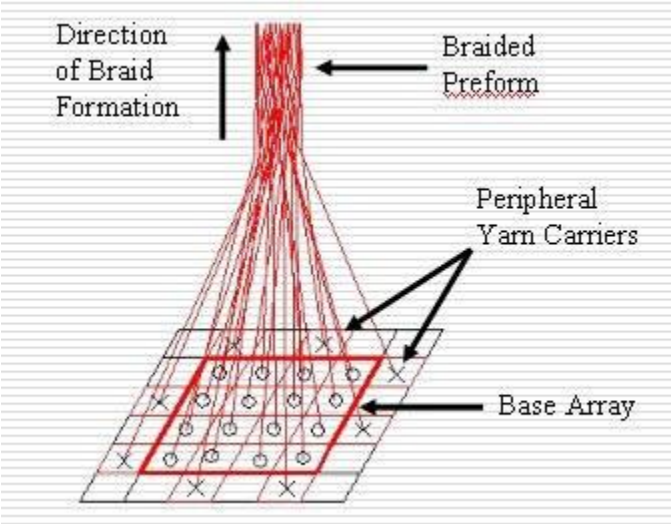


Figure 8: 3D Braiding [http://m-5.uml.edu/acmtrl/images/research/3D_braiding.jpg]

3.3.2.1.6 Surface Modification

The biological response to a biomaterial implant is highly dependent on the chemical and structural properties of the material surface [Garcia, 2008]. Upon exposure to physiologic conditions, almost instantaneous interactions occur between the material's outermost atoms/molecules and water, physiological ions, other solutes, proteins, or other macromolecules. The material/biological interface becomes a "heterogeneous biological conditioning film" [Andrade, 1985, Malmsten, 1998] of various

absorbed biologics. This initial surface layer sets the stage for many other physiologic interactions down the road. In this way, it is the surface properties which will eventually determine the success or failure of a device [McArthur, 2004].

Most clinically approved biomaterials lack the surface properties necessary to facilitate desirable interactions [Harbers, 2006]. Surface modification offers an opportunity to increase the biofunctionality of a material without losing any of the favorable bulk properties [Andrade, 1985]. Surface modification techniques can affect a wide range of properties including wettability, permeability, biostability, adhesion, or biocompatibility. In general, there are two ways to modify a material surface: either by alteration of the existing surface (physiochemical modification) or the application of some kind of coating [Garcia, 2008, McArthur, 2004].

Physiochemical modifications affect the atoms, compounds, or molecules on the surface of the material. This process can be accomplished through the use of chemical reactions (such as oxidation, reduction, and acetylation) or physical means such as etching and mechanical roughening or polishing. There are also highly ordered methods which create surface chemical patterns such as photolithography or micromachining [Garcia, 2008, Palsson, 2004]. One method that has recently been adapted for collagen microthread use is lyophilization (freeze drying). This phase separation technique involves freezing rehydrated threads so that ice crystals form, forcing precipitate between them. The frozen threads are then lyophilized and the ice is sublimated, leaving behind a perfect mirror image of the ice crystals in the threads. Initial experimentation with this technique yielded collagen microthreads with grooves and ridges that align with the axis of the thread. The mechanical and biological response to this microthread modification technique has yet to be tested [Carey, 2009b].

Surface coatings involve the creation of a film that either eliminates physiological recognition and reactivity of the implant or promotes rapid material integration [Harbers, 2006]. Often, these coatings incorporate biological molecules, such as proteins, peptides, or polysaccharides that will help to

generate precise, controlled, and predictable responses [McArthur, 2004]. There are three main methods for incorporating biomolecules into material surfaces: physical absorption, physical entrapment, or covalent immobilization. Physical absorption is a passive method involving van der Waals forces, hydrogen bonding, and other interactions that normally dictate protein absorption in the body. It is a simple and efficient method which can be strengthened through the use of crosslinking or high-affinity interactions (ex: antibody-antigen). Physical entrapment uses encapsulation or matrix systems to better control the stability, availability, and release kinetics of incorporated biomolecules. In covalent immobilization, biomolecules are tethered to a substrate which can then be formed into a network or solid support. Crosslinkers or coupling agents are often used to facilitate covalent biomaterial attachment [Garcia, 2008].

As has been shown, surface modification allows further flexibility in and control over the materials and implants used in tissue engineering. It is an important post-production modification with relevance to microthreads and uniaxial load bearing applications.

3.4 Summary and Need for Project

The purpose of this project is to design a device which facilitates efficient and precise fabrication and processing of biopolymer microthreads for uniaxial load bearing applications. Currently, methods for fabrication and processing are mainly done by hand in the laboratory. This requires a large amount of time and produces threads with varying dimensions, quality, and properties. As of 2009, there have been few products which worked toward achieving this goal. These products and their limitations were discussed in Section 3.3.1 Production. It is the hope of the project team that their device will help to automate the production and post-production modification of fibers. It will minimize variability due to human error, create fibers with uniform mechanical and physical properties, and include a variety of material and processing options.

4.0 Project Strategy: Problem Definition Phase

There are four major aspects to the problem definition phase of the design process: clarifying design objectives, developing design attributes, ranking each of these design attributes, and creating a revised client statement that focuses and defines the project. The following section outlines how the design team used research, discussions, interviews, pairwise comparison charts, and weighted objective trees to make informed decisions relating to the design of the device.

4.1 Design

According to Dym and Little, engineering design is the "thoughtful development of objects that perform desired functions within given limits [Dym, 2004]." This section will present an overview of the design process and demonstrate how the design team used this method to generate and evaluate design goals.

Before the design process can begin, it is important to identify parties who are involved in the design effort. The three stakeholders for this project are the designer, the client and the user. The role of the designers is to develop a product using design specifications which satisfy both the client and the user. The designers for this project consist of the Major Qualifying Project team: Corinna Ellis, Christopher Serafin, Paul Vasiliadis, and Carol Wood. The client, Professor George Pins, is the individual who wants the design to be conceived. He provided the design team with an initial client statement, a project description, and an expectation of deliverables. The user, Worcester Polytechnic Institute PhD candidate Jonathan Grassman, is the person who will actually be using the final device. Throughout this process, the design team used input from the client and user to make informed decisions pertaining to the final objectives of the device.

4.2 Clarification of Design Goals

At the beginning of this project, the design team was given the following initial client statement:

"Design and develop a multifunctional production system that will facilitate the reproducible production, processing, and scale-up of biopolymer microthreads for regenerative therapies."

To better understand this problem, the design team gathered background information about the field of tissue engineering, the role of biopolymer microthreads in this field, and various production and post production processes. The design team also read through the previously mentioned Major Qualifying Project to gain a better understanding of the needs, functions and limitations of this device and how the device could be improved upon.

They then conducted a series of interviews with the user and client. Both parties were able to provide information on what they needed and wanted to see in a new device, how the device would be implemented, what was especially difficult with the current methods, and why the device designed by the previous MQP project, which had a similar design goal, is not currently used. Next, the design team corresponded with Dr. Kevin Cornwell. Dr. Cornwell completed his PhD thesis working with biopolymer microthreads and was also a major contributor to the previous Major Qualifying Project. He was able to provide the design team with further insight into field of biopolymer microthreads and explained some of the advantages and disadvantages of the previously designed system. Finally, the team toured the client's professional workspace to view firsthand how the threads were made.

The design team used this information to form a list of needs, wants, and constraints for the design team, the client, and the user, which can be seen below. A need is necessary for the success of the device, a want is something desired, but not necessarily required, and a constraint is a limiting factor.

A. Design Team
    a. Needs:
        i. Needs to finish by end of D term
        ii. Needs to graduate
        iii. Needs to have a working prototype at the end of D term
        iv. Needs to follow design process

v. Needs input from client/user
vi. Needs to improve on previous designs
vii. Needs to keep accurate records
viii. Need to work effectively as a team
ix. Needs to validate final design through experimentation

b. Wants
i. Wants to get a good grade
ii. Wants to make a device that will be implemented
iii. Wants to satisfy client
iv. Wants device to be versatile

c. Constraints
i. Time limit (A-D term)
ii. Budget (624 USD)
iii. Client/User availability
iv. Limited Expertise

B. Client
a. Needs:
i. Needs an automated device that can be used in the lab to make microthreads
ii. Needs weekly and term reports
iii. Needs to grade the project
iv. Needs to guide the project
v. Needs consistent production

b. Wants:
i. Wants well designed device that will allow for processing and scale-up
ii. Wants versatile device for multiple applications/processes
iii. Wants project completed in a timely manner
iv. Wants device to integrate with current lab procedures

c. Constraints:
i. Availability
ii. Money
iii. Limited expertise of design team

C. User
a. Needs:
i. Needs to be able to use the device
ii. Needs to have threads automatically made
iii. Needs to have better threads made
iv. Needs to use threads made by device
v. Needs instruction on use of the device
vi. Needs device to be reprogrammed for specific functions
vii. Needs device to be reliable
viii. Needs device that is easily maintained

b. Wants:
i. Wants to contribute as little as possible to the thread process
ii. Wants device to be user friendly
iii. Wants a Low turnaround time and high production rate

c. Constraints
i. Time
ii. Knowledge of the device

The design team then consulted with the client and the user to evaluate this list. They discussed how clarification was necessary in regards to the aspects involved in an "automated system." The processing parameters involved in making threads from automated extrusion, such as stretching, crosslinking, and sterilizing, needed to be expanded upon so that they could be further developed and prioritized. However, the client and user still felt the design team's list adequately represented the needs, wants, and constraints of all three parties.

The design team used the feedback from the client and user to further develop their needs, wants, and constraints in to a list of objectives, functions, and constraints for the project. Objectives describe what the device must have or what it must be, functions describe what the device must do, and constrains tell what the limiting factors of the design are. This list can be seen below.

OBJECTIVES

- Efficient Turnaround-from batch to batch
- Easy to Clean
- Automated
- Easy to Use
- Versatile
- Minimize Human Contribution
- High Thread Production rate
- Accurate: how close to defined standard
- Consistent thread properties
- Reprogrammable for Different Functions/Types of Threads
- Able to be Scaled Up
- Reliable, does not break often
- Easily Maintained

CONSTRAINTS

- Time Limit (A-D Term)
- Money (624 USD)
- Must fit on lab bench
- Limited Expertise of Design Team
- Safe for User
- Made of Non-reactive Materials

FUNCTIONS

- Produce Collagen Threads
- Produce Fibrin Threads
- Sterilize Threads
- Seed Threads with Cells
- Crosslink Threads
- Bundle Threads
- Braid Threads
- Twist Threads
- Surface Modify Threads
- Stretch Threads
- Allow Threads to be Moved
- Hold Threads in Place
- Make Multiple Threads at Once
- Regulate Fabrication Speed
- Regulate Thread Diameter
- Regulate Thread Length
- Regulate Solution Temperature
- Regulate Solution pH
- Allow Threads to be Dried

4.2.1 Indented Objectives List

The design team organized the finalized objectives list into the following indented objective list. This allowed the design team to cluster similar objectives and arrange them into a hierarchical structure of main objectives and sub-objectives.

- **I. Versatile**
  - **a. Production**
    - **i. Sterile Threads**
    - **ii. Thread Materials**
    - **iii. Thread Diameter**
    - **iv. Formation Pattern**
    - **v. Temperature**
    - **vi. Anchor Threads**
    - **vii. Facilitates Thread Drying**
    - **viii. Facilitates Buffer Changes**
    - **ix. Uniformity and Precision of Thread Stretching**
    - **x. Seeding Cells in Threads**
  - **b. Post Production**
    - **i. Uniformity and Precision of Thread Stretching**
    - **ii. Sterilize Threads**
    - **iii. Seed Threads with Cells**
    - **iv. Crosslink Threads**
    - **v. Bundle Threads**
    - **vi. Braid Threads**
    - **vii. Twist Threads**
    - **viii. Surface Modify Threads**
- **II. Accuracy: How close to defined standard**
- **III. Precision or Reproducibility of Results**
  - **a. Thread Diameter**
  - **b. Thread Length**
  - **c. Thread Mechanical Properties**
  - **d. End Product matches Specified Parameters**
- **IV. User Friendly**
  - **a. Easy to Use**
  - **b. Easy to Clean**
  - **c. Efficient Turnaround –from batch to batch**
  - **d. Easily Maintained**
  - **e. Reliability**
- **V. Automated**
  - **a. Minimize Human Contribution**
  - **b. High Production rate**
- **VI. Able to be Scaled Up**

There were six level one objectives including versatility, accuracy, precision or reproducibility of results, user friendliness, automation, and the ability to be scaled-up. The design team established that versatility applied to production parameters and processes, such as thread material and seeding cells in threads as well as post-production processes such as crosslinking and bundling.

One of the major requirements of the device was to improve upon the current system and produce precise and reproducible threads. The design team understood that this applied to the thread

diameter, length, mechanical properties, and whether or not the end product of the device matched the parameters that were inputted into the machine. For example, if the temperature is set to 37°C, how close will the temperature be to that given parameter?

The next main objective was that the device be user friendly. This meant that it should be easy to use, easy to clean, have efficient turnaround from batch to batch, be easily maintained, and be reliable. One of the major issues with the device designed by the previous MQP team was that it is not user friendly enough to warrant use over the current system. The design team wanted their design to improve upon this flaw.

Automation of the device, another major requirement, was broken down into minimizing human contribution and having a high production rate. The final two level one objectives, accuracy and scale-up, did not have any clarifying sub-objectives.

4.2.2 Quantitative Assessment of Indented Objectives

With a better understanding of how each objective was related to one another, the design team could now make decisions about the relative importance of each. To add structure to this decision making process, the design team utilized pairwise comparison charts. A pairwise comparison chart allows the design team, client, and user to compare all objectives which are on the same level of the indented objective list and rank them in order of importance to the final design. Pairs of objectives are compared one by one and the objective in each pair that is more important receives a 1, while the less important objective receives a 0. In cases where both objectives had equal importance, a score of ½ was given to each.

The design team, client, and user filled out separate Pairwise comparison charts (for full charts, see Appendix A: Pairwise Comparison Charts), and the results were cumulated using a weighting system. Each stakeholder received a 1/3 weight. The scores of each stakeholder were first normalized by adding one to each score; this was to ensure no objective received a score of zero. Total scores for each

objective were determined by multiplying these normalize scores by the weight of each stakeholder and totaling the scores from each stakeholder. Final weighted scores were calculated by dividing each total score by the total possible points.

4.2.2.1 Results

In most cases, the design team, client, and user ranked the objectives in a similar manner. In cases where there was a marked disagreement, a discussion was held between the design team and the client in order to determine why this discrepancy had occurred. This facilitated a greater understanding of the decision process that each party had utilized. In all cases, the design team and the client felt that, despite initial scoring disagreements, the weighted scores were a fair reflection of the wants, needs, and desires of all three stakeholders. Table 6 shows the results of the scoring for the level one objectives.

Table 6: Results of Pairwise Comparison of Level One Objectives

Level 1 Objectives	Design Team	Client	User	Total	Weight
Versatile	3.50	5.50	5.50	4.83	0.23
Accurate	2.00	1.00	1.00	1.33	0.06
Precision or Reproducibility of Results	5.50	5.50	5.50	5.50	0.26
User Friendly	3.00	3.50	3.50	3.33	0.16
Automated	5.50	3.50	3.00	4.00	0.19
Able to be Scaled Up	1.50	2.00	2.50	2.00	0.10

The most important level one objective identified by the stakeholders was the Precision or Reproducibility of Results, closely followed by Versatility, and then Automation. During initial scoring of Versatility vs. Automation, the design team thought that Automation would be more important than Versatility, but the client and user felt the opposite way. In subsequent discussions, the client explained that he would rather be able to do more, diverse processes with the device than have a machine that can just make threads automatically. Furthermore, the client felt that the device would not necessarily need to be automated in order to make large improvements over the current system, such as minimizing human handling.

The following tables (Table 7-Table 12) show comparisons between the sub-objectives of each level one objective.

Table 7: Results of Pairwise Comparison of Level Two Versatility Sub-Objectives

Versatile Level 2	Design Team	Client	User	Total	Weight
Production	1.50	2.00	2.00	1.83	0.61
Post-Production	1.50	1.00	1.00	1.17	0.39

The client and user both felt Production processes were more important than Post-Production processes, while the design team felt they should be ranked evenly (Table 7). The client felt that the design would be more useful if it produced threads in a manner that would facilitate threads mobility (such as transport to and from different treatment baths) as opposed to a device which does not incorporate a platform for effective thread transport, but instead produces threads and performs one or two specific post-production processes. Threads need to be mobile when they are initially made so the user can work with them. The design team felt a large goal of the project was designing a device which would facilitate both steps, which is why they ranked both evenly. In the end however, Production received a bigger weighted score than Post-Production.

Table 8: Results of Pairwise Comparison of Level Two Versatility Sub-Objectives

Production Level 3	Design Team	Client	User	Total	Weight
Sterile Threads	3.00	1.00	1.00	1.67	0.03
Different Materials	6.00	5.00	5.00	5.33	0.10
Different Diameters	4.00	4.00	3.50	3.83	0.07
Formation Patterns	1.00	3.00	3.00	2.33	0.04
Temperature	8.50	8.00	8.00	8.17	0.15
Anchored Threads	8.50	8.00	8.00	8.17	0.15
Thread Drying	7.00	8.00	8.00	7.67	0.14
Buffer Changes	8.50	8.00	8.00	8.17	0.15
Uniformity and Precision of Thread Stretching	5.50	8.00	8.00	7.17	0.13
Seeding Cells in Threads	3.00	2.00	2.50	2.50	0.05

There was a general agreement among all three stakeholders when ranking the level two production sub-objectives (Table 8). There was a three way tie for the most important sub-objective between Temperature, Anchored Threads, and Buffer Changes. These three were very closely followed by Thread Drying and Uniform and Precision of Thread Stretching, but there was a gap between these and the rest of the sub-objectives. While there were some differences of opinion regarding the original rankings of Uniformity and Precision of Thread Stretching and Sterile Threads, the stakeholders felt no discussion was necessary as the weighted scores were logical. One comment the client made was that he felt a robust design could inherently be applied to anything, which is why different materials or diameters was ranked lower

Table 9: Results of Pairwise Comparison of Level Three Post-Production Sub-Objectives

Post-Production Level 3	Design Team	Client	User	Total	Weight
Uniformity and Precision of Thread Stretching	4.00	5.50	4.50	4.67	0.13
Sterilize	4.50	3.50	3.50	3.83	0.11
Seed Threads	2.00	3.00	2.00	2.33	0.06
Crosslink	7.00	7.00	6.50	6.83	0.19
Bundle	7.00	7.50	7.50	7.33	0.20
Braid	1.00	1.50	1.00	1.17	0.03
Twist	6.00	6.00	4.50	5.50	0.15
Surface Modification	4.50	2.00	6.50	4.33	0.12

When comparing most objectives, the importance of each could be determined easily by considering which was more relevant to the initial client statement and the desires of the stakeholders. In the case of the level three post-production assessments (Table 9), however, this proved to be ineffective in allowing the design team to determine relative importance. Therefore, a specialized set of metrics had to be developed in order to allow the design team to make informed decisions. Each level three post-production objective was ranked based on three criteria. The design team considered the feasibility of incorporating a particular objective into the device, how useful a particular objective was for uniaxial load bearing applications, and whether or not a system already existed in the laboratory for

achieving a particular objective. For example, cell seeding is already performed in biological hoods and incubators. Will people be more inclined to use a cell seeding feature of the device or the system already in place? This was the type of question considered when during assessment of the level three post-production objectives.

The most important level three post-production sub-objective was Bundling, closely followed by Crosslinking, and then Twisting. The design team felt this made sense, as those post-production modifications were especially important for preparing threads for uniaxial load bearing applications. The major point of discussion in this group was Surface Modification. The scores from the design team, client, and user were very different. The client clarified that the reason he and the user thought Surface Modification should be ranked lower was the feasibility of this process. The client imagined Surface Modification to include the attachment of biomolecules via solutions, which must be done in very small volumes because of cost. Performing this process in a large bath (such as the one that threads would likely be made in) would simply not be feasible. The design team was not aware of this fact when ranking, but understood that it would play a major role.

Table 10: Results of Pairwise Comparison of Level Two Precision/Reproducibility Sub-Objectives

Precision or Reproducibility of Results Level 2	Design Team	Client	User	Total	Weight
Thread Diameter	2.00	3.50	3.50	3.00	0.30
Thread Length	1.00	2.00	2.00	1.67	0.17
Mechanical Properties	3.00	3.50	3.50	3.33	0.33
Specified Parameters	4.00	1.00	1.00	2.00	0.20

The most important sub-objective for level two precision or reproducibility of results (Table 10) was Mechanical Properties closely followed by Thread Diameter. This grouping had a large amount of inconsistency among the scores of the three stakeholders because the client and user did not fully understand what was meant by the term “Specified Parameters.” The design team had meant this sub-objective to evaluate how well the device input parameters would match the device output parameters. For instance, if the user set the device to make threads that were 0.5 centimeters in diameter, how close

would the threads come to actually being that size? It should be noted that this is different from precision or reproducibility of thread diameter, which is evaluating how close the thread diameters are *to one another* and has nothing to do with the original device input. The design team and client decided that "Machine Production Precision" may be a better term for sub-objective.

However, even after clarification, the client and user still felt that Specified Parameters was a low level sub-objective. The client said he would not mind if the device output did not match the input as long as this happened in a consistent manner and the device did the same thing every time relative to the inputted settings. If the user put in X diameter and always got 6X diameter threads, it would be easy to simply set the device to X/6 and get the X diameter threads that were needed.

Table 11: Results of Pairwise Comparison of Level Two User Friendly Sub-Objectives

User Friendly Level 2	Design Team	Client	User	Total	Weight
Easy to Use	2.50	3.50	3.00	3.00	0.20
Easy to Clean	1.00	2.50	2.50	2.00	0.13
Efficient Turnaround	4.00	3.00	3.00	3.33	0.22
Easily Maintained	3.00	2.00	2.50	2.50	0.17
Reliability	4.50	4.00	4.00	4.17	0.28

The stakeholders ranked Reliability (a measure of how often the device breaks) highest among the level two user friendly sub-objectives (Table 11). This was followed by Efficient Turnaround and Easy to Use. There was no discussion necessary for this grouping as all three stakeholders were in relatively close agreement.

Table 12: Results of Pairwise Comparison of Level Two Automated Sub-Objectives

Automated Level 2	Design Team	Client	User	Total	Weight
Minimize Human Contribution	2.00	1.50	1.50	1.67	0.56
High Production Rate	1.00	1.50	1.50	1.33	0.44

Minimizing Human Contribution and High Production Rate were very evenly matched (Table 12). Though the design team had felt it was more important to Minimize Human Contribution, the client and user felt both sub-objectives had similar connotations and so were equally important. Both components would decrease the time that was necessary for workers to put into making threads.

The design team compiled the results from the indented objective tree and pairwise comparison charts into a Weighted Objective Tree (Figure 9), which provides a comprehensive visual representation of the design goals. The first number in each box (lefthand side) denotes the objective's weight in relation to other objectives on the same level, which have the same color and level of color shading. The second number in each box (righthand side, bold) denotes the objective's wieght in relation to all other device objectives.

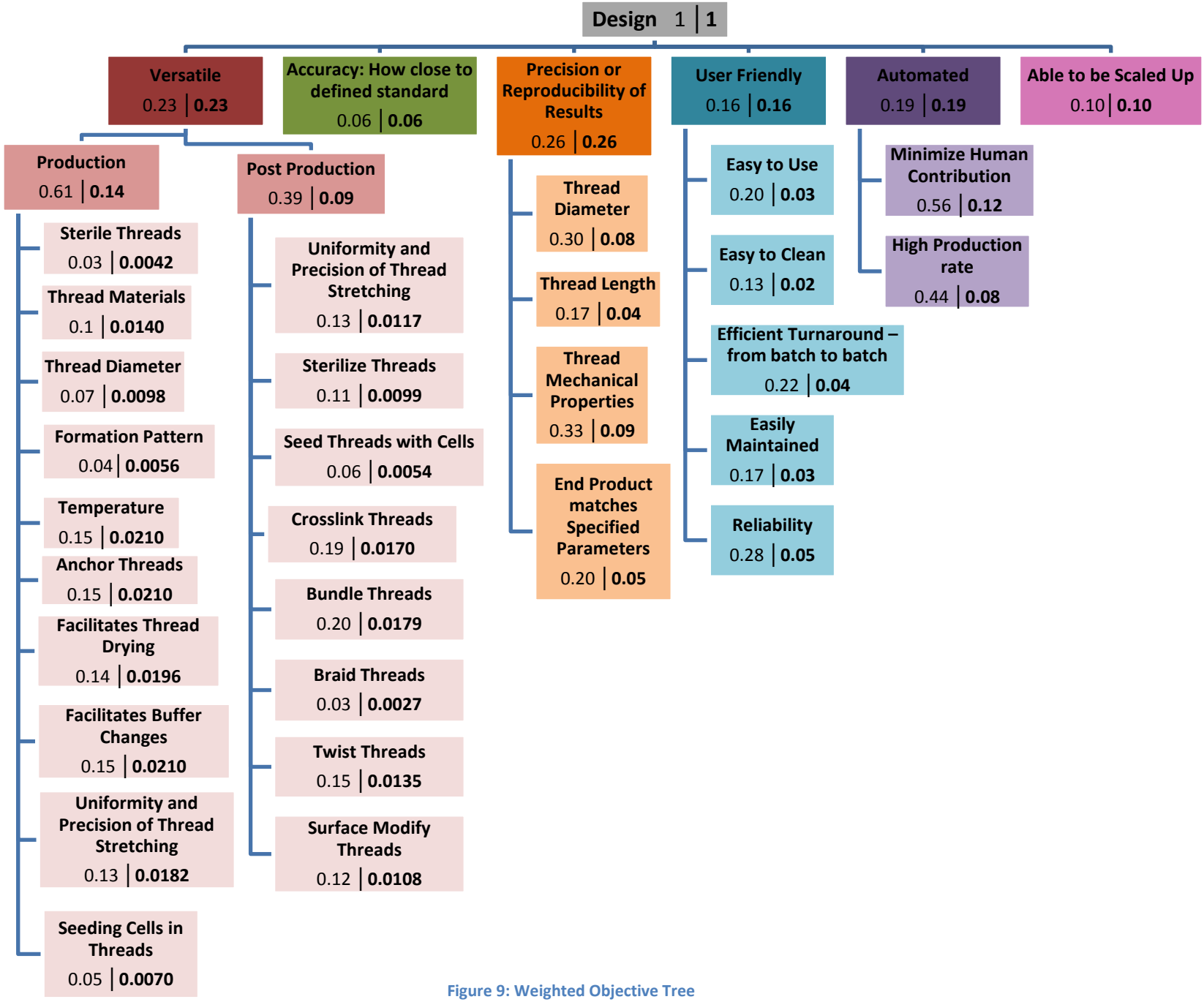


Figure 9: Weighted Objective Tree

4.2.3 Revised Client Statement

The original client statement given to the design team was to "Design and develop a multifunctional production system that will facilitate the reproducible production, processing, and scale-up of biopolymer microthreads for regenerative therapies." In order to clarify this statement and focus the project, the design team conducted background research, interviews, and developed a ranked list of design objectives. Based on the qualitative and quantitative analyses of the device objectives, the design team developed the following revised client statement:

> **Design and construct a reliable and precise system that produces at least 15 biopolymer microthreads for uniaxial load bearing regenerative therapies and incorporates 0-3 buffer changes and temperature control, as well as a removable thread anchoring system to facilitate drying, stretching and physical post-production modifications such as bundling, crosslinking, and twisting.**

This revised client statement focus the project toward a specific application, and also incorporated many of the upper level design objectives. The design team utilized a Gantt Chart (Appendix B: Gantt Chart) to plan out structure their design process.

5.0 Alternative Designs

5.1 Generation of Alternative Designs

Once the design team had fully defined and ranked the attributes necessary for the final device, they began to look at methods for satisfying the needs and wants of all the stakeholders. The team organized the functions and initial specifications of the design, conducted brainstorming sessions, and used laboratory experience, the literature review, and a previous MQP to create a set of alternative design solutions.

5.1.1 Pruned and Indented Functions List

In order to generate design ideas which effectively addressed the most important design considerations for the device, the design team created the following pruned and indented functions list (Figure 10) The list is arranged in order of importance, with the most important functions at the top. This list was based on the rankings developed using the pairwise comparison charts. The lowest design attributes were removed, and only the most important attributes were taken into account. All design attributes are significant, but the design team felt a pruned functions list would allow for the generation of alternative designs that were most relevant to the needs and wants of the design team, user, and client.

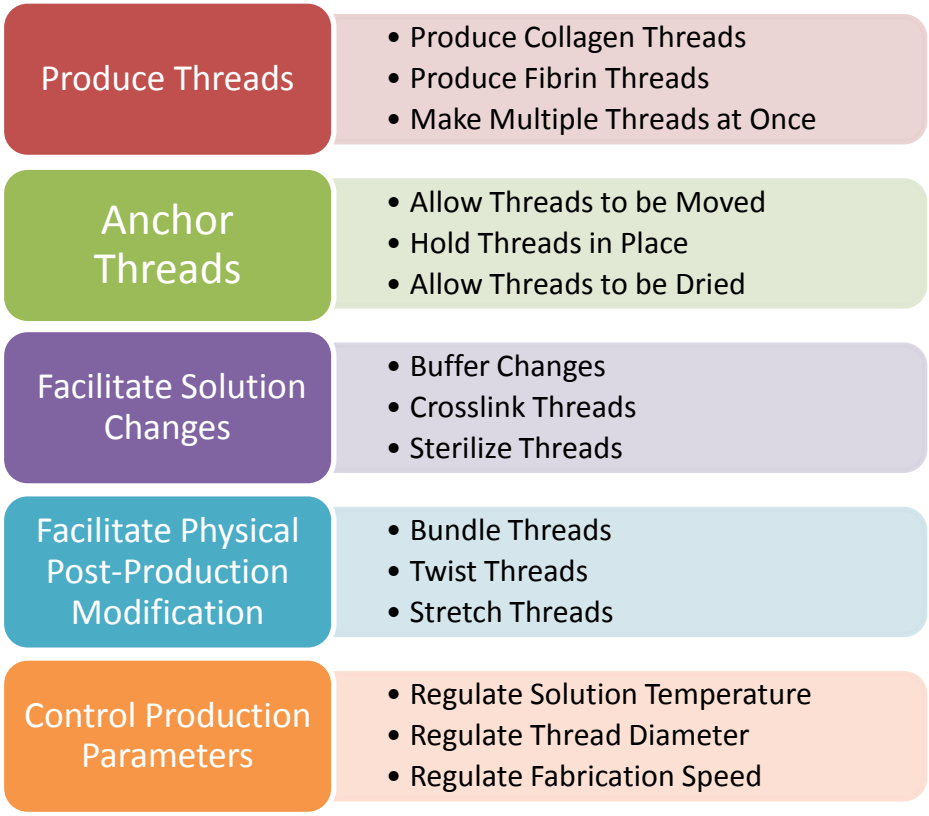


Figure 10: Pruned and Indented Functions List

There were five main functions necessary for the device to perform: produce threads, anchor threads, facilitate solution changes, facilitate physical post-production modifications, and control production parameters. Each main function also had several sub-functions which the design team took into consideration when developing alternative designs to satisfy each of these functions.

5.1.2 Initial Specifications

Using the pruned functions, the team developed a list of initial specifications (Table 1) for the device. Specifications are statements which outline the properties and attributes that the design must contain [Dym, 2004]. These specifications were based on knowledge gathered from the laboratory, the literature review, as well as the previous MQP. Firstly, from the literature, the design team knew that there would need to be approximately 0-2 bath changes needed to create the threads. Collagen requires two bath changes, but fibrin requires only one. Post production could require anywhere from 1-3 more bath changes after this in order to sterilize, crosslink, or surface modify the threads. The team came to the conclusion that each of these bath changes should take no more than ten minutes from start to finish, or the device would not be considered an improvement on the current system. Next, all of these bath changes should take place between room temperature and body temperature, 20°C (required for fibrin threads) and 37°C (required for collagen threads), respectively. The design team also knew the current thread production processes required from 350-700mL of buffer solution, so the team came to the conclusion that the bath design must accommodate at least this much liquid. Next, the threads would need to be soaked in solution for between 15 minutes and 24 hours; again this is dependent on the types of threads, 15 minutes for fibrin and 24 hours for collagen. A total of fifteen threads per batch was considered as an initial specification because it is the approximate amount that is made with one batch using the current system. Based on previous research, the group concluded that twelve inch threads at 50-70μm would be sufficient for the post-production procedures. The design team proposed

that the threads be dried for at least 24 hours, not touching one another, and under their own weight in order to ensure usability, as is currently done. Finally, it was concluded that the threads would need to be anchored at each end throughout this process.

Table 13: Initial Design Specifications

Parameter	Specifications
Buffer Changes	0-5+
	Less than 10 minutes
Temperature Range	20°C-37°C
Solution Volumes	350-700mL
Soak Times	15mins-24hours
Batch Size	15 threads
Thread Length	12 inches
Thread Diameter	50-70µm
Drying Time	24 hours
	Separated
	Under own weight
Attachment	Length of entire process
	Wet and dry conditions

Following the formation of these specifications, the design team discussed their results with the client and the user. From this meeting, it was concluded that these specifications were a good initial evaluation, but they may change as the project evolves.

5.1.3 Brainstorming Sessions

To satisfy the aforementioned functions and specifications, two brainstorming sessions were held to generate alternative designs. First, the design team met and brainstormed design solutions. The team went through each main function and generated a bulleted list of sub-functions that were necessary for each and possible means for satisfying each. The design team used the reviewed literature, the previous

MQP, and group discussion to generate alternative design ideas. The results of this session can be seen in the list below.

- Produce Threads
    - Get material from solution to thread morphology
        - Extrusion
            - through syringe
            - through spinneret
        - Molding
            - Injection molding
            - Set molding-pouring solution in and allow to set
        - Combination
            - Extruded into grooves
        - Drawing
        - Cookie Cutter
        - Rolling
    - Drawing
        - Motor
            - Fully automated 2D plotter
            - Motorized head, can only go straight
        - Guided
            - Slotted guide lid
            - Sliding tracks
- Control of Solutions
    - One bath vs. Multiple (Solutions move or Threads move)
    - Get solution in
        - Stock bottles connected to bath with tubing
            - Manual valve system
            - Computerized valve system
        - Paul's idea: completely filled compartments, water drains and fills until full
    - Get solution out
        - Valve drainage out into waste bottle
        - Aspirate solution into waste bottle
        - Move threads to new bath
- Physical Manipulation
    - Stretch
        - Crank mechanism
        - Vertical weights
    - Bundle
        - Sliding/scraping threads together
        - Moveable anchor points (slide together)

        - Folding rack (to place threads on top of one another)
    - Twisting
        - Clamped ends for manual twisting
        - Crank mechanism for twisting
        - Motorized twisting
- Anchoring System
    - Attach at ends vs. attach along the whole length
    - Clamps
    - Surface that threads will stick to (etched/porous)
    - Movable knobs
- Control System
    - Production Parameters
        - LabVIEW™
    - Temperature
        - Heat actual solution
        - Place solution in an heated outer bath
        - Oven or Entire lab is 37 degrees
        - Hot plate

The design team then presented this general list to the client and user who wished to contribute ideas as well. Therefore, a second brainstorming session was held between the design team, client, and user. Each main function was allotted 15 minutes for brainstorming, with 15 minutes left over to allow the design team, client, and user to revisit any function that they wanted to discuss in further detail. Each 15 minute slot began with a member of the design team drawing and presenting an idea which could then be modified or added to by any other party. Anyone could also choose to present a new idea and each new idea or modification was given a name. During this process, all design ideas were considered and negative comments were kept to an absolute minimum in order to generate as many ideas as possible. The figures below show the results of the brainstorming session.

For the Produce Threads function (Figure 11), the design team, client, and user focused on how to accomplish the motion necessary to extrude the threads: moving up and down the bath at a constant speed to form each thread and then moving from thread to thread. The group's ideas fell into two major categories: a moving bath or a moving extrusion head. For each idea, the group discussed how each type

of motion could be accomplished. These ideas included a conveyor belt or motorized cart. There was also some discussion based around the versatility of the moving extrusion head idea and how it could be adapted for use with different sized and patterned threads. The final discussion point for the Produce Threads function was the possibility of layering the extrusion system to allow for a greater number of threads to be made with each batch without having to increase the device's footprint.

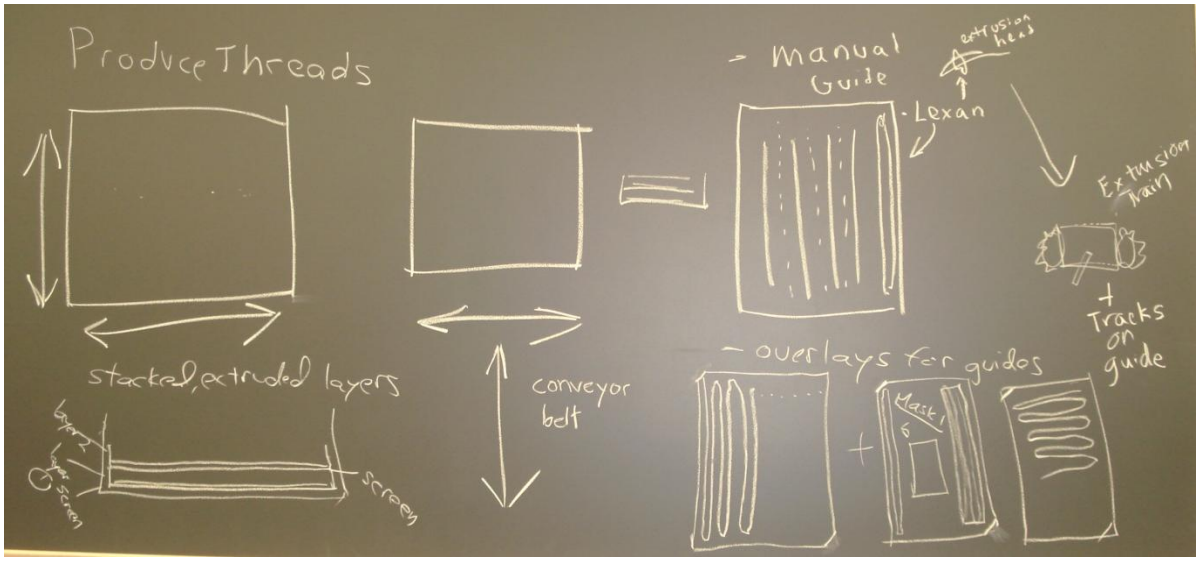


Figure 11: Brainstorm Board for Produce Threads Function

For the Anchor Threads function, (Figure 12) the group focused on which surface would be most effective for thread adhesion as well as how to incorporate this into a structural frame that would allow the threads to be moved easily and safely. This was a major design change from the previous MQP, which only focused on a surface which the threads would adhere to and not how they could be moved effectively after extrusion. To promote thread adhesion, the group thought roughened or patterned surfaces would be most effective because they would increase the area of contact between the thread and the material. The frame ideas included structures that would be versatile enough to allow for extrusion, drying, and stretching.

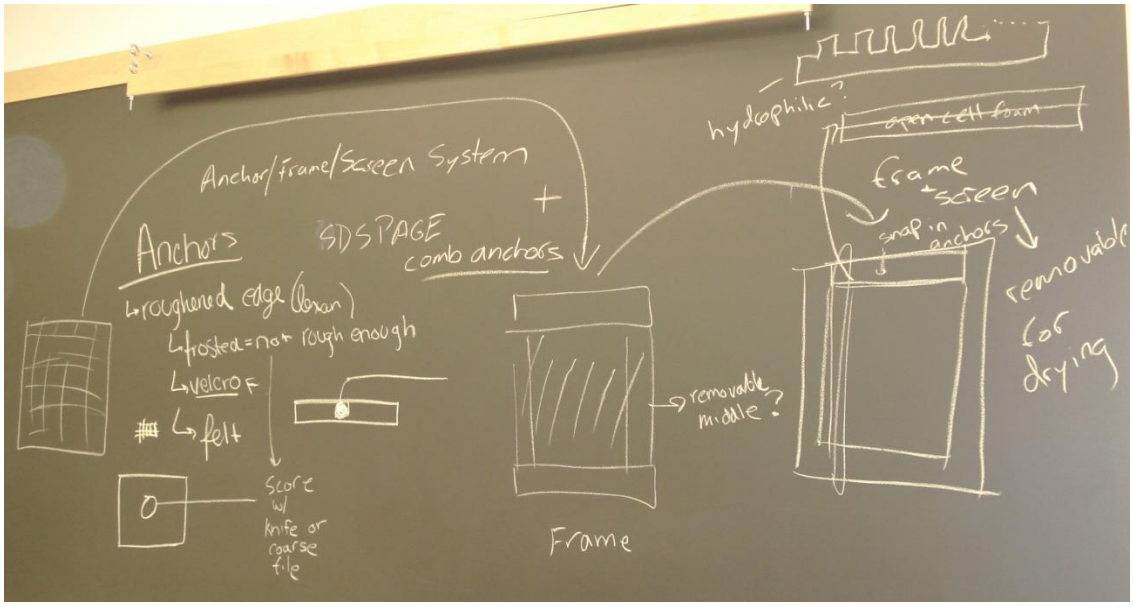


Figure 12: Brainstorm Board for Anchor Threads Function

For the Physical Modification function (Figure 13), the group focused on a system that would allow for bundling, twisting, and stretching. When presenting an idea, the design team, client, and user, tried to keep in mind that any system would need to allow for as many physical modifications as possible and also integrate with the anchor design. Most often, a design idea was initially focused on a specific physical modification and subsequent discussion centered on how to adapt this idea to allow for other types of modification.

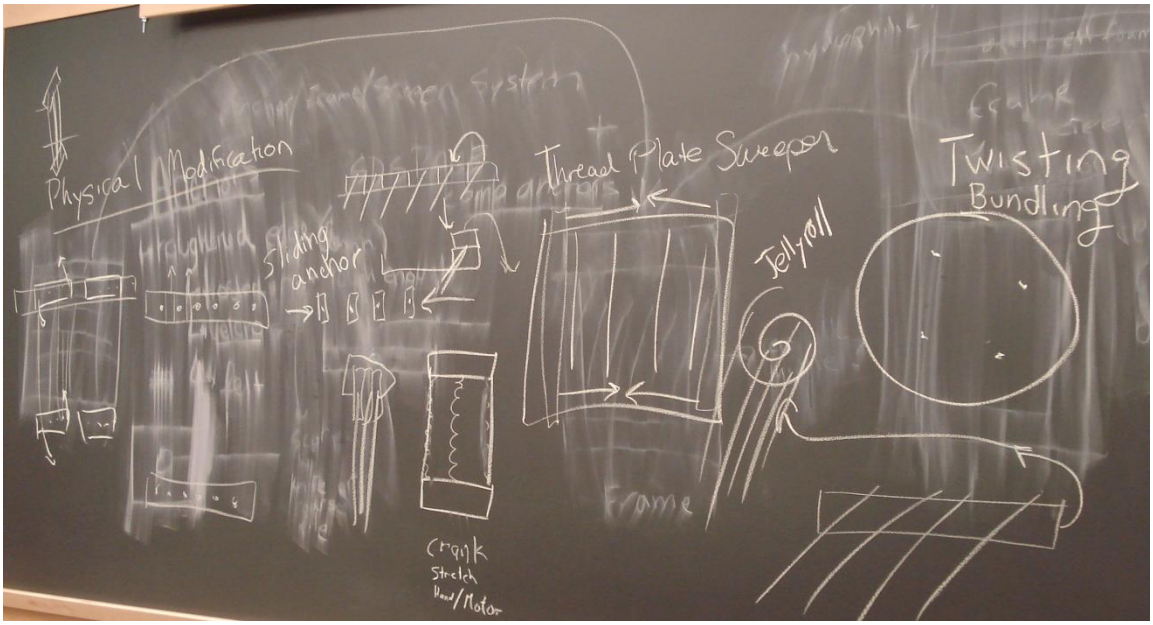


Figure 13: Brainstorm Board for Physical Modifications Function

For the Solution Changes function (Figure 14), the group discussed ideas for filling and draining the bath and how, potentially, these could be combined into one system. The proposed systems relied on external driving forces such as gravity or vacuums

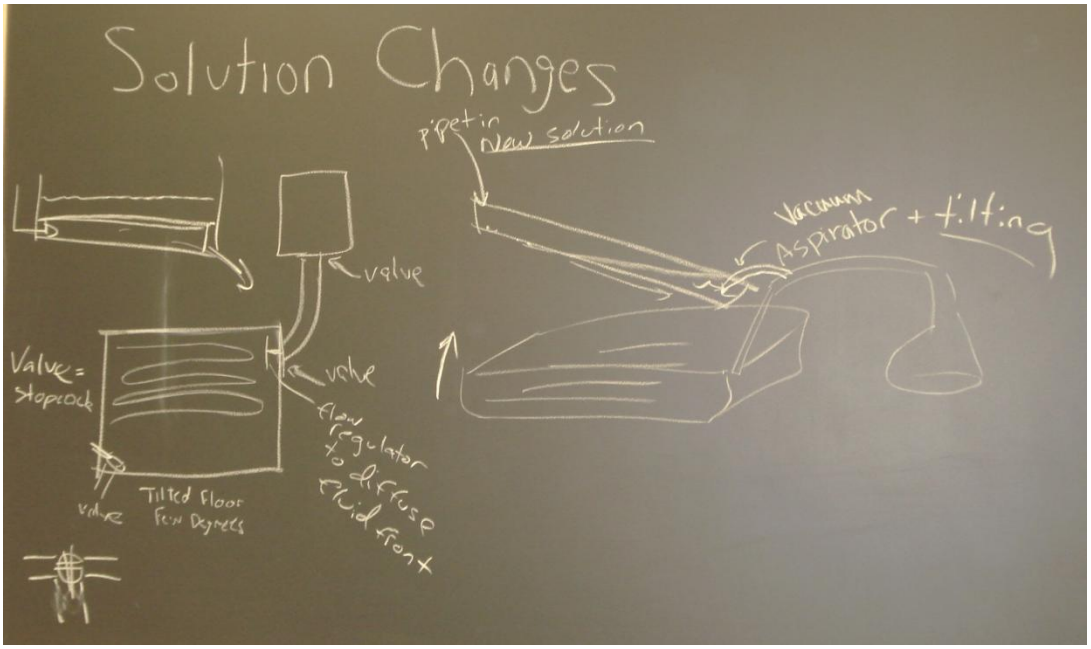


Figure 14: Brainstorm Board for Solution Changes Function

There were no drawings made for the Control Production Parameters function because a simple list was sufficient. The group did not require images to understand the proposed ideas or discuss how they would be executed.

From this final brainstorming session, the design team developed the following morphological chart (Table 14) which took into account all the design ideas and modifications.

Table 14: Morphological Chart

Function	Means									
Produce Threads	Extrusion Train	Bi-directional motor head	Conveyor Belt	Molding	Manual Guide	Cookie Cutter	Rolling			
Anchor Threads	Roughened surface	Combination anchor and platform	Fill holes	Filled grooved ends	Grooved Platform	Combs	Clamps	Mesh Screen	Solid Platform with Hole	Pegs
Facilitate Solution Changes	Hopper System	Titled Floor	Tipped Bath	Aspirate						
Facilitate Physical Post-Production Modifications	Sliding anchors	Jellyroll	Tilting Blocks	Thread Plate Sweeper	Stacking Anchors	Twisting Caps	Crank Mechanism	Vertical Weights		
Control Production Parameters	LabVIEW™	Manual	Heated bath	Heated Solutions	Incubator	Heated Room	Hot Plate			

5.1.4 Alternative Designs

Results from the brainstorming session were compiled into a number of alternative designs. These designs would be examined against a set of metrics for how well they meet the designs function requirements. The top designs for performing each specific function would be selected through this method and compiled into the final device. These alternative designs are discussed in detail using the figures and pro/con tables in the following section.

5.1.4.1 Produce Threads

5.1.4.1.1 Manual Guide

The manual guide consists of a sheet of material such as Lexan®, with a series of slots machined into it. A small bit that fits into the slots is attached to the end of the tubing of a manual extrusion system. The user would slide the bit down the slot at a steady pace, similar to the current system, but the guide would help keep the extrusion straight and level (Figure 15, Table 15).

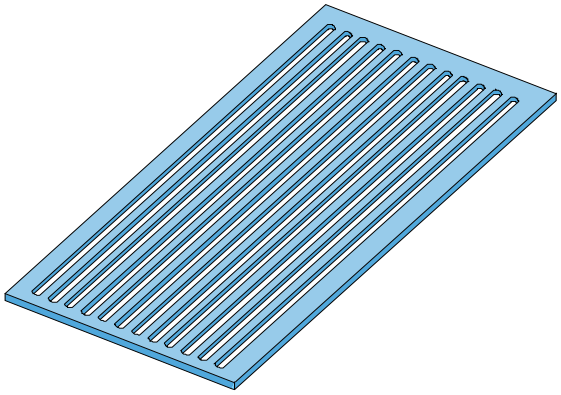


Figure 15: Schematic of Manual Guide

Table 15: Pros and Cons of Manual Guide

Pros	Cons
• Increased precision vs. current process • One part • Easy to make • Easy to use • Reliable	• Still some human contribution and error

5.1.4.1.2 Extrusion Train

The extrusion train implements the manual guide while removing the human element. The system consists of a track attached to the top of a manual guide to facilitate the movement of a small motorized vehicle along the slots. The device will hold the tubing of the extrusion system in place while moving it at a constant rate along the slots, allowing for more consistent extrusions (Figure 16, Table 16).

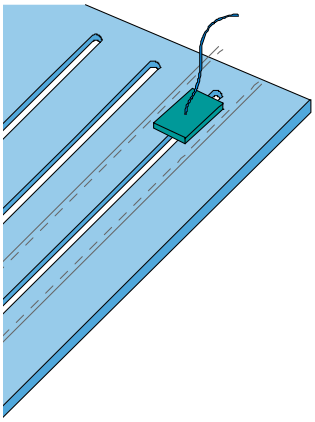


Figure 16: Schematic of Extrusion Train

Table 16: Pros and Cons of Extrusion Train

Pros	Cons
• Increased precision • Simple concept • Easy to use • Reliable	• Limitation making multiple threads

5.1.4.1.3 Molding

A molding system is comprised of two blocks that fold together to form a series of thread-shaped molds that can be filled and allowed to set. After the threads have solidified the blocks are separated and the molded threads removed (Figure 17, Table 17).

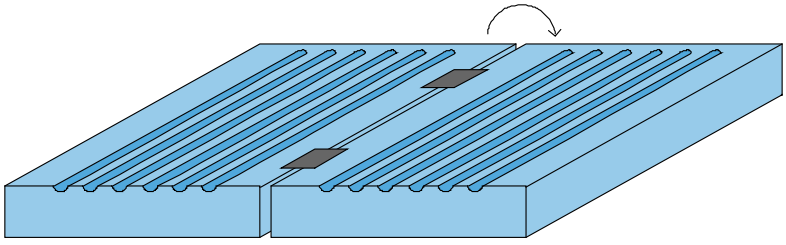


Figure 17: Schematic of Molding

Table 17: Pros and Cons of Molding

Pros	Cons
• Precise • Easy to use • Stationary system	• Minimal contact with solutions • Cannot see results • Air pockets • Precise machining

5.1.4.1.4 Cookie Cutter

A cookie cutter system involves pressing a block with a series of closely positioned blades onto a sheet of collagen. The blades will cut through the sheet and thread like strips of collagen will be formed (Figure 18, Table 18).

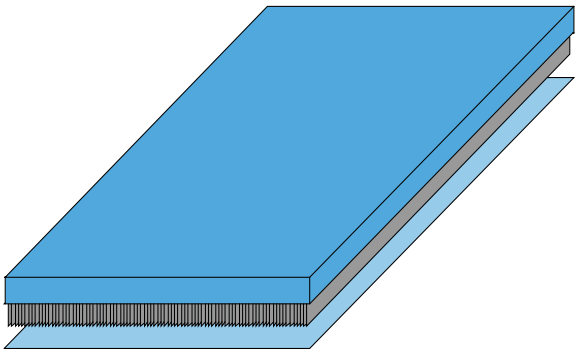


Figure 18: Schematic of Cookie Cutter

Table 18: Pros and Cons of Cookie Cutter

Pros	Cons
• Easy to use • Quick • Good precision • High production rate	• Waste • Undesirable thread shape • Human contribution needed • High shear stresses

5.1.4.1.5 Bi-directional Motor Head

A bi-directional motor head uses two motors mounted to a metal frame to move an extrusion head in two directions over a bath. Movement in two directions allows the device to extrude a thread along the length of the bath, shift to an adjacent position and begin extruding along the length of the bath again. This was the method chosen by a previous MQP group (Figure 19, Table 19).

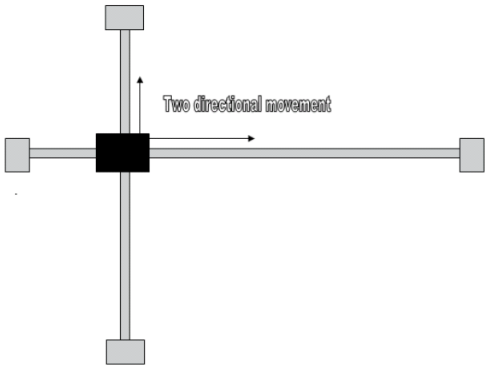


Figure 19: Schematic of Bi-directional Motor Head [Bishop, 2005]

Table 19: Pros and Cons of Bi-directional Motor Head

Pros	Cons
• Better range of motion/flexibility • Precise • Self-reliant • Automated • Reliable	• Complex • Many moving parts

5.1.4.1.6 Conveyor Belt

A conveyor belt system can be implemented that moves extruded threads from bath to bath. This system would allow for threads to be moved into static baths, requiring no bath changes (Figure 20, Table 20).

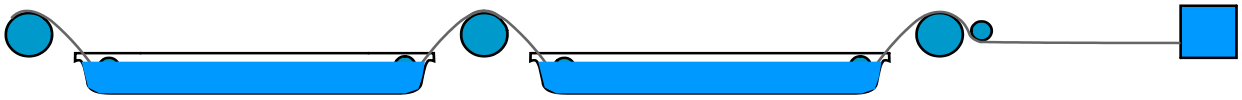


Figure 20: Schematic of Conveyor Belt

Table 20: Pros and Cons of Conveyor Belt

Pros	Cons
• High production rate • Staggered production • Precise • Automated • Reliable	• Large footprint • Many moving parts • Coordination of motors • Integration with solutions and heated bath • Thread break requires resetting extrusion system

5.1.4.1.7 Rolling

A system of rolling thread materials in the correct morphology could be established. First a broad, thin sheet of material would be produced and then this film would be cut into thin strips. Each of these strips would then be carefully rolled up lengthwise to create the thread-like morphology (Figure 21, Table 21).

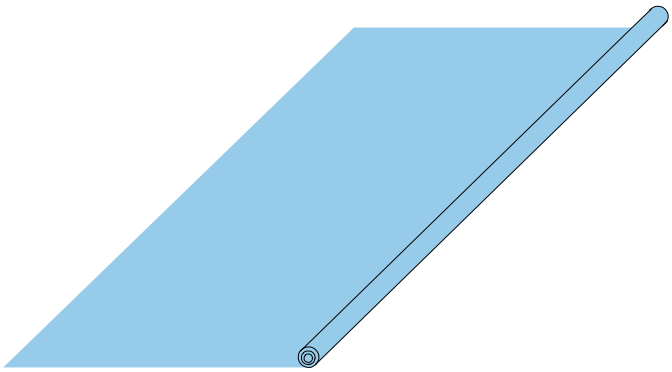


Figure 21: Schematic of Rolling

Table 21: Pros and Cons of Rolling

Pros	Cons
• Work well with cell seeding	• Size constraints • Complicated process

5.1.4.2 Anchor Threads

5.1.4.2.1 Fill-hole

The fill-hole system consists of a series of holes which serve as anchors for the ends of threads. A hole would be filled with thread material and extrusion would continue down a straight line to the anchor point at the other side of the bath where another hole would be filled. When the material sets, the filled holes will act as anchor points (Figure 22, Table 22).

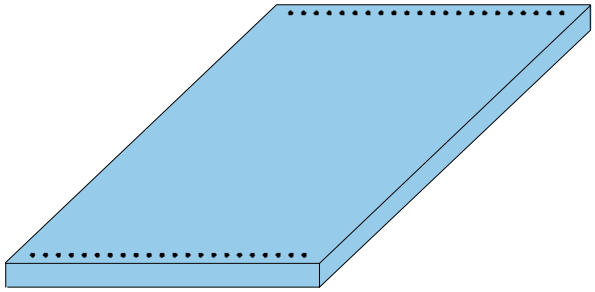


Figure 22: Schematic of Fill Hole

Table 22: Pros and Cons of Fill Hole

Pros	Cons
• Simple design • Could be combined with roughened surface	• Difficult to clean • Precise timing needed

5.1.4.2.2 Filled Grooved Ends

A grooved pattern at the ends of the platform for threads would allow for an indented area for thread material to attach to. The grooved area allows for a greater surface area and therefore, more attachment aiding in the anchoring process. The staggered pattern would help resist uniaxial forces that could cause detachment of the threads from the anchor system (Figure 23, Table 23).

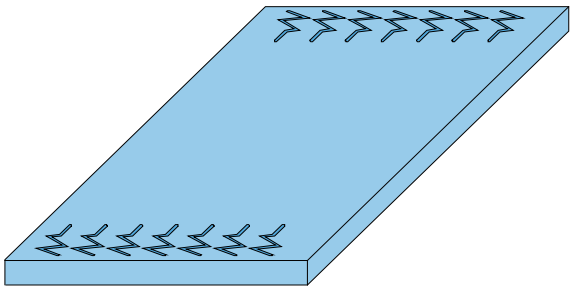


Figure 23: Schematic of Filled Grooved Ends

Table 23: Pros and Cons of Filled Grooved Ends

Pros	Cons
• Simple design • Could be combined with other methods	• Difficult to clean • Could require precise movements

5.1.4.2.3 Pegs

A peg system uses small pegs to serve as posts that threads can be formed around. The system would extrude a continuous thread weaved around the posts. After the extruded thread is set, it can be cut at the pegs to create a number of threads (Figure 24, Table 24).

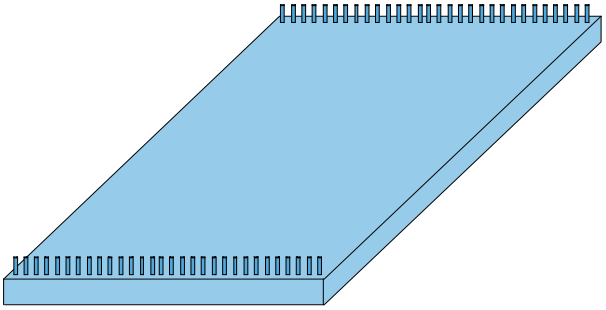


Figure 24: Schematic of Pegs

Table 24: Pros and Cons of Pegs

Pros	Cons
• Easy to make • Easy to clean	• Complicated to coordinate with production

5.1.4.2.4 Mesh Screen

A mesh screen can serve as an attachment platform for threads along their entirety while allowing the passage of liquid during buffer changes. The mesh can help reduce damage to the threads by alleviating any force exerted on the threads during buffer changes (Figure 25, Table 25).

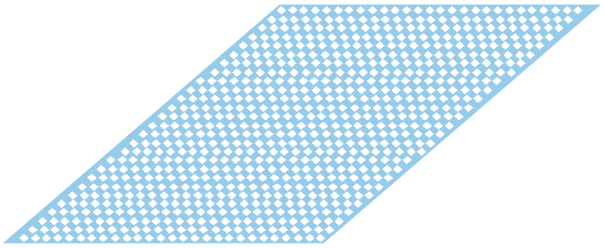


Figure 25: Schematic of Mesh Screen

Table 25: Pros and Cons of Mesh Screen

Pros	Cons
• Facilitates buffer changes • Anchors along entire thread	• Must have removable component to facilitate physical post-production modification • Hard to clean

5.1.4.2.5 Clamps

A clamp system consists of a hinged plate that clamps down onto the formed threads. Clamping would take place after the threads have been fully formed and would likely have to be combined with another initial anchoring system (Figure 26, Table 26).

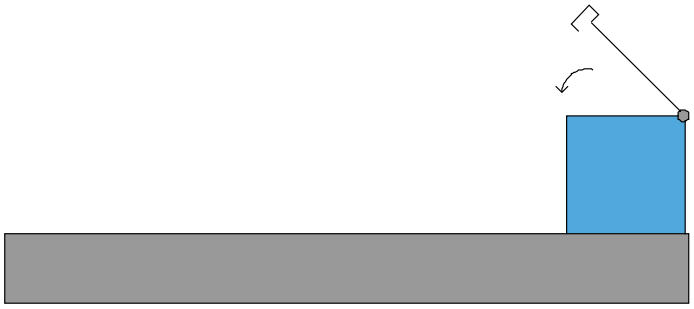


Figure 26: Schematic of Clamps

Table 26: Pros and Cons of Clamps

Pros	Cons
• Secure • Simple design • Easy to clean	• Will damage threads • Weakened contact points

5.1.4.2.6 Roughened Surface

The roughened surface anchor system will consist of anchor points with a surface that is roughened or scored in order to promote thread attachment (Figure 27, Table 27). A previous MQP found that scored Lexan® worked well for this [Bishop, 2005], but the design team also considered using Velcro® or felt.

Figure 27: Schematic of Roughened Surface

Table 27: Pros and Cons of Roughened Surface

Pros	Cons
• Simple design • Easy to use • No thread damage	• Hard to clean •

5.1.4.2.7 Combination Anchor and Platform

The combination anchor and platform system would combine the advantages of using discrete anchor points with the security and full length attachment of a platform anchor system (Figure 28, Table 28). The platform would be removable to facilitate post-production modifications such as stretching and bundling.

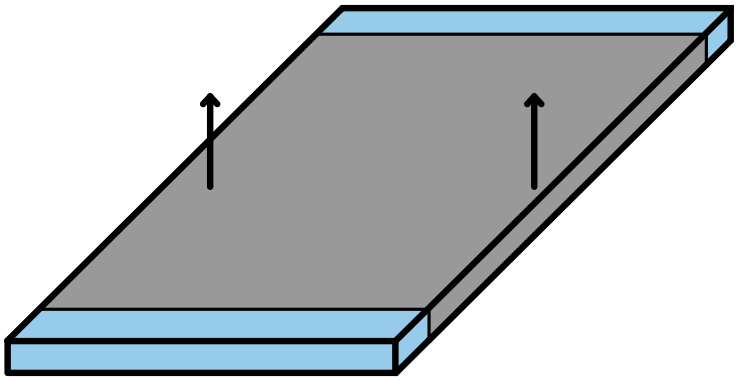


Figure 28: Schematic of Combination Anchor and Platform

Table 28: Pros and Cons of Combination Anchor and Platform

Pros	Cons
• Facilitates buffer changes • Anchors along entire length • Easy to use • No thread damage	• Hard to clean • Platform must be removable to facilitate physical post-production modification

5.1.4.2.8 Grooved Platform

The grooved platform anchor system consists of a solid platform with semi-circular grooves along the length. Threads would be extruded directly into these grooves which would allow for separation, alignment, and better formation (Figure 29, Table 29).

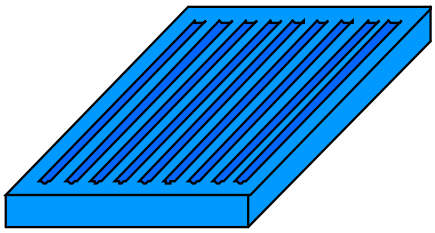


Figure 29: Schematic of Grooved Platform

Table 29: Pros and Cons of Grooved Platform

Pros	Cons
• More surface area for thread adhesion • Less thread movement side-to-side • Help to guide manual extrusion • Better thread morphology	• Precise machining • Hard to clean • Platform must be removable to facilitate physical post-production modification

5.1.4.2.9 Combs

A comb system works similarly to pegs, but are available pre-manufactured. The thread extrusion would take place in a similar manner as the pegs; there may be options for more interchangeability with the comb system (Figure 30, Table 30).

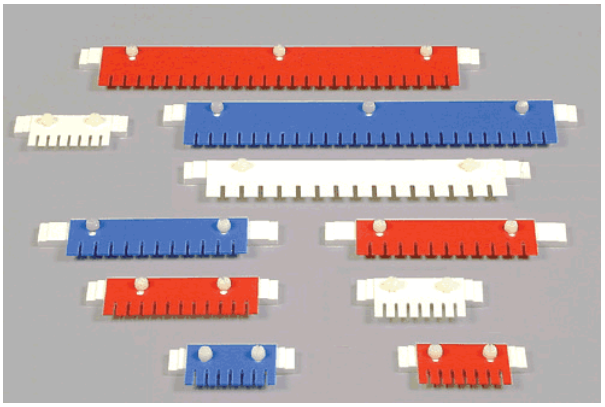


Figure 30: Picture of Combs Currently Used for Electrophoreses [http://www.topac.com/E80000.gif]

Table 30: Pros and Cons of Combs

Pros	Cons
• Commercially available • Facilitates thread alignment during formation	• Requires precise machining • Threads may slip out of position • Requires precise extrusion

5.1.4.2.10 Solid Platform With Hole

A solid platform with a hole works similarly to the mesh system, but instead has a hole positioned on the platform to assist with drainage (Figure 31, Table 31). The main difference from the mesh system is a more solid attachment among the length of the thread.

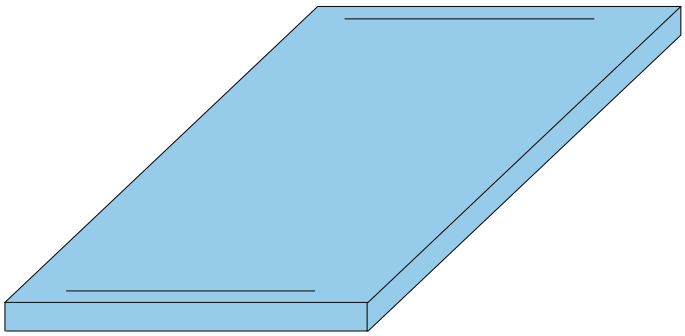


Figure 31: Schematic of Solid Platform with Holes

Table 31: Pros and Cons of Solid Platform with Holes

Pros	Cons
• Facilitates buffer changes • Anchors along entire thread	• Must have removable component to facilitate physical post-production modification

5.1.4.3 Facilitate Buffer Changes

5.1.4.3.1 Hopper System

The hopper system is a two chamber unit with valves after each chamber. The first chamber serves as a large reservoir for a solution. The second chamber fills to the exact volume needed for the bath and delivers solution to the bath. The process begins by opening the valve separating the two chambers and allowing the second chamber to completely fill, after which the valve is closed again. The second valve is opened after the second chamber is filled, filling the bath with the appropriate amount of solution (Figure 32, Table 32).

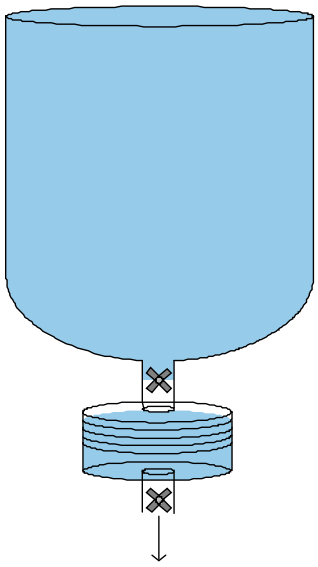


Figure 32: Schematic of Hopper System

Table 32: Pros and Cons of Hopper System

Pros	Cons
• Easy to use • Minimizes human contribution • Quick to use	• Need flow regulator to prevent thread damage

5.1.4.3.2 Tilted Floor Bath

A tilted floor design consists of a bath with a tilted bottom to facilitate drainage. A platform for thread formation will be placed above this tilt to serve as a level platform for thread formation as well as separating the threads from the forces associated with solution changes (Figure 33, Table 33).

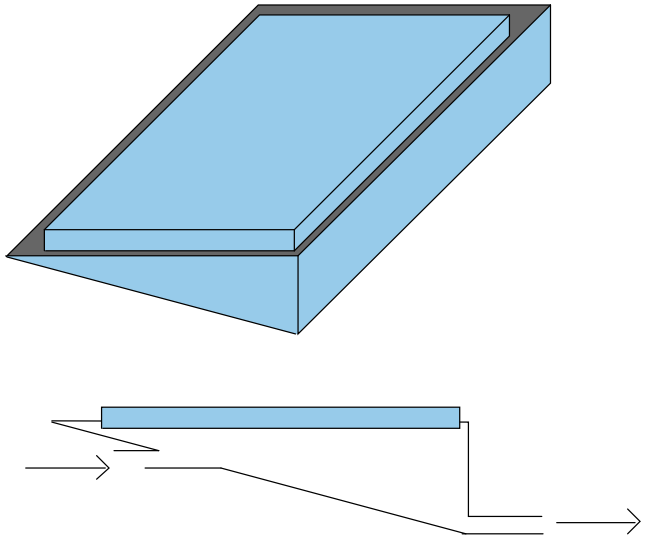


Figure 33: Schematic of Tilted Floor Bath

Table 33: Pros and Cons of Tilted Floor Bath

Pros	Cons
• Gravity driven (no outside power source) • Easy to use • Quick to use • Minimizes human contribution	• Uses more solution than a level bath

5.1.4.3.3 Aspiration

An aspirator could be used to remove buffer solution from the bath system. Aspirating the solution would eliminate the need to move the threads, but risks damaging them. An aspirator is a device which creates a vacuum capable of drawing up liquid. With this method, the bath would be given a slight tilt and the aspirator's intake tube would be placed into the solution to drain away the buffer (Figure 34, Table 34).

Figure 34: Schematic of Commercial Aspirator [http://www.gpsupplies.com/images/Products/L_Flaem_Suction_Pro_Professional_Aspirator.jpg]

Table 34: Pros and Cons of Aspiration

Pros	Cons
• Quick to use • Easy to use • Reliable drainage	• Requires additional equipment • May damage threads

5.1.4.3.4 Tipped Bath

A method for facilitating drainage could involve the use of a tilted bath. A tilted bath will allow for a simple method of drainage of solution out of the bath (Figure 35, Table 35).

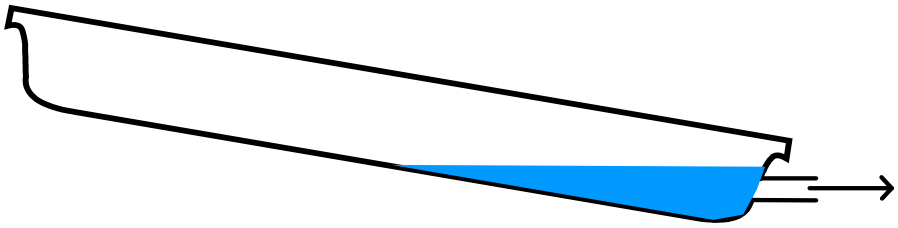


Figure 35: Schematic of Tipped Bath

Table 35: Pros and Cons of Tipped Bath

Pros	Cons
• Gravity driven • Simple procedure	• Takes time • Requires good anchoring • Inconsistent

5.1.4.4 Physical Modification

5.1.4.4.1 Tilting Blocks

A system of tilting blocks to bring threads closer together can be implemented to assist in bundling. After the threads are formed, the pegs on the anchor system tilt so that the horizontal distance between the threads is reduced, allowing for easier bundling (Figure 36, Table 36).

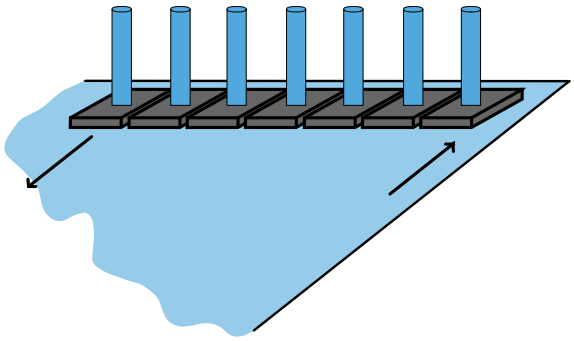


Figure 36: Schematic of Tilting Blocks

Table 36: Pros and Cons of Tilting Blocks

Pros	Cons
• Simple design • Easy to make	• Wasted materials • Requires long threads

5.1.4.4.2 Sliding Anchors

The sliding anchor system involves anchor points sliding together to aid in bundling. During thread extrusion and formation, the anchor points would be separated by a spacer on each end, which would be removed after thread production. With the spacers removed, the anchor points would then slide together allowing for easier bundling of threads (Figure 37, Table 37).

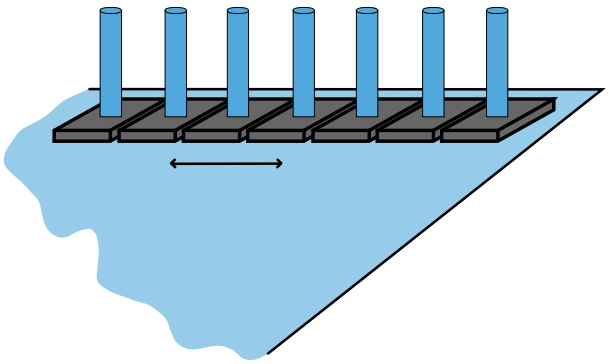


Figure 37: Schematic of Sliding Anchors

Table 37: Pros and Cons of Sliding Anchors

Pros	Cons
• Less wasted material • Easy to use • Quick to use • Customizable	• More complex system required • Requires accurate placement of threads

5.1.4.4.3 Jelly Roll

The jelly roll is a method of bundling where a flexible anchor system is implemented. An anchor would be constructed from a flexible material that could be rolled up. Threads attached to the anchor system would be gathered together as the anchor is rolled up, placing them in a semi-bundled formation (Figure 38, Table 38).

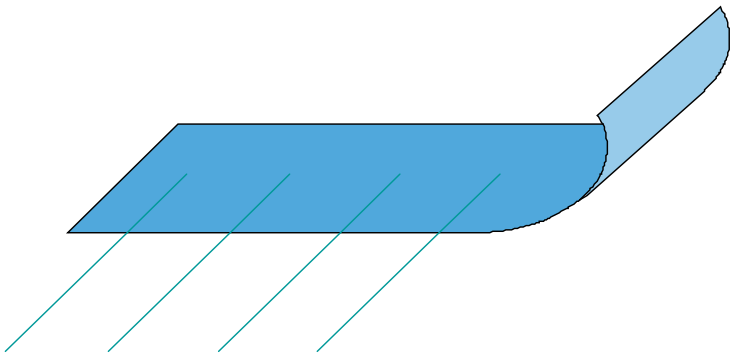


Figure 38: Schematic of Jelly Roll

Table 38: Pros and Cons of Jelly Roll

Pros	Cons
• Easy to use • Facilitates multiple physical modifications	• Hard to combine with anchor designs • Human error • Coordination of two different ends

5.1.4.4.4 Thread Plate Sweeper

The thread plate sweeper system uses two plates that sweep the formed threads from each side bringing them closer together (Figure 39, Table 39). This system will help to bundle threads closer together.

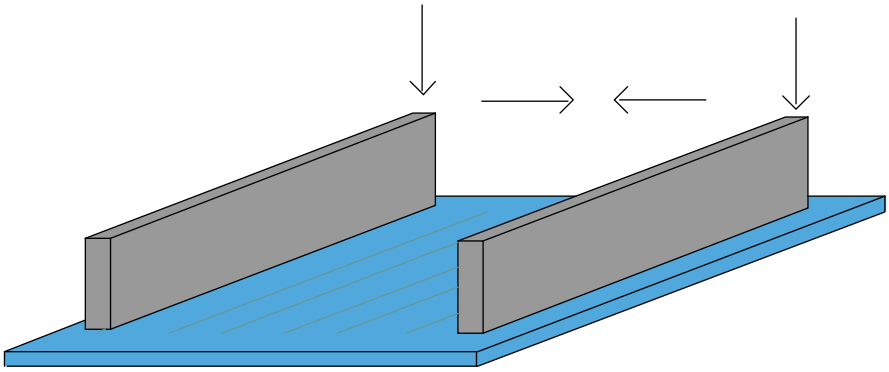


Figure 39: Schematic of Thread Plate Sweeper

Table 39: Pros and Cons of Thread Plate Sweeper

Pros	Cons
• Easy to make • Quick to use	• Extra components • Threads are no longer anchored • Thread damage

5.1.4.4.5 Crank Mechanism

A crank mechanism can be used as a method to stretch threads. The mechanism would consist of two ends that integrate with the anchor system and whose distance apart can be adjusted by a turn dial (Figure 40, Table 40).

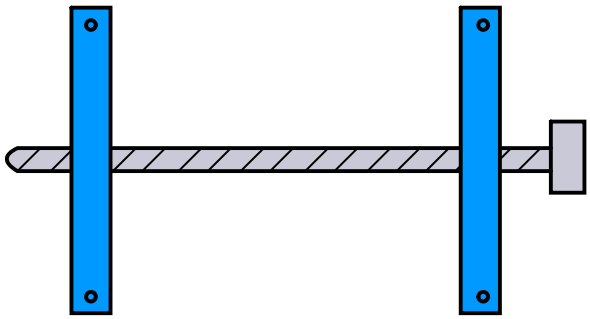


Figure 40: Schematic of Crank Mechanism

Table 40: Pros and Cons of Crank Mechanism

Pros	Cons
• Reliable • Consistent results • Easy to use	• Requires precise machining • Thread breakage

5.1.4.4.6 Vertical Weights

Vertical weights can be used as a method for stretching threads. The device would consist of a hook on one end of the anchor system that weights can be latched onto. The anchor system would be suspended vertically, and the added weight would stretch the threads (Figure 41, Table 41).

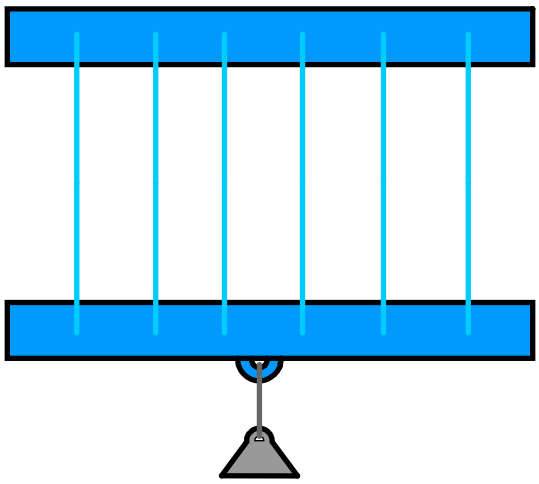


Figure 41: Schematic of Vertical Weights

Table 41: Pros and Cons of Vertical Weights

Pros	Cons
• Easy to use • No separate components needed	• Thread breakage • Inconsistent • Limited by weight amounts

5.1.4.4.7 Stacking Anchors

A stacking anchor system would be an anchor with a series sections connected by hinges. Each section would have the end of a tread drawn on it. After the threads have fully formed the anchor can be folded onto itself, bunching the separate threads together (Figure 42, Table 42).

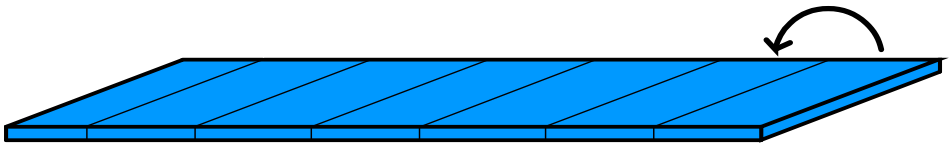


Figure 42: Schematic of Stacking Anchors

Table 42: Pros and Cons of Stacking Anchors

Pros	Cons
• Consistent • Easy to use • Easy to make	• Requires accurate placement of threads • Requires effective detachment of threads • Threads would no longer be anchored

5.1.4.4.8 Twisting Caps

Twisting caps would be used in conjunction with a thread bundling system. Assuming the threads are still attached to their anchor points, a twisting cap (such as a C-clamp) could be placed over the grouped anchor points and used to hold them together while a twisting motion is applied (Figure 43, Table 43).

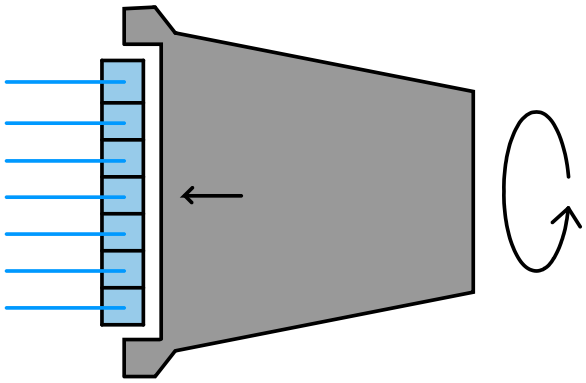


Figure 43: Schematic of Twisting Caps

Table 43: Pros and Cons of Twisting Caps

Pros	Cons
• Could be mechanized or done by hand • Easy to use • Simple design	• Thread breakage • Must integrate with bundling system

5.1.4.5 Control Parameters

5.1.4.5.1 LabVIEW™

LabVIEW™ is a control program used for a number of instrumentation purposes in a laboratory setting. The program can be used to design a virtual instrument that can control and monitor a wide variety of parameters, such as temperature, velocity, distance, and force, provided the correct tools are attached to the system (Table 44).

Table 44: Pros and Cons of LabVIEW™

Pros	Cons
• Been used previously • Reliable • Versatile • Good user interface • Consistent results	• Requires knowledge to reprogram

5.1.4.5.2 Manual

A manual control system is currently used in the lab. Laboratory workers control the speed of the extrusion head as well as the buffer changes and thread handling (Table 45).

Table 45: Pros and Cons of Manual Control

Pros	Cons
• Familiar system	• Inconsistent • Inefficient • Low production rate • Requires lab workers

5.1.4.5.3 Heated Bath

A heated bath can be used to heat the solution in an inner bath. The system would consist of an immersion heater coupled with an outer bath filled with water that an inner bath would sit in the inner bath's solution would be brought to temperature through the outer heated bath (Figure 44, Table 46).

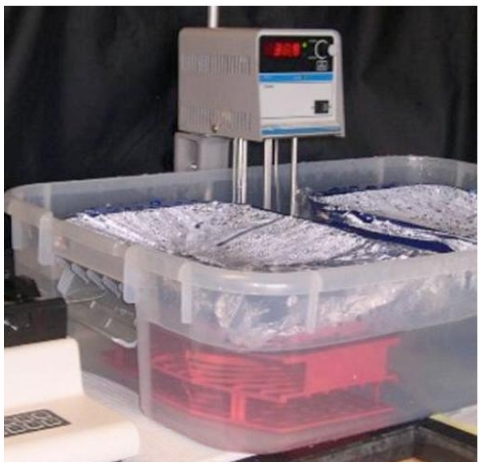


Figure 44: Photograph of Heated Water Bath System Currently Used in Client's Lab [Bishop, 2005]

Table 46: Pros and Cons of Heated Water Bath

Pros	Cons
• Reliable • Consistent • Easy to use • Easily maintained	• Energy wasted

5.1.4.5.4 Heated Solutions

Heating the buffer solution directly is also an option that could be implemented with the use of an immersion heater (Table 47).

Table 47: Pros and Cons of Heating Solutions

Pros	Cons
• Simple design	• Shelf Life • Long term effects • Evaporation • Temperature Consistency

5.1.4.5.5 Incubator

Housing the system in an incubator unit set to a target temperature would heat the bath to the corresponding temperature Figure 45: Photograph of One Type of Incubator [http://websites.labx.com/rankin/pics/44507.jpg].

Figure 45: Photograph of One Type of Incubator [http://websites.labx.com/rankin/pics/44507.jpg]

Table 48: Pros and Cons of Incubator

Pros	Cons
• Consistent • Reliable • Easy to use	• Energy waste • Expensive

5.1.4.5.6 Heated Room

The heated room works similarly to an incubator, but on a larger scale (Table 49).

Table 49: Pros and Cons of Heated Room

Pros	Cons
• Consistent • Reliable • Easy to use	• Energy waste • Uncomfortable work environment • Expensive

5.1.4.5.7 Hot Place

A hot plate can be used to heat the solution from underneath (Figure 46: Photograph of Commercially Available Hot Plate [http://www.made-in-china.com/image/2f0j00reBEHvyGqYpQM/Hot-Plate-Magnetic-Stirrer-MS300-400-.jpg]).

Figure 46: Photograph of Commercially Available Hot Plate [http://www.made-in-china.com/image/2f0j00reBEHvyGqYpQM/Hot-Plate-Magnetic-Stirrer-MS300-400-.jpg]

Table 50: Pros and Cons of Hot Plate

Pros	Cons
• Easy to use	• Uneven heat distribution • Improper temperature range • Dangerous

5.2 Evaluation of Alternative Designs

Once a list of alternative designs was established, the design team needed a systematic way to evaluate each and choose the one from each functional category which would best satisfy the

requirements of the stakeholders. To do this, the design team developed a set of metrics (Appendix C: Metrics) and quantitatively assessed each alternative design based on how well it satisfied each of the objectives originally set forth by the design team. All the scores were added up and the winning design from each category was combined into a final conceptual design. The following section is a detailed description of this process.

5.2.1 Development of Metrics

A metric is a numeric scale which allows the design team to determine if and how well the alternative designs achieve the objectives set forth for the project [Dym, 2004]. A metric not only outlines what is necessary for each design to satisfy an objective, but it allows the design team to make quantitative assessments of each alternative design. Each objective that the device must perform (See Section 4.2.1 Indented Objectives List) was assigned a metric which had a score ranging from 1 (lowest score) to 2, 3, or 4 (highest score).A full list of the metrics developed by the design team can be viewed in Appendix C: Metrics. Since each metric was not based on the same scale (some were 1-2, others 1-4), objectives with a larger score range had the possibility of receiving more points, which would add an undesired weight to that objective. To avoid this, each score that an alternative design received was normalized by dividing the final score by the highest possible score. For example, a production mechanism which allowed for the use of collagen, fibrin, and other threads would receive a 3 out of 3 ranking, while a production mechanism which allowed for the use of only collagen or fibrin threads would receive a 1 out of 3 ranking. When each of these scores was normalized and divided by the highest possible score of 3, the final scores given to these design components would be 1 and 1/3 respectively. This effectively standardized all metrics on a 0-1 scale and ensured the metric system would not affect the relative importance of each objective.

5.2.2 Decision Matrix

After defining metrics, the design team developed a set of decision matrices (Appendix D: Old Decision Matrix and Appendix E: New Decision Matrix) to create a numerically ordered final ranking for each component. Each function had a corresponding decision matrix and each alternative design was evaluated using two different weighting systems. The compared results of each helped the design team to verify the alternative design rankings and ensure that the best designs were chosen.

The decision matrix for each function was set up in the same way. Each row of the decision matrix contained an objective or constraint and each column contained one of the alternative designs for that function. Each alternative design was first evaluated on a Yes (Y) or No (N) basis to ensure that it satisfied the design constraints. Any alternative designs that did not satisfy a constraint were eliminated. Only two alternative designs, the heated room and the hot plate, did not satisfy the design constraints. The design team then went through and ranked each design using the developed metrics and the list of design objectives. Not all objectives were relevant to each main function so a different set of objectives was used for each function. For example, the designs meant to produce threads could not be evaluated on their ability to modify threads post-production. Similarly, designs meant to control temperature could not be evaluated on their ability to produce threads. Additionally, the Precision and Reproducibility of Thread Mechanical Properties objective could not be evaluated because preliminary testing is needed to determine how well a given design would satisfy this objective. Although this meant different objectives were used for each main function, the alternative designs within each main function were still ranked by the same system so effective comparisons could still be made. Once the scores were normalized, as previously described, they were weighted using two different scales.

5.2.2.1 Old Decision Matrix

The first matrix system (Appendix D: Old Decision Matrix) weighted the scores by multiplying the normalized metric score by the weight percent that each objective received. The weight percent for

each objective was based on the weighted objective tree, and reflected the importance of each objective in comparison to all other objectives, not just those under the same high level objectives. These were the scores that were denoted in bold in the weighted objective tree. For example, “Easy to Use” was a Level 2 objective under the Level 1 objective ‘User Friendly.” “Easy to Use” received a 20% weight compared to the other Level 2 objectives under “User Friendly,” but “User Friendly” received a 16% weight compared to the other Level 1 objectives so “Easy to Use” received a 3% weight overall (0.20 x 0.16 x 100).

One problem associated with this weighting system was that the design team was comparing both upper and lower level objectives at the same time. Sub-objectives, such as Formation Pattern, generally had very small weight percents because they had so many weighted objective levels above them. The more times a weight percent had to be multiplied, the smaller it became. Upper level objectives, such as Able to Be Scaled Up did not have sub-objectives so it retained its original, very high weight percent and was able to effect alternative design rankings in a much larger way. The design team, client, and user felt this weight accumulation system may not accurately reflect how the overall importance of some sub-objectives would actually compare to upper level objectives.

5.2.2.2 New Decision Matrix

In the second matrix system (Appendix E: New Decision Matrix), scores were weighted based on the weight percent of the highest level objective related to each set of sub-objectives, as opposed to the previous system which assigned a weight to each sub-objective. To do this, all the normalized scores assigned to the sub-objectives under a given higher level objective were added up and multiplied by the weight percent of that higher level objective. For example, Sterile Threads, Thread Materials, Thread Diameter, Formation Pattern, Facilitates Buffer Changes, and Seeding Cells in Threads were all sub-objectives under Production. So the normalized scores from each of those objectives were added up and

multiplied by the 14% weight that Production received. The summed and weighted scores from each high level objective were then added up to give each alternative design its final score.

While this new system was advantageous because it eliminated problems comparing sub and upper level objectives, it was flawed in that it did not take into account the individual weights of each sub-objective when their scores were added together. By simply adding all the sub-objectives and multiplying by the upper-level objective, each sub-objective was essentially given the same weight, which was not necessarily accurate. For example, the Bundle Threads Post-Production Modification receive a weight of 20% during pairwise comparisons, nearly twice that of the Sterilize Threads Post-Production Modification. However, with the weighting system of the new decision matrix, this ranking was eliminated.

5.2.2 Results

Overall, there were no changes to the highest ranked alternative designs in each functional category, regardless of which weighting system was used. When comparing the results between both weighting system, the design team, client, and user agreed that this helped to verify the winning design in each functional category. The results from each decision matrix and functional category can be seen in the tables below (Table 51-Table 67). Old decision matrix scores are shown on the left and new decision matrix scores on the right.

The maximum possible score for each objective varied depending on which function was being examined and which weighting system was used. These maximum scores can be seen in Table 51 below.

Table 51: Maximum Scores Possible for Decision Matrix Rankings

Function		Old System	New System
Produce Threads		76.16	296.00
Anchor Threads		51.46	359.00
Facilitate Solution Changes		43.42	126.00
Facilitate Physical Post-production Modifications		48.48	242.00
Control Production Parameters	**Production Parameters**	62.63	229.00
	Temperature	39.00	83.00

It is important to note that the comparative scoring of each design is important, and not the actual score that was received. Because of the different systems of adding and weighting, scores from the second weighting system were higher, but this does not necessarily reflect a better score than was received from the first weighting system. What is important is where the alternative design falls in relation to other alternative designs in that category.

5.2.2.1 Produce Threads

Table 52: Old Matrix Produce Threads Scores

Design	Score
Extrusion Train	68.76
Bi-directional motor head	66.61
Molding	65.07
Conveyor Belt	61.48
Manual Guide	59.17
Cookie Cutter	45.57
Rolling	42.17

Table 53: New Matrix Produce Threads Scores

Design	Score
Extrusion Train	275.92
Bi-directional motor head	271.33
Conveyor Belt	257.67
Molding	243.67
Manual Guide	239.08
Cookie Cutter	204.42
Rolling	198.42

The first category of designs being ranked was the various methods for the production of biopolymer microthreads. Each component in this category was ranked (Table 52; Table 53) based on objectives such as the ability, ease of use, and quality of threads produced. The highest scored design was the Extrusion Train, with new and old weighted scores of 275.92 and 68.79, followed by the Bi-directional Motor Head. Both designs had a high degree of automation, and they would be able to

produce threads with a high degree of precision and reproducibility, but the extrusion train is a slightly simpler design and more user friendly and reliable. The rest of the alternative designs suffered greatly from problems relating to precision and reproducibility of the formed threads.

5.2.2.2 Anchor Threads

Table 54: Old Matrix Anchor Threads Scores

Design	Score
Roughened Surface	50.10
Fill Holes	48.10
Filled Grooved Ends	48.10
Pegs	48.10
Mesh Screen	46.20
Solid Platform with Hole	46.20
Combs	44.76
Combination Anchor and Platform	44.70
Grooved Platform	43.09
Clamps	39.60

Table 55: New Matrix Anchor Threads Scores

Design	Score
Roughened Surface	331.33
Combination Anchor and Platform	321.42
Fill Holes	320.67
Filled Grooved Ends	320.67
Pegs	320.67
Combs	317.33
Clamps	289.33
Mesh Screen	275.50
Solid Platform with Hole	275.50
Grooved Platform	243.83

The next category of design ideas consisted of the different ways for anchoring the biopolymer microthreads to the device during various production and post-production processes. Each design was ranked (Table 54; Table 55) based on the ability to secure each microthread in place, as well as the potential for the design to damage the threads and the ease of use of the design. The highest ranked design was the Roughened Surface anchor system because it had been an effective method in the previous MQP, and had little chance of damaging the threads. This design idea received new and old weighted scores of 331.333 and 50.10. It was followed mostly by design such as Fill Holes or Pegs which also anchored threads on the ends. These designs offered not only effective results, but a high level of versatility that would allow for many kinds of post-production modifications to take place. Their scoring differences mainly arose from issues with ease of use. The Mesh Screen or Solid Platform designs would

anchor threads along the entire length and would have to be adapted to allow for post-production modifications, which is why they received lower scores.

5.2.2.3 Facilitate Solution Changes

Table 56: Old Matrix Solution Changes Scores

Design	Score
Hopper System	42.42
Tilted Floor	41.67
Aspirate	29.67
Tipped Bath	28.84

Table 57: New Matrix Solution Changes Scores

Design	Score
Hopper System	120.67
Tilted Floor	118.00
Tipped Bath	99.08
Aspirate	87.75

The third category was the list of design ideas for performing solution changes for draining and filling the bath. These design ideas were ranked (Table 56; Table 57) based on the ease of use of the design and the likelihood that the design would damage the threads. The hopper system ranked highest with a new and old weighted score of 120.667 and 42.42 due to its ease of use and minimal amount of human contribution. The tilted floor design came in second place. Because one is a method for filling the bath and one is a method for draining the bath, the design team decided to combine both designs into one system that would facilitate solution changes. The Aspirator and Tipped Bath designs were not as user friendly and could potentially damage threads.

5.2.2.4 Facilitate Physical Post-production Modification

Table 58: Old Matrix Bundling Scores

Design (Bundling)	Score
Sliding Anchors	37.87
Jellyroll	37.37
Stacking Anchors	35.48
Thread Plate Sweeper	34.31
Tilting Blocks	34.04

Table 59: Old Matrix Twisting Score

Design (Twisting)	Score
Twisting Caps	38.37

Table 60: Old Matrix Stretching Score

Design (Stretching)	Score
Crank Mechanism	36.12
Vertical Weights	35.37

Table 61: New Matrix Bundling Scores

Design (Bundling)	Score
Sliding Anchors	206.42
Jellyroll	205.08
Tilting Blocks	201.75
Thread Plate Sweeper	190.08
Stacking Anchors	187.08

Table 62: New Matrix Twisting Score

Design (Twisting)	Score
Twisting Caps	210.42

Table 63: New Matrix Stretching Score

Design (Stretching)	Score
Crank Mechanism	194.58
Vertical Weights	177.25

The next category was made up of design ideas for the physical post-production modifications of the threads such as bundling, twisting, and stretching. The main objectives were to provide a system for moving and managing the biopolymer microthreads that would be easy to use and allow for many different kinds of physical modifications. The results of this functional category (Table 58 - Table 63) were divided into three groups because the alternative designs addressed the needs of bundling, twisting, or stretching. The final design for allowing physical post-production modification will either combine or allow for each of these three separate functions.

Sliding Anchors won in the bundling category, with scores of 206.42 and 37.87, because it offered the most versatile and controlled way to create thread bundles. It also should not damage the threads, like the Thread Plate Sweeper design. The Crank Mechanism design won in the stretching

category for a similar reason; it would offer better, more precise control over stretching rate and distance. Though Twisting Caps was not competing against any other designs, it received a high percent of the totals points possible so the design team felt it had sufficiently satisfied the design objectives.

5.2.2.5 Control Production Parameters

Table 64: Old Matrix Production Parameters Scores

Design (Production Parameters)	Score
LabVIEW™	57.63
Manual	42.44

Table 65: Old Matrix Heating Scores

Design (Temperature)	Score
Heated Bath	36.75
Incubator	35.75
Heat Solutions	34.00

Table 66: New Matrix Production Parameter Scores

Design (Production Parameters)	Score
LabVIEW™	212.83
Manual	169.08

Table 67: New Matrix Heating Scores

Design (Temperature)	Score
Heated Bath	73.67
Incubator	70.92
Heat Solutions	68.33

The results in the final design category of device control (Table 64 - Table 67) were also divided into separate groups because the alternative designs addressed controlling production parameters and temperature. LabVIEW™ was chosen for controlling production parameters, with scores of 212.83 and 57.63, because it will offer far better control than a manual system, as has already been demonstrated in a previous MQP. For temperature control, the heated room design was disqualified because it did not meet the following constraints: time limit, budget, and must fit on lab bench. The hot plate design was disqualified because it did not meet the constraint which specified that the device must be safe for the user, and it was also noted that it would be difficult to find a hot plate that accurately worked at 37 °C. The heated bath design won with scores of 73.67 and 36.75 because it is simple, effective, and also currently used in the client's lab.

5.3 Final Conceptual Design

By combining the winners from each category, a final conceptual design (Figure 47) was formed which the client, the design team, and the user felt had the best chance at achieving the wants and needs of the original problem statement. The final conceptual design is made up of the following: 1) an extrusion head which is controlled by a motor that guides it along a predetermined path, 2) a double walled bath which circulates water through an immersion heater, 3) a sliding anchor system composed of roughened surface anchor points which would secure the biopolymer microthreads in place as well as allow for the threads to be easily moved together for bundling or twisting, 4) a hopper solution system and a tilted floor for solution changes. In addition, the design contains a twisting mechanism, a stretching mechanism, and LabVIEW™ as a control system for the device (not shown).

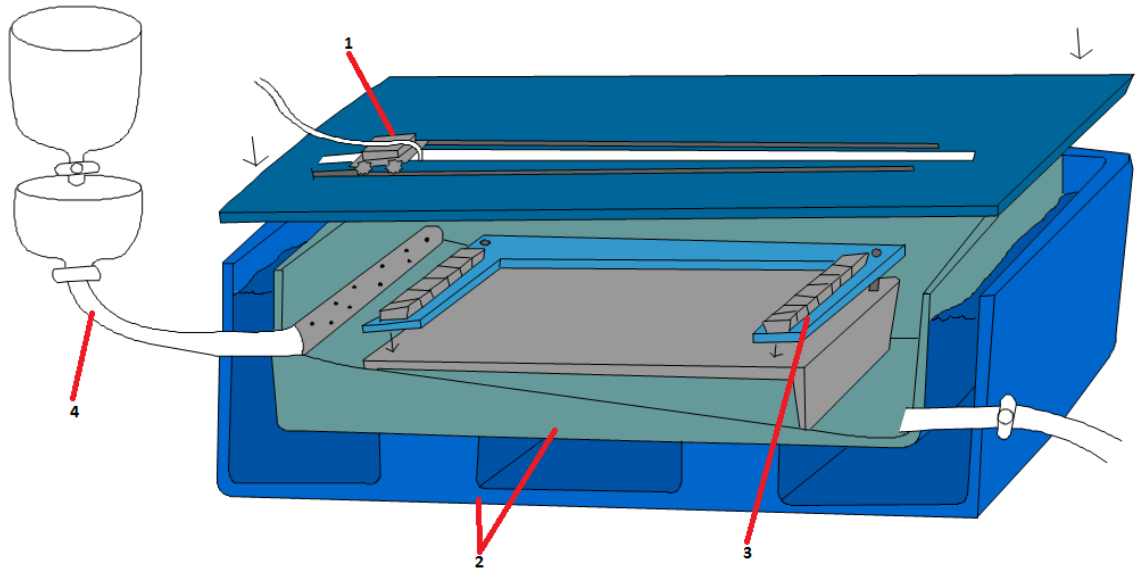


Figure 47: Schematic of Final Conceptual Design

The extrusion train (Figure 48) would increase both the consistency of the diameter of the threads as well as the production rate, and it minimizes the amount of human contribution necessary for the device to function. The motorized component would receive electrical input from LabVIEW™ to regulate the speed of the device, and would travel along a guided track which will most likely resemble a

rack and pinion system. A syringe pump would be connected to the device and microthreads would be extruded along the guided pathway.

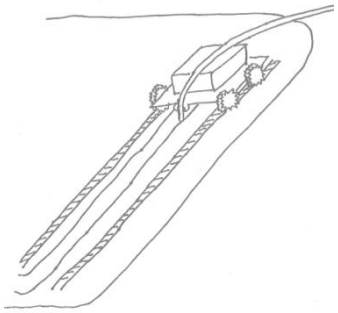


Figure 48: Drawing of Extrusion Train

The double walled, heated bath system (Figure 49) would use circulating hot water to heat the contents of a smaller bath containing the microthreads in a buffer solution. The bath will be heated with the use of an immersion heater, and will be constructed out of Lexan®, which has been shown to exhibit no significant amount of leaching when used in collagen microthread production [Bishop, 2005]. A methylene chloride adhesive will be used to secure the bath system.

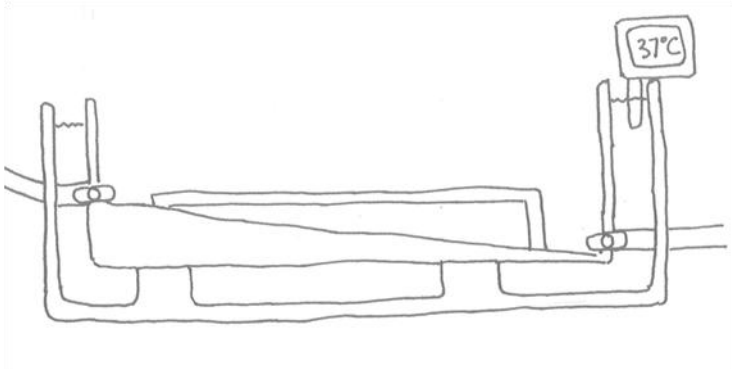


Figure 49: Drawing of Heated Water Bath with Immersion Heater

The hopper system (See Figure 32) will allow for easy measuring and filling of new solutions without damaging the threads, and will most likely be composed of Pyrex® lab containers and polyethylene tubing. Because both of these materials are often used in laboratory procedures, it is unlikely that they will react with buffer solutions. Once the desired amount of solution has been drained from the upper container into the lower container, the solution can be emptied into the bath system

through a tube with a series of increasingly large holes to allow for gentle filling of the bath. The drainage system would allow for the removal of solution through the use of a tilted floor and a valve for drainage. Extrusion of the microthreads will occur on a nearly level surface which will be supported above the tilted floor. This will reduce any negative effects due to fluid movement as the solution is drained from the bath.

Once extruded, with the help of the roughened sliding anchor system, the threads would remain in place during production and solution changes. Preliminary anchor testing (See Section Initial Anchor Design Testing) was successful in proving the effectiveness of a roughened polycarbonate anchor. By being able to slide the anchor points back and forth (Figure 50), each thread can be moved closer to one another in order to bundle groups of threads together, or to allow for bundles of threads to be twisted.

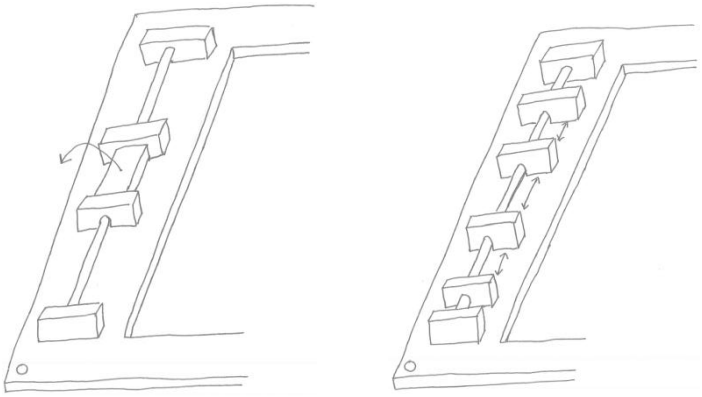


Figure 50: Drawing of Sliding Anchor System

This twisting can be accomplished with the use of a twisting cap, which will be created from Lexan®, cable ties, C-clamps, or another method. Because uniform stretching of microthreads is important, a mechanism for stretching has been designed which will attach to the sliding anchor system and allow for precise stretching to be performed on the microthreads. This mechanism (Figure 51) will be composed of a crankshaft which rotates through a threaded hole that extends or contracts to stretch the threads, and two guide rods on either side maintain parallel motion. The crankshaft and guide rods will most likely be composed of a metal component, and the rest of the component will be made of

Lexan®. Finally, the device would be controlled by LabVIEW™ in order to maximize precision and to increase production rate of biopolymer microthreads.

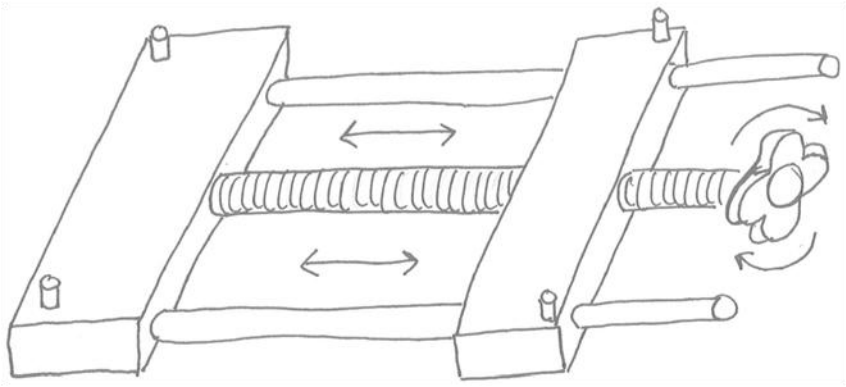


Figure 51: Drawing of Crank Mechanism

6.0 Design Verification

Immediately following the development of the final conceptual design, the project team proceeded to test some of the initial components. This proof-of-concept testing is an extremely important aspect of the design process. It determines whether a specific portion of the project is able to meet the design requirements [Dym, 2004]. In order to help clarify which components of the device are being referred to in the upcoming sections, a table was made which includes the names and descriptions of all individual components and can be found in Appendix F: Parts List In addition, a budget with the costs of each of the parts in the device was created and is located in Appendix G: Budget List.

6.1 Initial Anchor Design Testing

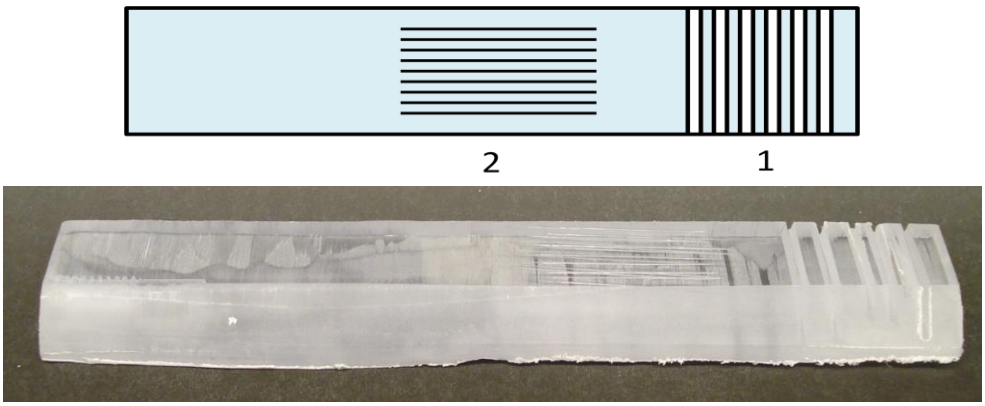
One important design function is that the anchors need to properly secure the threads. Therefore, the first test that was conducted involved evaluating the anchor point designs. The project team decided that these should be made out of Lexan®. This decision was based on the fact that it is available in multiple thicknesses, it will not leach toxic chemicals into the bathwater, and is also easy to machine and relatively inexpensive. Additionally, a Lexan® anchor system was used by a previous MQP

and demonstrated effective thread attachment [Bishop, 2005]. When obtaining materials the design team opted to not limit polycarbonate components to be built from brand name Lexan®, but instead obtained any polycarbonate brand that was readily available to them. First, strips of polycarbonate were cut into the shape seen in Figure 52 below using a vertical band saw.

Figure 52: Side View of Tested Anchor Points

The project team believed that this sloped shape would facilitate thread viability by creating a ledge which would prevent breakage as they were being extruded. Originally, these anchors were made with ½" thick polycarbonate, but this proved to be too high for the level of solution in the bath. As a result, the design team needed to plane the anchors down to a thickness of approximately ¼" with a vertical band saw before use.

Next, in order to determine which type of anchor would be most conducive to attachment, the project team used a vertical band saw, Leatherman® pocket knife, drill press, and hack saw to score rectangular polycarbonate pieces into different patterns. Top-down views of these can be seen in Figure 53. They are also described in detail in Table 68 below.



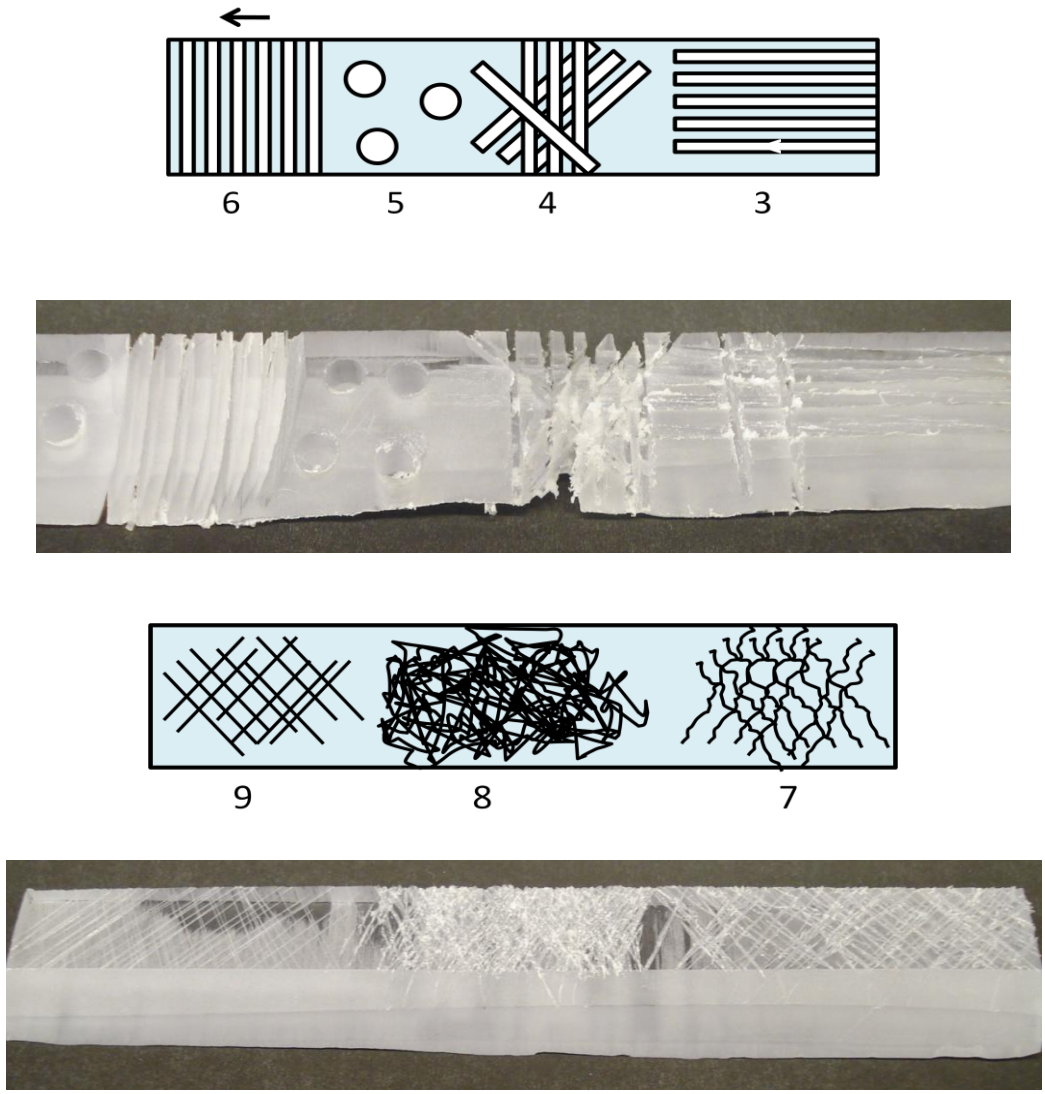


Figure 53: Schematics and Pictures of Different Anchor Patterns

Table 68: Description of Different Anchor Patterns

NUMBER	TYPE	MACHINE/METHOD USED
1	Vertical Deep Slits	Vertical Band Saw
2	Horizontal Knife Scoring-Shallow and Straight	Leatherman® Straight Blade
3	Horizontal Deep Slits	Vertical Band Saw
4	ZigZag Deep Cut	Vertical Band Saw and Hacksaw
5	Fill Hole	Drill Press
6	Angled Vertical Slits-Deep Cut	Vertical Band Saw
7	Roughened Criss Cross	Leatherman® Saw Blade
8	Roughened	Leatherman® Saw Blade
9	Small Criss Cross	Leatherman® Straight Blade

The design team made the decision to use collagen threads as a test model. However, it is important to note that the device will be designed to accommodate both collagen and fibrin thread production and post production. Collagen was chosen as a model for several reasons. First, the design team was constrained by their budget and could not afford to test their prototype with other thread materials. Second, collagen formation only requires one extrusion solution, and this greatly decreases the cost associated with testing as opposed to the two solutions needed for fibrin. In addition, collagen thread production has more requirements such as multiple bath changes. Therefore, if the device can accommodate and support the production and post production of collagen threads, than it will also do so for fibrin threads.

Sets of four collagen threads were extruded onto each pattern. The process for production of collagen threads can be seen in Section 3.3.1.1.1. A total of 36 small threads (approximately 2.5 inches) were extruded onto the polycarbonate anchors and into the bath. At each buffer change, they were picked up by hand from the edges of the polycarbonate in order to move them from bath to bath as well as to test their attachment strength on each anchor point (See Supplemental Video 1).

The general observations noted by the design team were that the polycarbonate pieces need to be low profile so that they can be completely submerged in the least amount of solution as possible. They must also be stable as the thread is being extruded, which includes remaining properly submerged in solution throughout the process. Overall, it was noted that the threads stuck well on the inclined platform, which means that this is an effective shape. If the threads broke during extrusion, they generally broke at the edge between the platform and the bath. A photograph of the threads extruded on a set of anchor points can be seen in Figure 54 below.

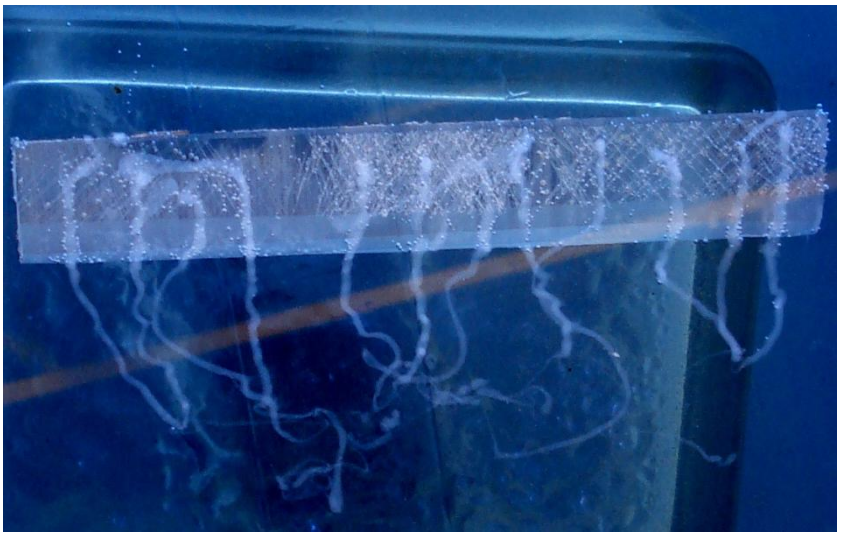


Figure 54: Photograph of Collagen Threads Attached to an Experimental Anchor

After the collagen threads were fully formed as shown above, the project team further investigated the effectiveness of each method for anchoring collagen threads by conducting pull tests. The purpose of these tests was to examine how much force could be applied to threads before thread detachment. The threads were stretched at a constant rate while hydrated in the last buffer solution, DI water. The pull tests were done by hand using tweezers to examine uniaxial loading. Threads were pulled along the bottom of the dish in the direction of the arrow displayed in Figure 55 below (See Supplemental Video 2).

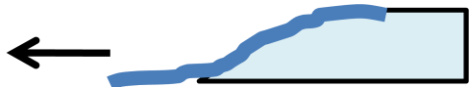


Figure 55: Schematic of Pull Testing Procedure

Because the pull tests were done by hand, a set of metrics was developed in order to quantify the strength of the attachment between each thread and the anchor points, which can be seen in Table 69 below.

Table 69: Metrics Developed for Pull Testing

Metric	Score
Broke during bath changes	1
Broke right away during pull testing	2
Broke after slight tension applied	3
Broke only after significant tension applied	4

Three out of the four threads on each anchor design were pulled using the pull test described above. The design team took observational notes regarding the behavior of the pulled threads by anchor design. A summary table of this information can be seen in

Table 70 below.

Table 70: Qualitative Results of Pull Testing

Pattern	Observation
1	• Noticeable stretching before breaking • 2 threads broke just before incline slope • Begins to pull off incline • 1 thread broken before testing at the end of the anchor
2	• More tension than previous • There is resistance, but broke at the end of the anchor
3	• Broke within thread, not as a result of anchor point • One thread was too thin initially to evaluate
4	• Could not withstand much tension • Broke off at edge of anchor point
5	• Collagen did not fill in holes completely • Thread broke after pulling it out of the hole • Thread itself broke, not near anchor
6	• Pulls itself off the anchor then breaks

	• Minimal stretching
7	• Very good tension • 1 thread broke at the edge of the incline • 1 thread broke halfway up the incline
8	• Visible weak point in thread • 1 thread broke at the edge of the incline • 1 thread broke halfway up the incline
9	• Much greater tension than most others • Broke at the edge of anchor point • Break is in the thread, does not come off anchor

In addition to observational notes, data from each thread was quantified using the metric system. The data that is missing is from threads that broke or were too thinly extruded to evaluate. The results from these tests can be seen below in Table 71.

Table 71: Quantitative Results of Pull Testing

Pattern →	1	2	3	4	5	6	7	8	9
Metric Results	3	4	4	2	3	2	3	-	4
	3	3	-	2	2	2	4	3	3
	1	1	1	1	-	-	-	-	3

The team used these results to arrive at several conclusions regarding the anchoring system. Most importantly, all of the threads were able to adhere to the anchors. This is why a pull test was performed, to better establish which anchor system would be most effective. In addition, the team noticed that most of the threads were breaking along their length, as opposed to coming off of the anchor points, which again proved that the anchors successfully facilitated thread adhesion. In regards to pattern numbers two and three, the design team noticed that horizontal slits did not pull off the anchor points. During the test for number 5, the fill holes, the collagen didn't fill into the hole and form

an anchor as was expected. It merely wrapped itself around the edge of the hole, causing the thread to be improperly secured. The deep angled vertical slits, number 6, were too wide for the threads. Therefore, during the pull tests, the threads tended to pull off the anchors because they did not fit. Number 7, the deeper criss-cross, generally seemed too jagged for proper adherence. Therefore, in general, it was concluded that shallower scored polycarbonate displayed better adhesion.

Following the pull test, the last thread at each anchor point was dried for 24 hours and rehydrated overnight in DI water to test if the threads continued to stick. Each thread successfully passed this test. Therefore, the team concluded that the preliminary tests of the anchor points were successful.

6.2 Inner Bath Dimensions

Before construction of the tilted bath system could begin, approximate dimensions of the bath would need to be chosen and a final angle of tilt for the floor was necessary. In order to control expenses, the bath system would need to allow for production of a large number of threads using the least amount of fiber formation buffer solution. Currently, the client uses 700mL of buffer for each 5mL amount of collagen extrusion solution (50mg total collagen), which makes approximately 13 threads. For the design team, the client said a 10mg of collagen: 100mL ratio would be sufficient. Based on these needs, the group created a spreadsheet using Microsoft® Excel to aid in selecting final dimensions for the bath (See Supplemental Spreadsheet 1). The spreadsheet allowed the design team to select a length, a width, an angle of tilt, and the amount of extra space above the top of the bath. Next, the user would select the dimensions of the inner wedge of the bath on top of which the microthreads would be extruded. The first calculation performed by Microsoft® Excel was the total volume of solution necessary to fill the entire bath, and was calculated in parts as follows: the total volume of the bath, which was the

sum of the volume of the triangular wedge created by the tilted floor and the volume of the extra space above the wedge, and the total volume of the inner wedge, which was based off the length, width, and angle of tilt selected by the user. By subtracting the volume of the inner wedge from the rest of the bath, the total amount of solution necessary to fill the entire bath was calculated. A sample calculation can be seen below in Figure 56.

	A	B	C	D
1	Dimensions and Volume of Platform		Volume of Liquid Necessary for Soaking Threads	
2	Length (in)	12.000000	Volume (in3)	59.845029
3	Width (in)	7.500000	Volume (mL)	980.680497
4	Angle Deg	10.000000	Volume (L)	0.980680
5	Angle Rads	0.174533	Volume (ft3)	0.034633
6	Height Platform (in)	2.115924	Volume (gallon)	0.259068
7	Extra Height (in)	0.500000		
8	Volume (in3)	140.216570		
9	Volume (mL)	2297.728926		
10	Volume (L)	2.297729		
11				
12	Dimensions and Volume of Wedge			
13	Length (in)	11.500000		
14	Width (in)	7.000000		
15	Angle Deg	10.000000		
16	Angle Rads	0.174520		
17	Height Platform (in)	1.996808		
18	Volume (in3)	80.371540		
19	Volume (mL)	1317.048429		
20	Volume (L)	1.317048		

Figure 56: Screen Shot of Sample Calculation performed in the Excel Spreadsheet which was used to calculate total volume and approximate drain time of the inner bath

For example, with a length of 12 inches, a width of 7.5 inches, extra height of 0.6 inches, and an angle of tilt of 5 degrees for the large component of the bath, and inner wedge dimensions of 11.5 inches in length, 7 inches in width, and 5 degrees in angle of tilt, the volume would be approximately 1 L. This was calculated by performing the following calculation for the large component of the bath:

$$\left(Length * Width * (0.5 * \tan(\Theta) * Length)\right) + (Extra\ Height * Length * Width)$$

and subtracting out the volume of the inner wedge:

$$(Length * Width * \tan(\Theta) * \ Length * 0.5)$$

The final equation for the given dimensions would be:

$$\left(12\ inches * 7.5\ inches * (0.5 * \tan(5) * 12\ inches)\right) + 0.5\ inches * 12\ inches * 7.5\ inches - \ (11.5\ inches * 7\ inches *$$

$$\tan(5) * \ 11.5\ inches * 0.5) = 60.90\ \text{in}^3 * \left[\frac{16.386\ mL}{1in^3}\right] * \left[\frac{1\ L}{1000\ mL}\right] = 0.998\ \text{L}.$$

With the aid of the spreadsheet created using Microsoft® Excel, final dimensions were calculated for the bath system and can be seen in Table 72. The angle of tilt was chosen based on preliminary testing which was performed by the design team. The experiment involved the use of a thin, smooth metal surface, and water. With the aid of a protractor, the metal surface was secured at a given angle and a small amount of water was poured onto the metal surface. Once this was complete, the design team observed whether or not the water would trickle down the metal surface or whether, partly due to surface tension, the water remained on the surface and did not move. At 5 degrees of tilt, it was observed that the water had a tendency to remain on the surface, but when this angle was increased to 10 degrees, runoff occurred. Because of this, it was initially decided that 10 degrees would be sufficient for use in the bath system. Based on these final dimensions, a total volume of approximately 1 L was calculated as necessary to fill the bath.

Table 72: Proposed Final Bath Dimensions

	Length (in)	Width (in)	Angle of Tilt (°)	Extra Height (in)
Outer	12	7.5	5	0.65

Wedge				
Inner Wedge	11.5	7	5	

6.3 Immersion Heater Experimentation

In order to begin construction and testing of the water bath system, the design team obtained a HAAKE E12 immersion heater from the Department of Biology and Biotechnology at Worcester Polytechnic Institute. Initial tests (Figure 57) were performed on this heater to ensure that the temperature of the bath water would be able to reach 37°C.

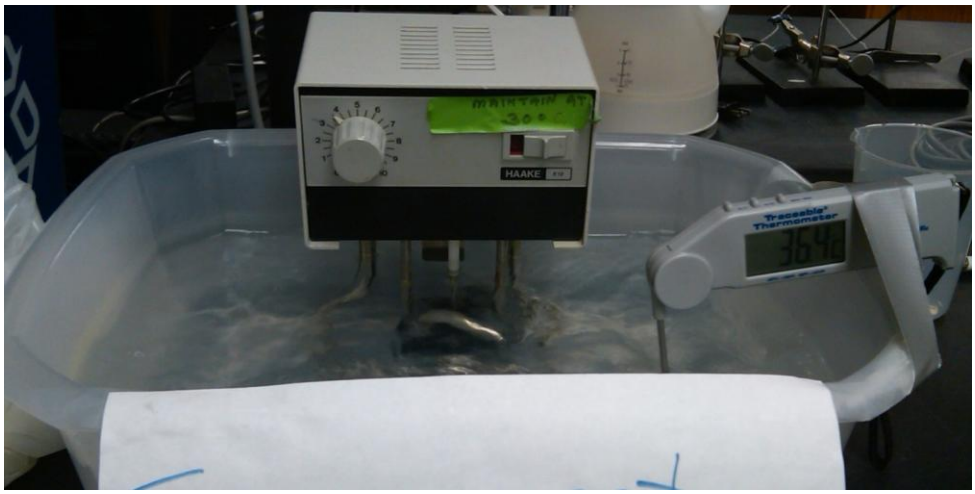


Figure 57: Initial Heater Setup

A plastic tub was filled with tap water and the immersion heater was placed inside. The dial was set to "4" and both a glass and electronic thermometer were used to monitor the temperature of the bath water in 10 minute increments until a relatively constant temperature was reached. Results from this preliminary test can be seen in Table 73 below.

Table 73: Results from Initial Tests of Immersion Heater

Time	Temperature (°C)
0	27.5
10	35.5
20	40.6

The test was successful and it was found that the increments on the dial, 1,2,3,4, corresponded to 10, 20, 30, and 40 degrees Celsius, respectively. Therefore, it was concluded that this immersion heater would be sufficient to properly heat the final design. Additionally, the design team noted that the immersion heater had a very powerful water circulator pump which sufficiently moved the water around the entirely of the experimental bath. Furthermore, the heating element was quite powerful, as it was able to heat the water in the plastic tub about 13 degrees in 20 minutes.

After verifying the viability of using an immersion heater system, the design team needed to perform further testing to quantify the equipment's performance. This included testing: 1) the heating profile of the immersion heater, 2) its heating performance over time, and 3) how the temperature of the water in the outer bath would affect the temperature of the solutions in the inner bath. From discussions with the client and user, the design team decided that the temperature of the solution within the inner bath must between 36.0°C and 37.0°C at all times. A temperature slightly (less than 0.5°C) below this range was acceptable, but anything above 37.0°C would cause denaturing of the threads.

6.3.1 Heating Profile Initial Trial

The design team began by testing the heating profile of the immersion heater. Three separate trials were performed and each was separated by at least 5 hours to prevent any residual heat affecting the results of subsequent trials. Using a similar set-up to initial viability testing, a large plastic tub was filled with water and the immersion heater was placed inside. A digital Fisher Scientific thermometer was placed inside the tub to monitor temperature. Each trial lasted for two hours, to allow the

immersion heater to heat the bath up fully and allow the design team to observe the short term precision of the immersion heater. During Trial 1, temperature measurements were taken every ten minutes, but because of the immersion heater's powerful heating element, this proved to be too infrequent to generate an accurate heating profile. Therefore, measurements were taken every five minutes in subsequent trials. The design team also used a slightly different temperature setting in each trial to try and determine which immersion heater setting corresponded to 37°C. Results from the three trials can be seen in Figure 58 and Table 74 below.

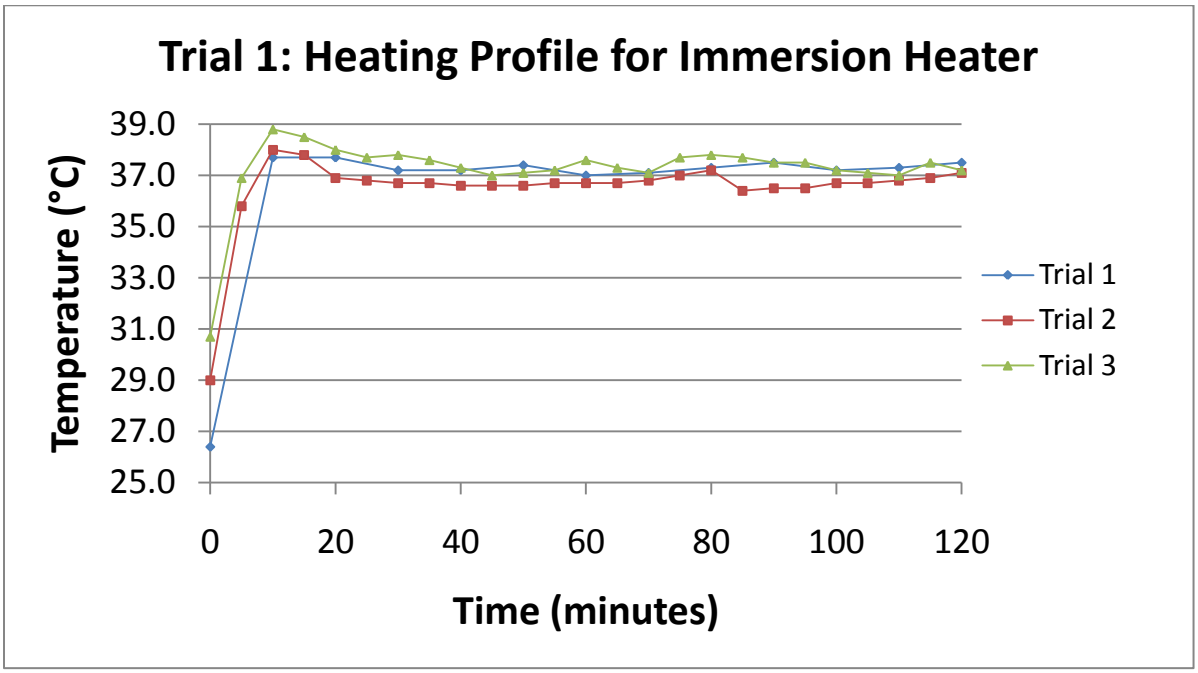


Figure 58: Graph of first three heating profile trials using immersion heater

Table 74: Results from first three heating profile tests using immersion heater

Time (minutes)	Temperature (°C)		
	Trial 1	Trial 2	Trial 3
0	26.4	29.0	30.7
5	-	35.8	36.9
10	37.7	38.0	38.8
15	-	37.8	38.5
20	37.7	36.9	38.0
25	-	36.8	37.7
30	37.2	36.7	37.8
35	-	36.7	37.6
40	37.2	36.6	37.3
45	-	36.6	37.0
50	37.4	36.6	37.1
55	-	36.7	37.2
60	37.0	36.7	37.6
65	-	36.7	37.3
70	37.1	36.8	37.1
75	-	37.0	37.7
80	37.3	37.2	37.8
85	-	36.4	37.7
90	37.5	36.5	37.5
95	-	36.5	37.5
100	37.2	36.7	37.2
105	-	36.7	37.1
110	37.3	36.8	37.0
115	-	36.9	37.5
120	37.5	37.1	37.2

Based on the three heating profile trials, the design team concluded that it takes approximately 20 minutes for the immersion heater to bring the outer bath to temperature. With an acceptable temperature range of 36.0-37.0°C, the design team decided that outer bath could be considered fully heated to a consistent temperature when the temperature measurements taken over the various time

intervals did not vary by more than 0.5°C. In Trials 2 and 3, this point occurred after 20 minutes. In Trial 1, this point was achieved at the 30 minute mark, but Trial 1 had longer time intervals between measurements so it is possible that the outer bath was heated a short time before that. Additionally, the design team noted that this consistent temperature state was maintained over the entire two hour period, with the exception of one time interval, around the 70-80 minute mark, in Trials 2 and 3. In terms of the settings on the immersion heater, temperatures above 37.0°C were achieved in all three trials; however, in the final bath system, heat would need to be transferred from the outer bath, through the polycarbonate inner bath, and into the buffer solution in the inner bath. This means that an outer bath temperature of slightly higher than 37.0°C may be acceptable or even necessary to achieve an inner bath temperature within the range of 36.0-37.0°C. The correct immersion heater setting could not be determined from this experiment alone.

6.3.2 Heating Profile Long Term Trial

While the initial heating profile experiments demonstrated that the immersion heater was able to maintain a consistent (temperature fluctuations of no more than 0.5°C) bath temperature for two hours, collagen microthread production is a 72 hour process, so the immersion heater must be able to maintain a relatively constant temperature over this entire time frame to avoid damage or denaturing of the threads. To test this ability, the design team set up a long term heating experiment. The immersion heater and a thermometer were placed in a filled plastic tub exactly like the previous experiments. Because the experiment was taking place over several days, it was necessary to place plastic wrap over the tub to prevent evaporation. Temperature measurements were taken every 5 minutes for the first hour and then every hour for the next 12 hours and every 12 hours for the next 72 hours. This allowed the design team to observe the initial heating profile of the immersion heater as well as its ability to maintain a precise water temperature. Results from this experiment can be seen in Figure 59 and Table 75 below.

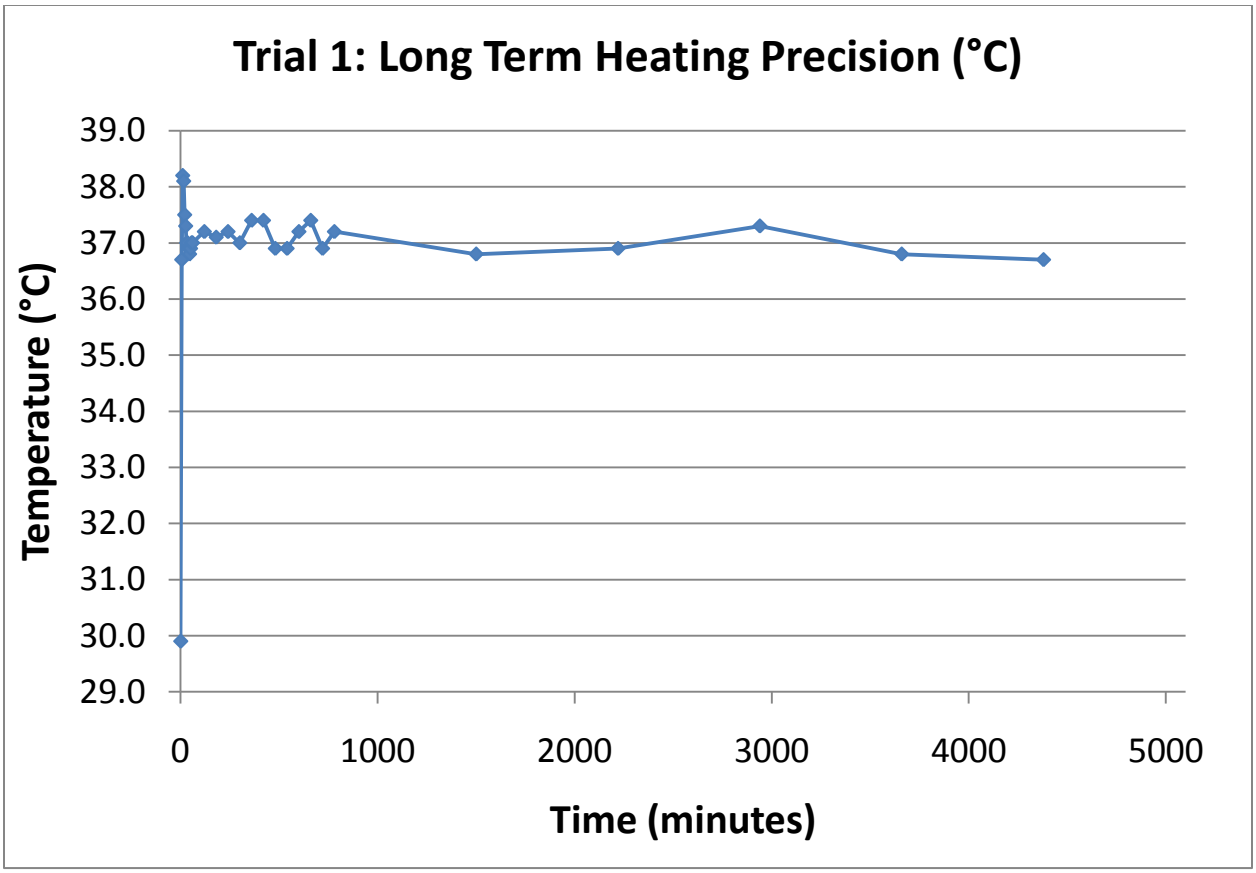


Figure 59: Graph showing results from long term heating test

Table 75: Detailed results from long term heating test

Time (minutes)	Temperature (°C)	Time (minutes)	Temperature (°C)
0	29.9	**300**	37.0
5	36.7	**360**	37.4
10	38.2	**420**	37.4
15	38.1	**480**	36.9
20	37.5	**540**	36.9
25	37.3	**600**	37.2
30	37.0	**660**	37.4
35	37.0	**720**	36.9
40	36.9	**780**	37.2
45	36.8	**1500**	36.8
50	36.9	**2220**	36.9
55	37.0	**2940**	37.3
60	37.0	**3660**	36.8
120	37.2	**4380**	36.7
180	37.1	**5100**	36.9
240	37.2		

By the design team's definition of a consistent bath temperature, fluctuations of no more than 0.5°C, the immersion heater was able to maintain a consistent bath temperature over the entire 85 hour period. Also, the same heating profile was observed as in the previous trials. While once again the bath temperature did rise above the 37.0°C limit, as previously stated that limit does not apply to the temperature of the outer bath, but rather only the solution within the inner bath.

6.4 Outer Bath Construction and Leak Testing

In order to maintain the microthreads at 37°C, the design or purchase of an outer bath heating system was required which the inner bath could be placed into, as can be seen in the model below in Figure 60.

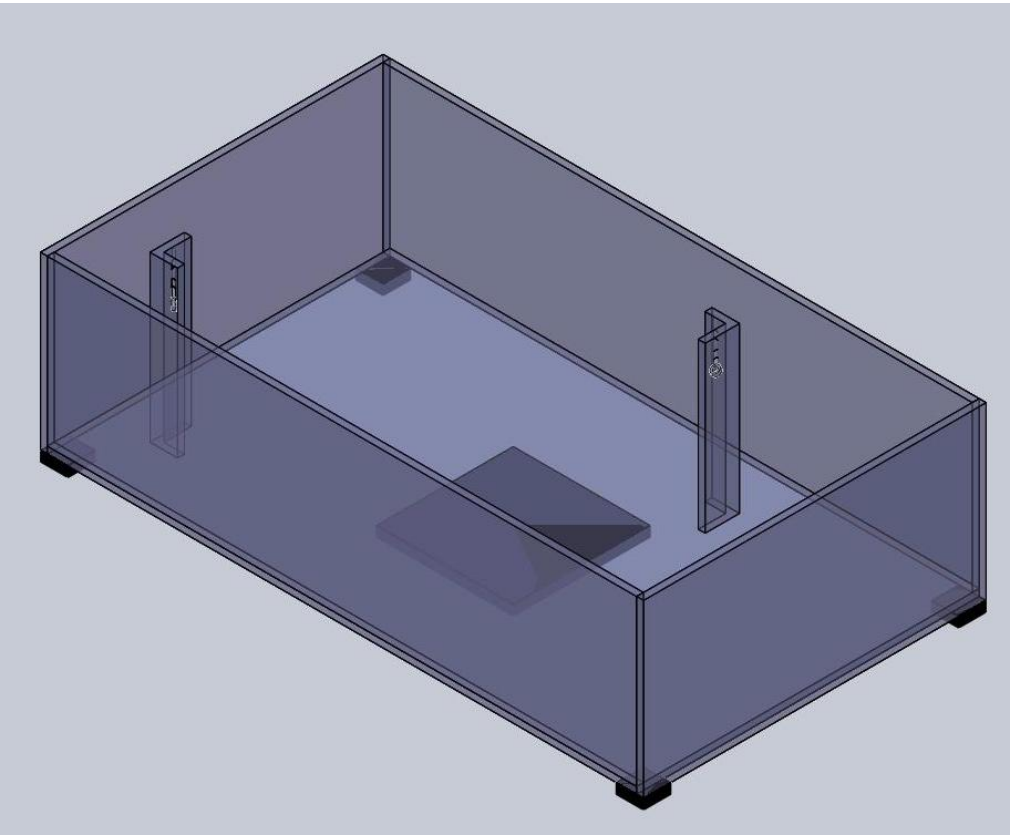


Figure 60: Model of Outer Bath

Initially, the design team did not feel this component needed to be custom built because the client currently uses a commercially bought system in his laboratory and the dimensions of the outer bath did not need to be precise as long as it provided adequate heating. However, as per the client's suggestion, it was decided that building the bath from polycarbonate was a feasible option, as it was in the team's budget and would allow for greater control of bath dimensions. The final design for the outer bath consisted of five polycarbonate sheets, cut to size, and glued using methylene chloride glue. The final dimensions of the outer bath were 21.5" x 12" x 6". After allowing the glue to cure for 24 hours, the bath was filled with water and the immersion heater was attached to the side. The heater was adjusted until a temperature of 37°C was attained in the bath. The bath was allowed to run overnight, under conditions that would be similar to its use in a laboratory setting, to test for leaks. In order to improve visual observation and in case of evaporation, the water was dyed blue and paper towels were placed around the bath's outside edges. Had any leakage occurred and evaporated, the dye would still be present on the paper towels. However, no leaks were present and testing with the inner bath began.

During heating profile testing with the inner bath, it was discovered that there was a considerable amount of heat loss into the bench top. To alleviate this issue, high density foam feet were cut and attached to the bottom of the bath (Figure 61), raising it above the lab bench.

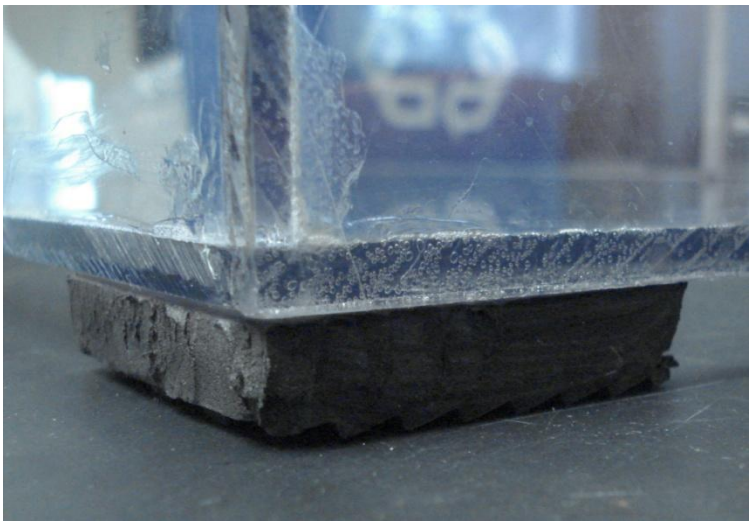


Figure 61: Rubber Feet on Bath System

Additionally, evaporation and the resulting heat loss were found to be major issues in heating the inner bath efficiently using the outer bath system. To help eliminate heat and water loss from evaporation, lids were made out of acrylic for both the inner and outer bath. Both lids had holes drilled into them to allow thermometer probes to fit into each bath, and a space was cut from the outer bath's lid to allow the immersion heater to fit. The team observed an issue with the lid for the outer bath. The heat combined with the absorption of evaporate by the acrylic caused the lid to deform so that it no longer laid flat on the top of the bath. The lids were also made out of black acrylic which did not allow for adequate observation of the bath while the lids were in place. These two issues prompted the team to remake the lids using transparent polycarbonate.

6.5 Anchor System Design Changes and Experimentation

In order to secure the threads so that they do not float in the bath during extrusion, an anchor system was needed. The initial anchor design was composed of 12 small anchor points which fit into a larger comb as can be seen in Figure 30 in Section 5.3. During thread extrusion, the comb would be placed between the anchor points, but once the threads were fully formed, the comb could be removed and the anchor points slid together to facilitate bundling. To accomplish this, the comb would need to slide easily on and off the anchor points. Each ½" x ¼" x ¼" anchor was machined out of polycarbonate using a band saw and a drill press. A #33 drill bit was used to drill a 0.113" diameter hole into the middle of each anchor point and an aluminum rod was fit through them in order to connect the anchors to one another. Next, a comb device, as seen in Figure 30 in Section 5.1.4.2.9 Combs, was cut to fit over the sliding anchor points using the band saw. A temporary assembly of the system was made by taping the sliding anchor points to a piece of scrap polycarbonate, which can be seen in Figure 62 below. This allowed the design team to identify the advantages and disadvantages of the sliding anchor system before implementing it in the final design.

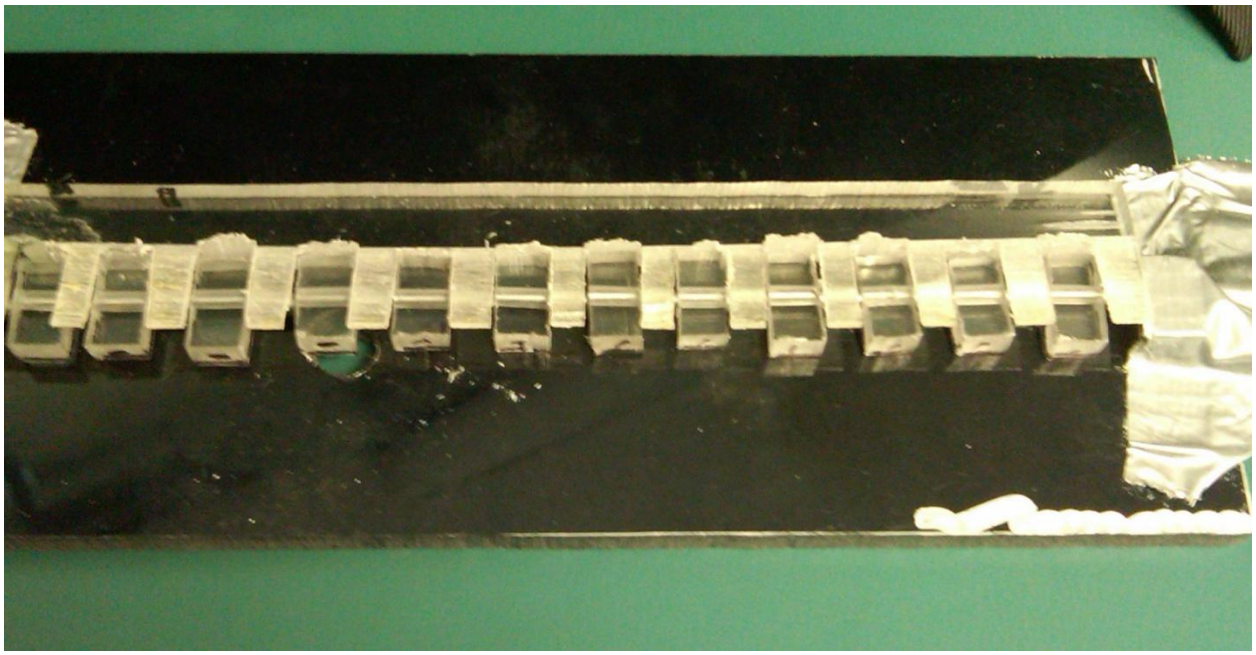


Figure 62: Temporary Assembly of Sliding Anchor Points

Based on this initial experiment, the design team determined two major flaws with this system. Because the anchor points and anchor comb were drilled and cut individually by hand, they were not as precise as was necessary. Each anchor point and each notch in the anchor comb was a slightly different size, which made it nearly impossible to accurately fit all the pieces together. Furthermore, while the anchors could have been more precisely machined using acrylic materials and a laser cutter, the design team felt this would not significantly increase the ease of use of the system. Even with pieces that fit perfectly together, the process of alignment would still be very time consuming, and in the end would not have significantly affected the bundling ability of the device. As a result, the design team decided to move towards an alternative design.

6.5.1 Alternate Design: Roughened Anchor Blocks

Due to the difficulties associated with the use of sliding anchor points, the new anchor system involved no moving parts. Rather, two ¼" polycarbonate strips were cut, using the band saw, to be 7.125" x 0.75". The new anchor points, seen below in Figure 63, were made to be removable in order to

accommodate future design changes, and their surfaces were roughened using a hacksaw and a pocket knife. The hacksaw was used to make deep cuts in the anchor points, and the pocket knife was used to score the surface of the anchor points. By analyzing the results from the anchor tests in Section 6.1, the project team believed that this pattern would produce maximum thread adhesion. With the accuracy of the extrusion head and the versatility of the device coding, the design team decided that bundling could be implemented easily even without the use of sliding anchor points. This could be done by extruding the threads closer together in a partially bundled formation.

Figure 63: New Roughened Anchor Points

The anchor points were attached by pegs to the anchor sides. These C-shaped pieces provided a support for the anchor points to attach to. After extrusion and thread formation, the anchor sides would be detached and serve as a platform for moving the anchor points to the stretching mechanism. In order to move both halves of the anchor system simultaneously out of the bath for post production procedures, removable anchor brackets, which can be seen below in Figure 66 were machined out of ¼" polycarbonate using a band saw. This formed a frame with the anchor points and the anchor sides, shown below in Figure 64 and Figure 65.

Figure 64: Picture of Anchor System

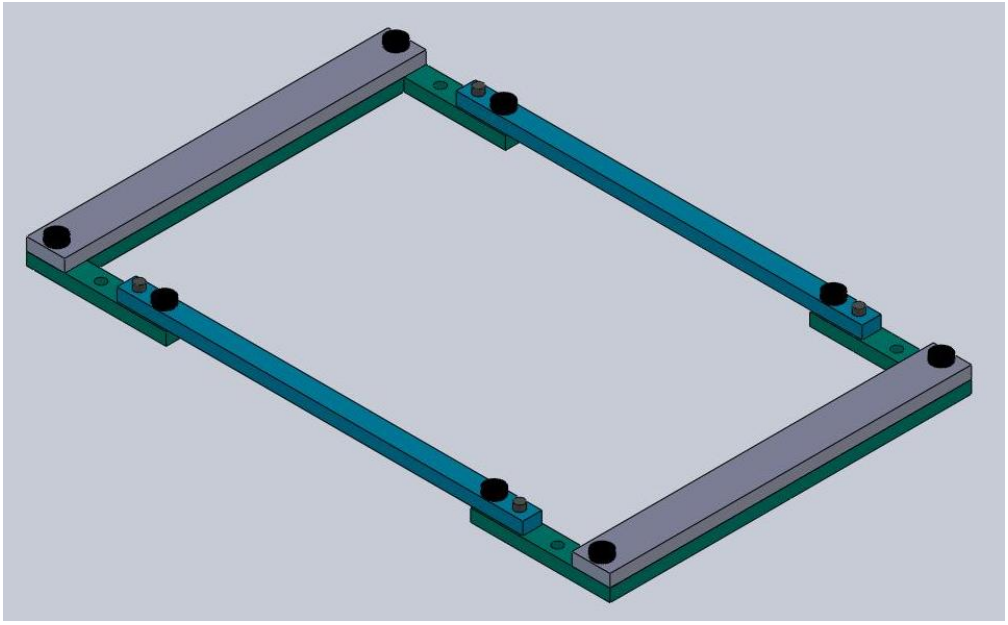


Figure 65: Model of Anchor System

Because the entire anchor system was made to be easily disassembled, Nylatron® screws were used to attach the central components of this frame. Initially, the frame was not sturdy and tilted from side-to-side as force was applied. To resolve this problem, one 3/16” diameter x 0.5” high post was mounted at each end of the anchor sides, creating a secondary attachment point at each end of the anchor brackets. This helped keep the frame rigid.

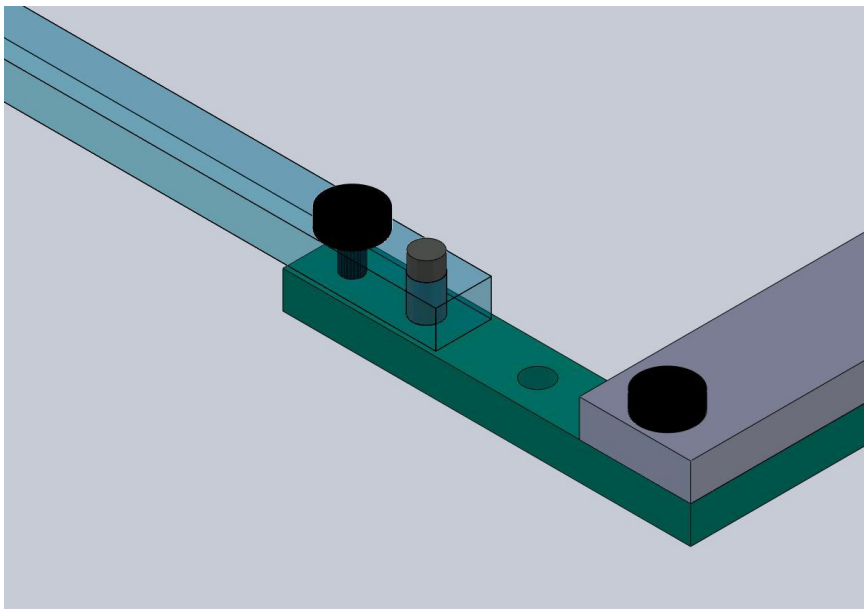


Figure 66: Model of Anti-Tilting Anchor Posts

Next, an anchor block, to be placed in the middle opening of the anchor frame, was made out of black acrylic. This piece allowed extrusion to take place at a constant height and also facilitated easy viewing of the newly formed threads.

6.5.2 Testing of the New Anchor System

After the anchor frame was constructed, threads were made using the current by-hand method and materials, in order to test the efficacy of the anchor design. Because the anchor system was not designed to integrate with the Pyrex® dish currently used by the client, the anchor frame did not line up with the anchor block. This was because the bottom of the dish was curved upward slightly. However, in

order to complete the experiment, it was necessary to use these materials, and so plastic wrap and parafilm were used in order to keep the anchor frame level with the anchor block.

The first trial was performed using insoluble bovine collagen extruded by hand. 5.0mL of 10mg/mL bovine collagen was loaded into the syringe pump at extruded at 0.225 mL/min into a bath of 700mL of Fiber Formation Buffer, which is the standard procedure used by the client. Initially, these threads seemed to form properly, as can be seen in Figure 67 below.

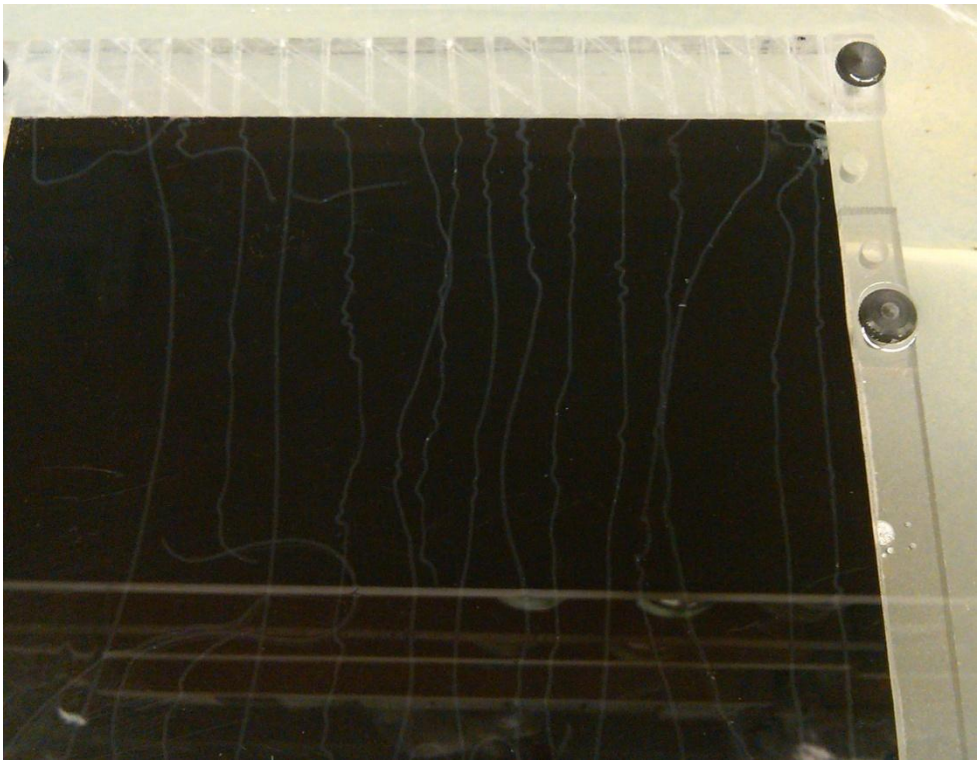


Figure 67: Initial Bovine on Anchors Before

After extrusion, the threads were left in the buffer solution to form overnight. However, 24 hours later, the threads were found to have disintegrated, shown in Figure 68 below.

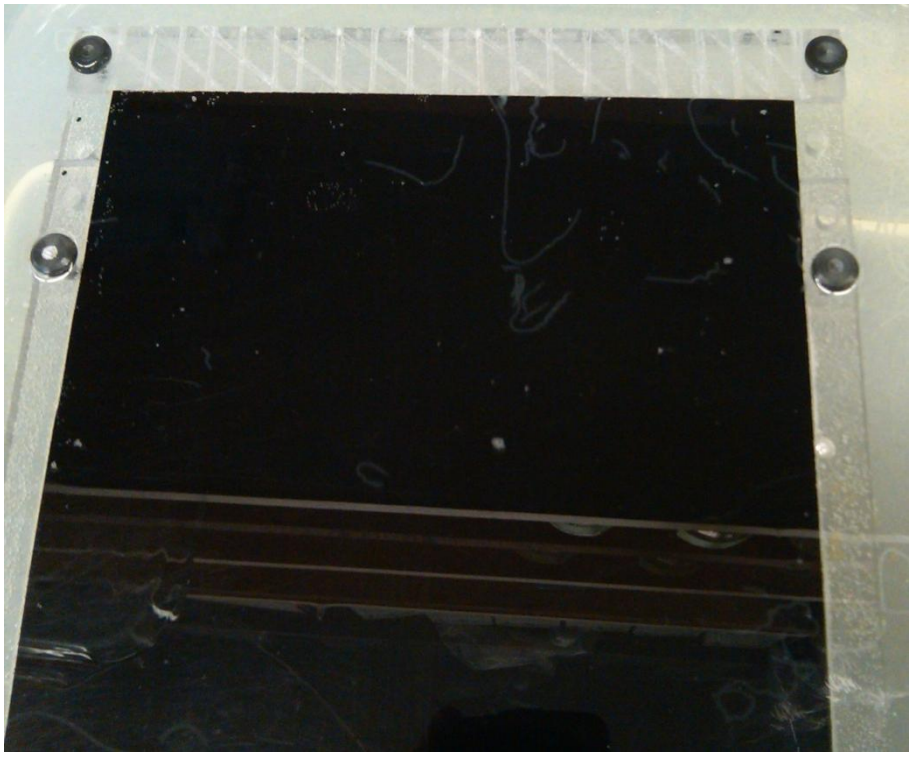


Figure 68: Initial Bovine on Anchors After

Initially, the design team postulated that the pH of the solution might have caused the threads to break down; however, it was found that the pH was approximately 7.4, which was accurate. The client was unsure as to why this had occurred, and advised that the experiment be repeated. Two possible explanations are: 1) because the bath was heated using an immersion heater it was possible that the temperature exceeded 37°C, and this may have denatured the threads or 2) that the threads were disturbed in the bath during formation.

In order to determine the cause of this problem and examine the anchor points, the bovine collagen was extruded again by hand using the same procedure stated above, but this time on the bench top. During this second trial, the threads did not completely disintegrate as they did before, but they were extremely fragile and broke with even the slightest touch by forceps. Therefore, the project group was unable to determine the efficacy of the anchor system.

The third trial was performed using type I collagen from rat tails, which is the type currently used by the client. The standard procedure for extrusion was used in this test, and yielded more favorable results. During this trial, the amount of time required to extrude one thread by hand was recorded and can be seen in Table 76 below.

Table 76: Time Trial results from by-hand extrusion

Trial	Time (sec)
1	51
2	47
3	51
4	47
5	52
6	52
7	58
8	47
9	46
10	46
11	50

The average time for extrusion of one thread was 50 seconds, with a standard deviation of 3.6 seconds. These results were used as reference points for programming the speed of the motor. The threads formed from this test were later used to compare thread geometry to those extruded by the final design. When extruded, the threads adhered to the anchor points, and remained this way through two bath changes (Figure 69), both Fiber Incubation Buffer and deionized water.

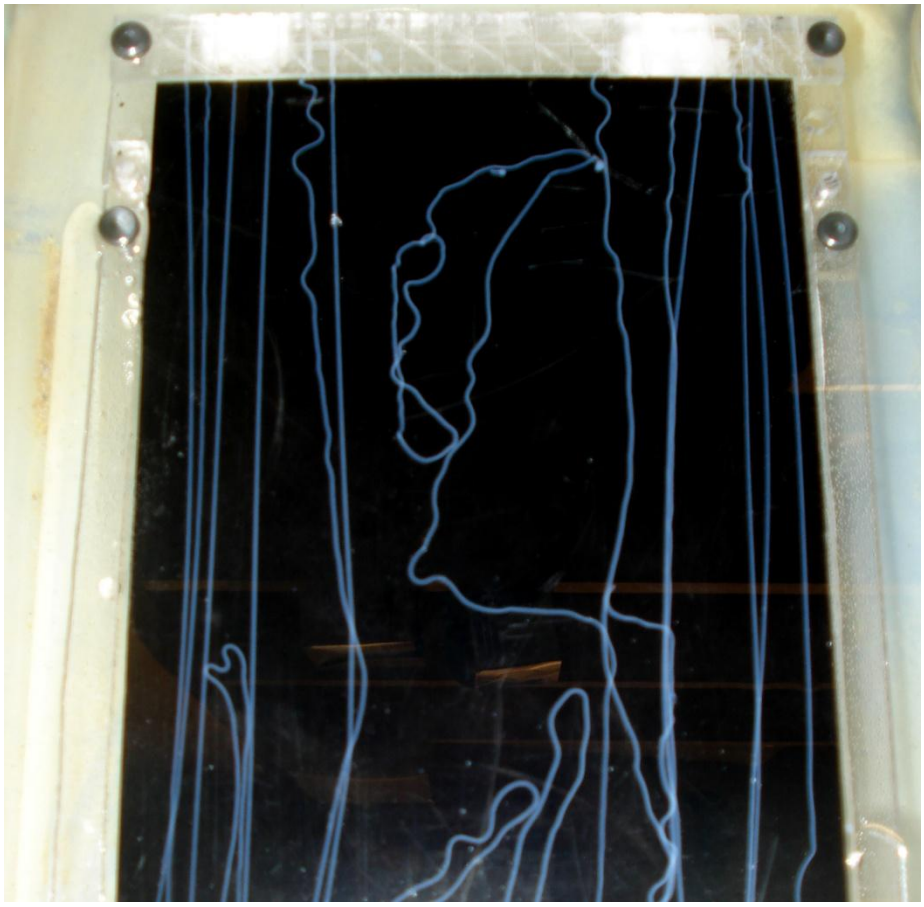


Figure 69: Rat Tail Collagen Extruded on Anchors

As the anchor frame was lifted out of the bath the threads hung without detaching from the anchor points, which can be seen in Supplemental Video 3 attached to this paper and Figure 70 below. Therefore, the roughened anchor system was found to be successful and this new component became a part of the final design.

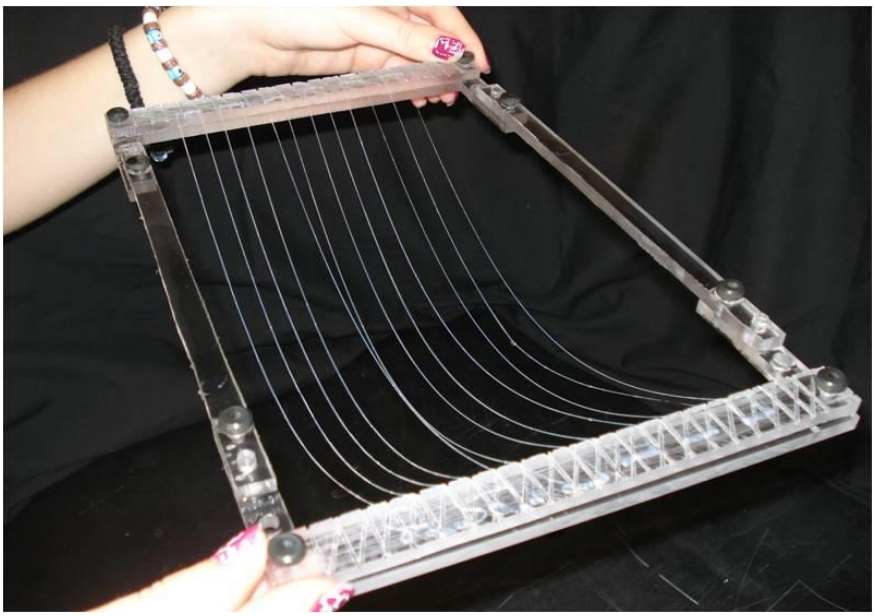


Figure 70: Lifting of Anchor System from Inner Bath

6.6 Physical Modification

In order to facilitate the post production bundling and stretching of the microthreads, experiments and design changes will be made to various components in D-Term. As was previously mentioned, the initial sliding anchor design was not feasible because it was too time consuming to assemble in the inner bath system. In order to continue to aid bundling, the design team will perform experiments by varying the distance between the threads during extrusion. If the threads can be extruded within 0.25" of one another, then the new anchor system would place the threads the same distance apart as the sliding anchor system, and so the user will still be able to bundle them.

6.7 Construction and Testing of Inner Bath

Based on the previous calculations performed by the design team (see Section 6.2), the final dimensions of the inner bath were 12.0" long x 7.5" wide x 4.0" high (inner dimensions) with an inner wedge 11.5" long x 7.0" wide x 0.25" high above the inner bath incline to facilitate water flow

underneath. This would allow for a sufficient number of threads to be made while conserving buffer solution. Though the angle of tilt was initially planned for 10°, when water runoff tests were performed with polycarbonate material instead of aluminum, it was found that 5° of tilt was more than adequate, which can be seen in Supplemental Video 4 attached to this paper. One quarter inch polycarbonate pieces for the inner bath and inner wedge were obtained from Plastics Unlimited Incorporated (Worcester, MA). The design team had the choice to buy cheaper scrap material and cut it to size using a band saw or buy new material and have the pieces cut by the company. In the end, the design team chose to have the pieces cut by Plastics Unlimited because it would be far more accurate and it was very important that the inner bath and inner wedge be completely water-tight. However, the design team did have to cut the sides of the inner wedge because Plastics Unlimited Inc. does not have the ability to make angled or complex cuts, both of which were required for these pieces. However, this was done using a Haas Tool Room Mill TM-1 Computer Numerical Control (CNC) machine to ensure accuracy.

6.7.1 Testing of Methylene Chloride Adhesive

The employees at Plastics Unlimited Incorporated were able to provide the design team with methylene chloride glue specifically designed to bond polycarbonate and create a water-tight seal. The design team was confident that this glue could be used without interacting with the buffer solutions because the employees at Plastics Unlimited Inc. said the glue was used to seal saltwater tanks. The glue sets within 5 minutes and takes about 1 hour to fully cure. To ensure a proper seal, the design team let all glued parts cure overnight.

Initial glue tests involved creating polycarbonate corners which the design team could then fill with water and leave overnight to check for leaking. The first corner was glued by squirting a thin line of glue on one polycarbonate test piece and then attaching a second piece and allowing the glue to cure, which can be seen in Figure 71 below.

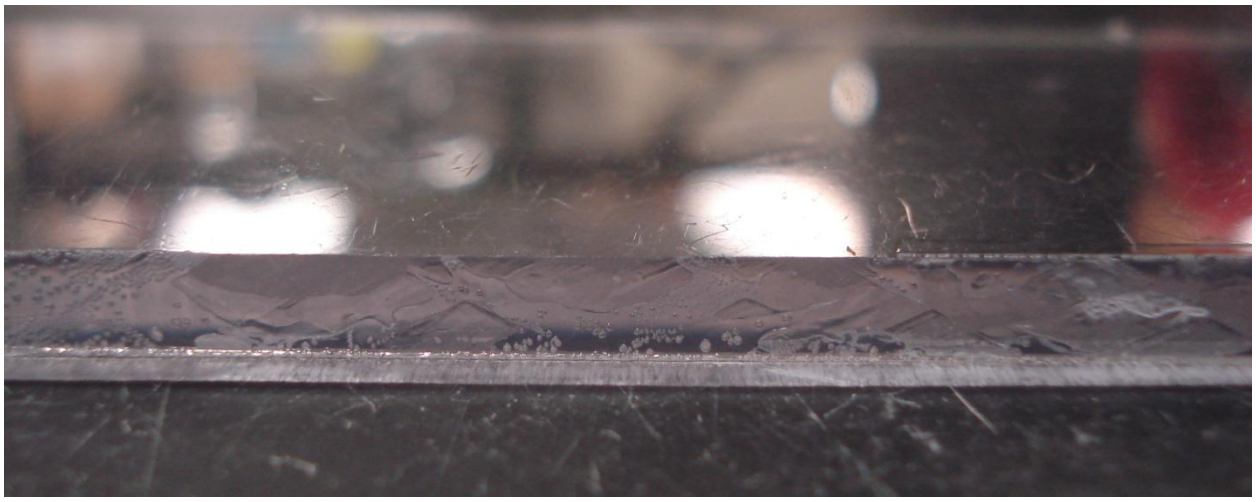


Figure 71: Incorrect Gluing

However, this was found to be ineffective at creating a water-tight seal. Next the design time tried the method that had been suggested at Plastics Unlimited Inc.: both polycarbonate pieces were placed together and the glue was squirted along the joint. Capillary action allowed the glue to flow into the joint and completely cover the entire surface. The design team could see a chemical reaction taking place which fused the pieces together over the entire joint area, unlike with the previous glue method (Figure 72). Once the glue cured, tests showed that it was able to hold water without leaking for several days.

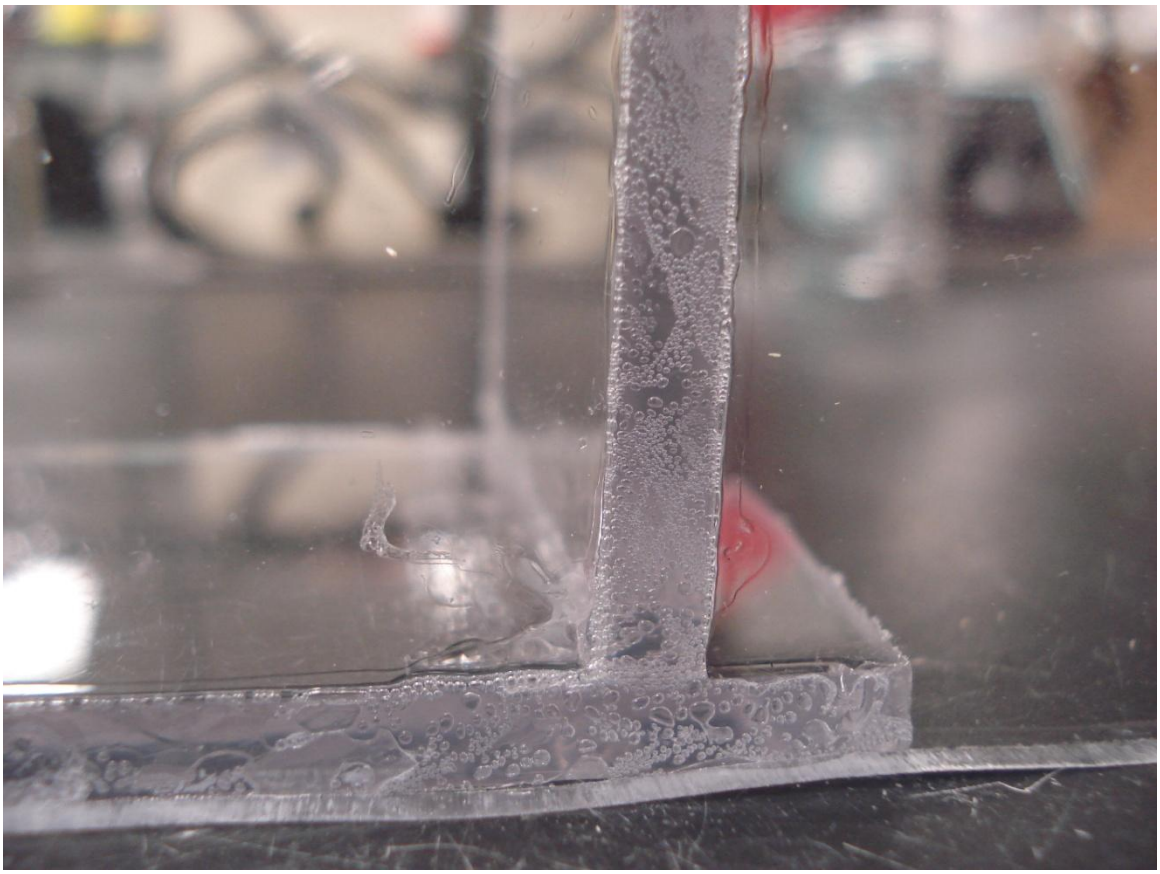


Figure 72: Correct Gluing

6.7.2 Construction of Inner Bath and Anchor Attachment System

Based on these trials, the design team was confident that the inner bath and inner wedge could be sealed with this glue method. Assembly began by securing the two inner bath short sides to the inner bath bottom. Wood blocks and a protractor (Figure 73) were used to ensure a correct 90° angle.

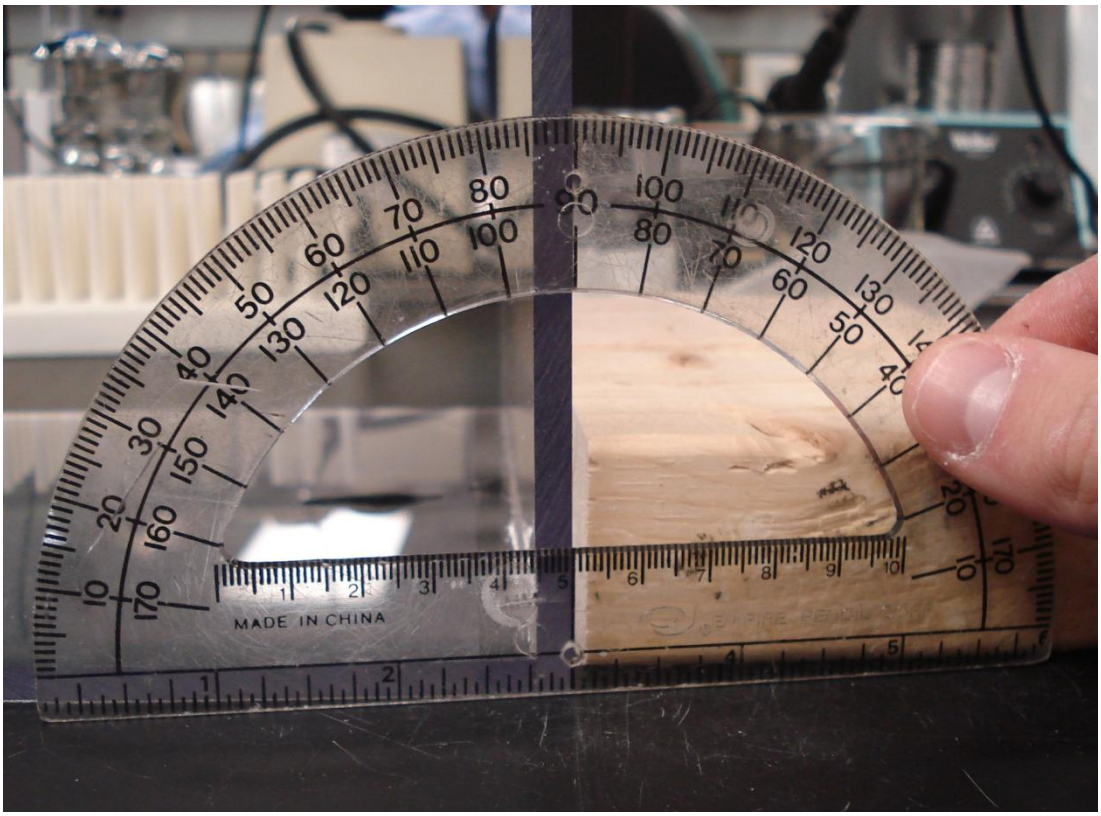


Figure 73: Wooden Block and Protractor

After curing, the inner bath incline was added. A metal protractor with a rotating arm was used to align the inner bath incline at the correct 5° angle. The inner wedge was then assembled and glued to the inner bath incline and the angle of the top was checked with a level to ensure that it was flat, shown in Figure 74 below.

Figure 74: Level Testing

Next, the anchor frame and anchor block were placed on the inner wedge, lined up, and marked to ensure correct placement of the anchor posts.

Initially, four polycarbonate anchor posts, 3/16” diameter x 0.5” high, were used to hold the anchor frame and were simply glued onto the inner wedge. Eventually the design team found that this was not a stable enough bond. Many of the anchor posts would simply fall off if subjected to any force in the x or y direction. To fix this problem, shallow holes were drilled into the inner wedge. The anchor posts were then press fit into these holes and glued. This combination of mechanical and adhesive fixation gave the anchor posts the necessary stability. Also, only two diagonally placed anchor posts were necessary to prevent motion of the anchor frame. Initially, there was a very tight fit between the anchor frame and the anchor posts, but this was not desirable because it could prevent the threads from being effectively lifted out the bath. A file was used to widen the post holes in the anchor frame until the anchor frame could slide on and off easily.

To secure the anchor block, the design team had initially intended to simply glue this component to the inner wedge. However, the client suggested that another post system might be more advantageous and versatile. Initially, four polycarbonate block posts, 3/16” diameter x 1/8” high, were

glued to the inner wedge. However, this was found to be a poorly designed system because it was exceedingly difficult manufacture a component where four separate block posts were aligned with four separate holes in the bottom of the anchor block while still maintaining correct alignment with the anchor frame. To increase the manufacturability, only two diagonally placed block posts were used and the holes in the bottom of the anchor block were made wider than the posts to allow for easy placement and removal.

After finalizing the placement and alignment of the anchor system, the inner bath long sides were glued. To facilitate a correct water seal and improve the heating profile of the inner bath, the long sides were modified slightly into trapezoid shapes, seen in Figure 75 below.

Figure 75: Cut Sides

This left the bottom wedge of the inner bath open to water flow from the heated outer bath and also eliminated a large area of trapped air. After final gluing of the inner bath, it was allowed to cure overnight and then filled with water and left for several days to test water-tightness. No leaks were found. A picture and model of the finished component can be seen below in Figure 76 and Figure 77, respectively.

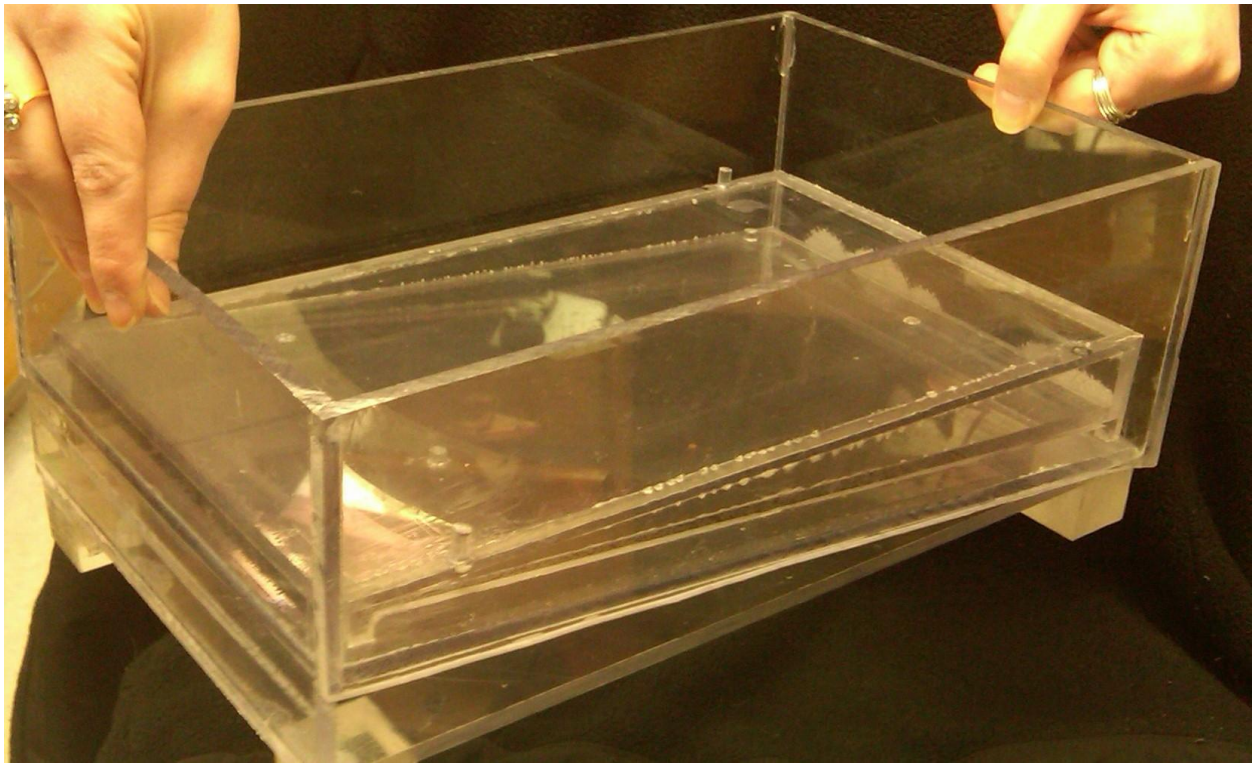


Figure 76: Picture of Inner Bath

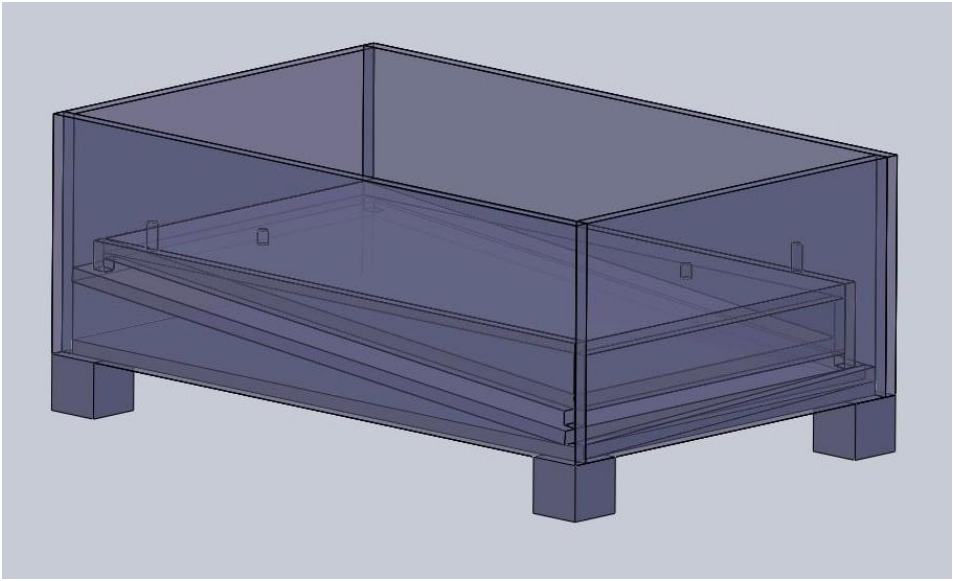


Figure 77: Model of Inner Bath

6.7.3 Buoyancy of Inner Bath

When the inner bath was first placed into the filled outer bath, it would not sit flush with the bottom of the inner bath. Because of the air trapped inside the inner wedge, the inner bath floated in

the water of the outer bath. The design team brainstormed various solutions to this problem. One way would have been to add weights to the open bottom wedge of the inner bath. By adding weights on the top of the inner bath until buoyancy was eliminated, the design team determined that approximately 1250g was need to ensure the inner bath did not float. However, because of the triangular nature of this bottom wedge, it was difficult to find weights that would fit into that space. It was also very difficult to hold the bath down in a level manner. From initial experimentation, the design team saw that placing weights into the bottom wedge would only effectively weight down one end of the bath and the other would still float freely. The design team's second idea was to drill into the inner wedge and channel water from the outer bath into this space. This would eliminate the large air pocket inside the inner wedge and could also further improve the heating profile of the inner bath. However, this would be difficult to fabricate and could compromise the water-tight nature of the inner bath and inner wedge.

Because of the difficulties surrounding these first two design ideas, the project team decided to go with an alternate solution: floater wedges. The completed design can be seen in Figure 78 below.

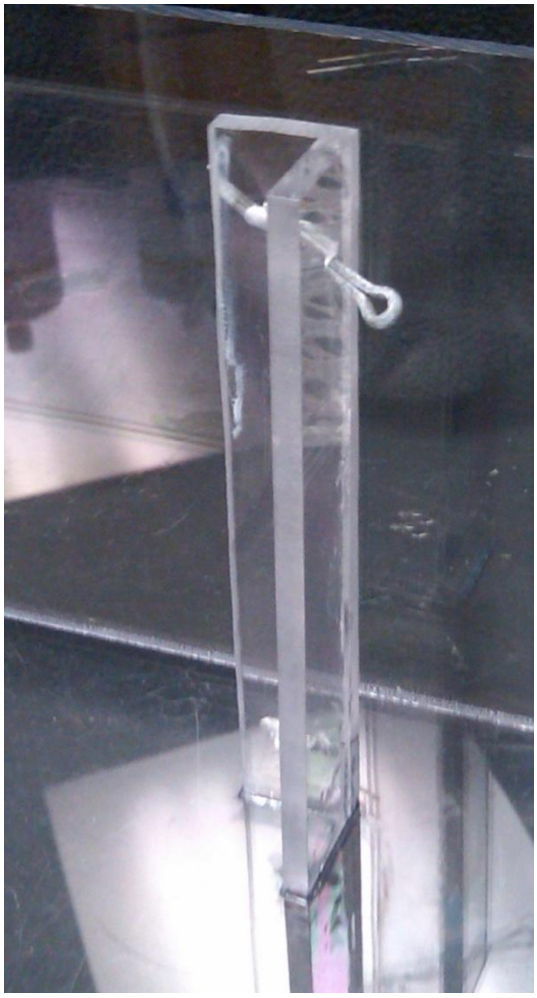


Figure 78: Floater Wedges

Two 90 ° corner pieces of polycarbonate would be glued to the bottom of the outer bath and aligned diagonally so that the inner bath fit between them. Holes would be drilled into these floater wedges just above the level of the inner bath. Pins would slide in and out of these holes to hold the inner bath down and prevent flotation. Four pieces of clear ¼" polycarbonate 1.0" wide x 6.0" tall were cut with a band saw. Floater wedges were made by gluing two pieces together at a 90°angle. After curing, 0.136" diameter holes were drilled with a #29 drill bit and steel wedge pins were inserted to keep the bath from floating. With this floater wedge system in place, the inner bath sat flush with the bottom of the outer bath and buoyancy was no longer an issue. Over time, some corrosion and calcification was seen

on the wedge pins. Replacing these with plastic or stainless steel wedge pins would eliminate this problem.

6.8 Second Round Immersion Heater Trials

While both initial immersion heater trials were successful and demonstrated the ability of the immersion heater to quickly and precisely heat a water bath, the experimental set-up was slightly different than that of the actual bath system. Firstly, the plastic tub used was a very thin polyethylene material, while the actual outer bath was made of ¼" polycarbonate. Also, the plastic tub had a smaller volume than the actual outer bath. Furthermore, the inner bath had not yet been constructed at the time of the first immersion heater trials so there was no indication of how effectively heat would transfer from the outer bath to the inner bath. While the previous trials were a good indication of the abilities of the immersion heater, the design team felt that subsequent testing was needed with the actual outer and inner baths to fully assure correct performance.

6.8.1 Long Term Test of Immersion Heater with Outer and Inner Baths

The goal of the new immersion heater trials shown in Figure 79 below was to verify the heating profile of the immersion heater, determine how quickly the solution inner bath would reach a consistent temperature with a fully heated bath, and assess the relationship between the temperature of the outer and inner baths.

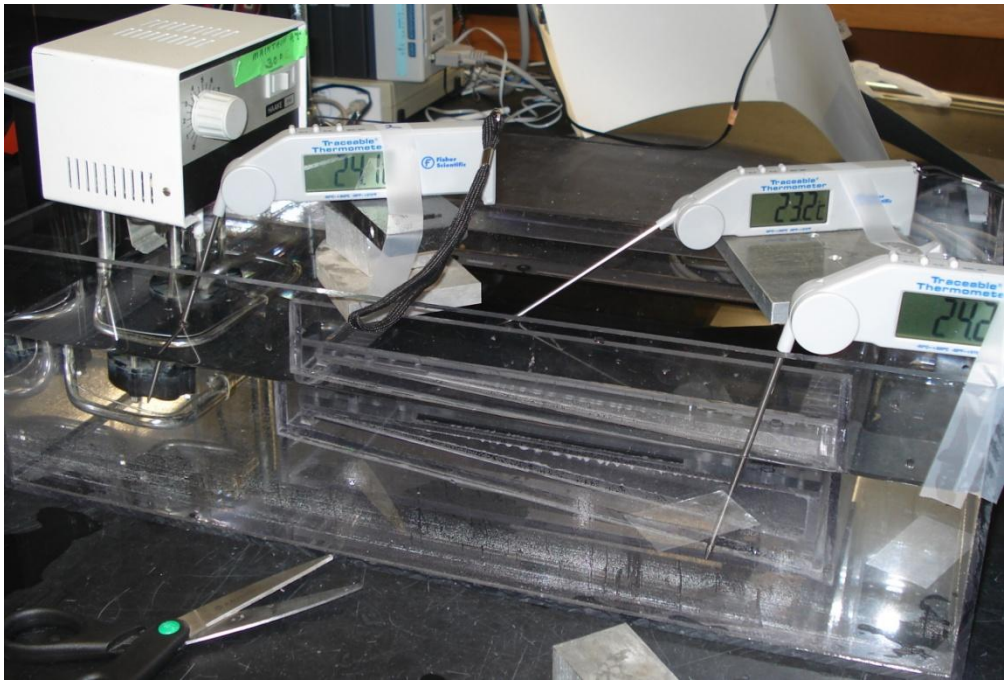


Figure 79: Set up of Subsequent Heating Trials with Inner and Outer Bath

The outer bath was first filled with approximately 12 L of tap water and the immersion heater and inner bath were placed inside. Once the immersion heater was turned on, temperature measurements were taken every 5 minutes until the outer bath maintained a consistent temperature for 20 minutes. Results from this experiment can be seen in Figure 80 and Table 77 below. Once the outer bath was up to temperature, the inner bath was filled with 1 L of tap water. Because the inner bath does not have a circulation system, two thermometers were placed inside, one at the anchor points and one at the center of the anchor block, to observe how even the heat distribution was. Temperature measurements were taken every 5 minutes for the first hour and then once an hour for the next three hours. The bath system was then covered and left overnight (10 hours) and the next day temperature measurements were again taken every hour for three hours. Results from this experiment can be seen in Figure 81 and Table 78 below.

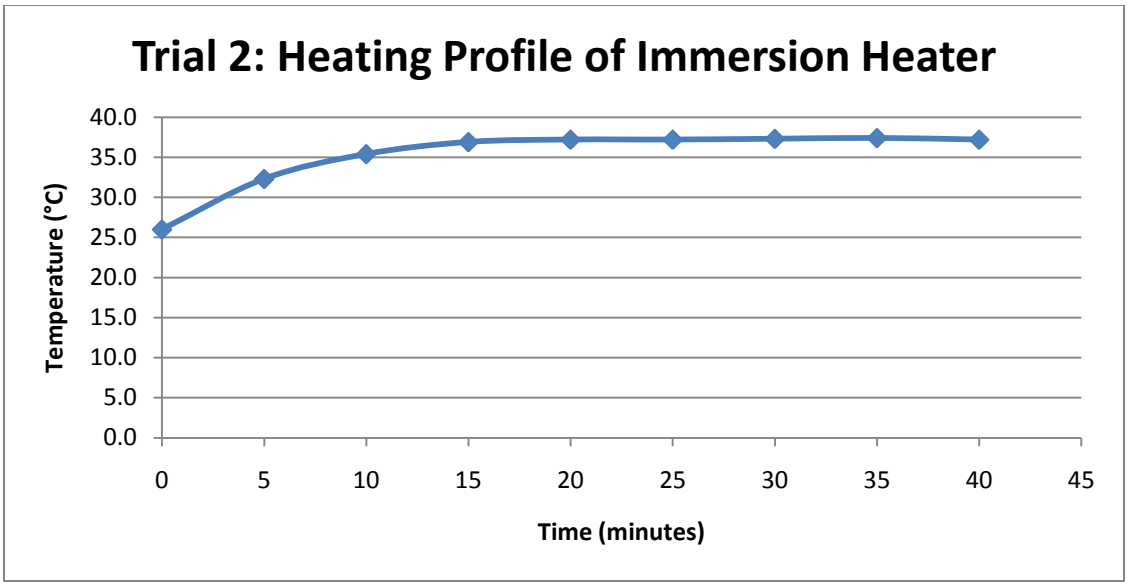


Figure 80: Short term trial of immersion heater. Time to reach constant temperature

Table 77: Detailed results from short term trial of immersion heater: Time to reach constant temperature

Time (minutes)	Temperature (°C)
0	26.0
5	32.3
10	35.4
15	36.9
20	37.2
25	37.2
30	37.3
35	37.4
40	37.2

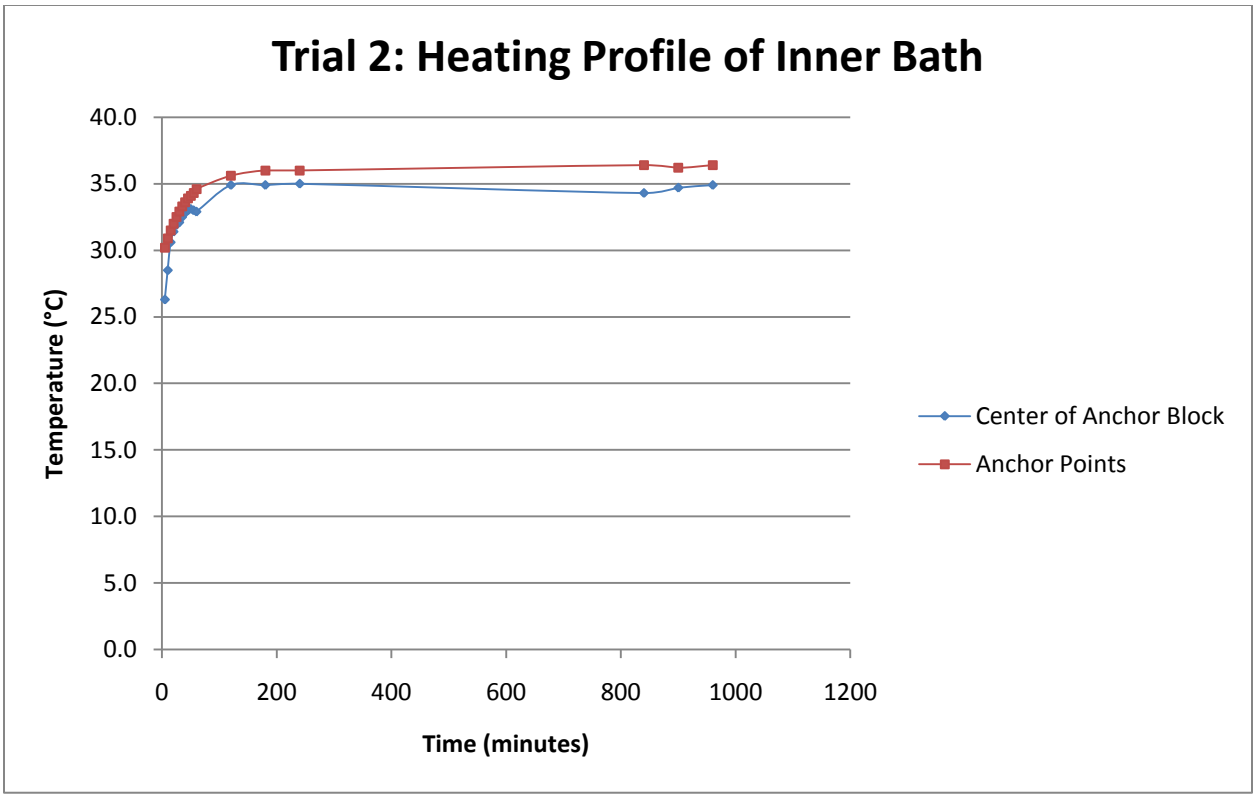


Figure 81: Long term immersion heater trial results

Table 78: Detailed results from long term immersion heater trial

Time (minutes)	Anchor Block Temperature (°C)	Anchor Point Temperature (°C)
5	26.3	30.2
10	28.5	30.9
15	30.6	31.5
20	31.4	32.0
25	31.9	32.5
30	32.1	32.9
35	32.5	33.3
40	32.8	33.6
45	33.0	33.9
50	33.1	34.1
55	33.0	34.3
60	32.9	34.6
120	34.9	35.6
180	34.9	36.0
240	35.0	36.0
840	34.3	36.4
900	34.7	36.2
960	34.9	36.4

6.8.2 Discussion of Results from Immersion Heater Trials

As expected, the outer bath took approximately 20 minutes to achieve a consistent temperature. However, the results for the inner bath temperatures were not as promising. Most problematic was that a constant temperature was not achieved throughout the inner bath. During most of the experiment, the design team noticed a 1-2°C difference between the temperature at the anchor points and the anchor block. This means the inner bath was not able to achieve a temperature within the acceptable limit of 36.0-37.0°C. There are several reasons this could have been taking place. As previously mentioned, the inner bath does not have a circulation system, so naturally the water above the anchor points, which are closer to the sides of the inner bath and therefore closer to the heated outer bath, would remain slightly warmer. It is possible that over a longer amount of time, this temperature difference would equilibrate, but this was not seen within the 13 hour experimental time limit. Also, the anchor block is made of ½" thick acrylic and was located directly above the air-filled inner wedge. With both of these barriers to transfer through, it is understandable that less heat might reach the solution in the anchor block area. One additional problem was that the inner bath heated up very slowly, which the design team felt could be problematic for correct thread extrusion. In order to remedy this problem, the design team decided that both baths would need a thick plastic cover. While this was necessary to prevent evaporation anyway, a thick layer of material sealing the bath systems would also trap heat and maintain consistent temperatures.

6.9 Third Round Immersion Heater Trials

A third heating profile test was performed to examine the functionality of the bath system with acrylic lids. These lids were cut to fit the inner and outer baths to prevent evaporation and heat loss, with holes drilled in for thermometer probes. The testing protocol was similar to the second round immersion heater trials and began with the outer bath water at room temperature with the lid on and no liquid present in the inner bath. The immersion heater was started and temperature readings were

taken every 5 minutes. After 20 minutes the outer baths temperature stabilized within 36.0-37.0°C. At the 45 minute mark, when it was insured that the outer bath was maintaining its temperature, the inner bath was filled and the lid placed on. Temperature readings from both baths continued to be recorded and the data was compiled into a chart (Figure 82).The inner bath's temperature reached a stable point in 100 minutes, and was within 1 degree of the outer bath at approximately 60 minutes.

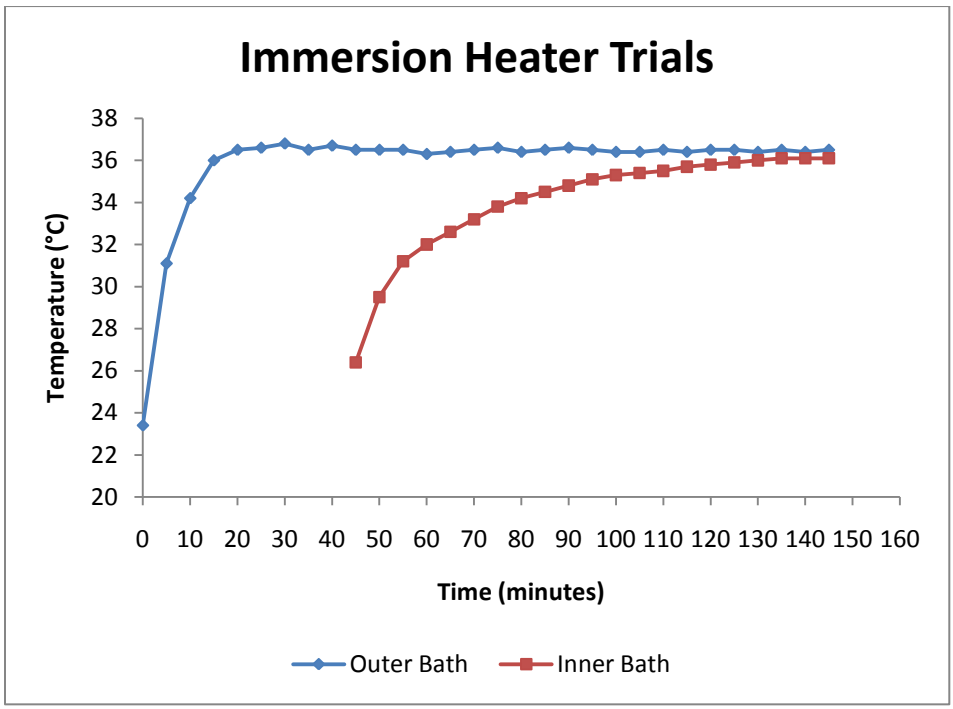


Figure 82: Heating Profile with Lids

From these final heating trials, the design team observed that a covered system was indeed more effective at transferring heat to the inner bath faster and more effectively but also maintaining a constant temperature throughout the inner bath. While the inner bath still took longer than the outer bath to reach temperature, the heating profile of the lidded system did not adversely affect thread production and was comparable to the current heating method.

6.10 Construction and Testing of the Hopper System

In order to more easily perform solution changes, a custom system was created which allowed for both the filling and draining of buffer solutions. Initially, to drain the bath the design team was planning on using a gravity-driven method described in Section 5.1.4.3.1 Hopper System and shown in Figure 32. A valve in one of the two lower corners of the inner bath could be opened and would allow for drainage to occur through a pipe that led from the inner bath system, through the outer bath system, and into a container for disposal. The design team purchased ¼ inch tubing and matching valves in order to facilitate this. However, it was found that this method would produce problems regarding gravitational drainage, particularly when integrating with the outer bath. This is because the water flow would not have any way to move against gravity and over the bath into a Waste Flask. The drainage system would need to flow through the outer bath, creating additional points at which leakage could occur.

6.10.1 Drainage of the Hopper System

In order to avoid the problems associated with gravitational drainage explained above, a new plan was implemented involving aspiration. Preliminary tests were conducted using tubing, a Pasteur pipette, and a Welch Model # 2522B-01 Aspiration Pump, which can be seen in Figure 83 below, in order to determine the feasibility of aspiration and proper positioning for the aspirator tubing.

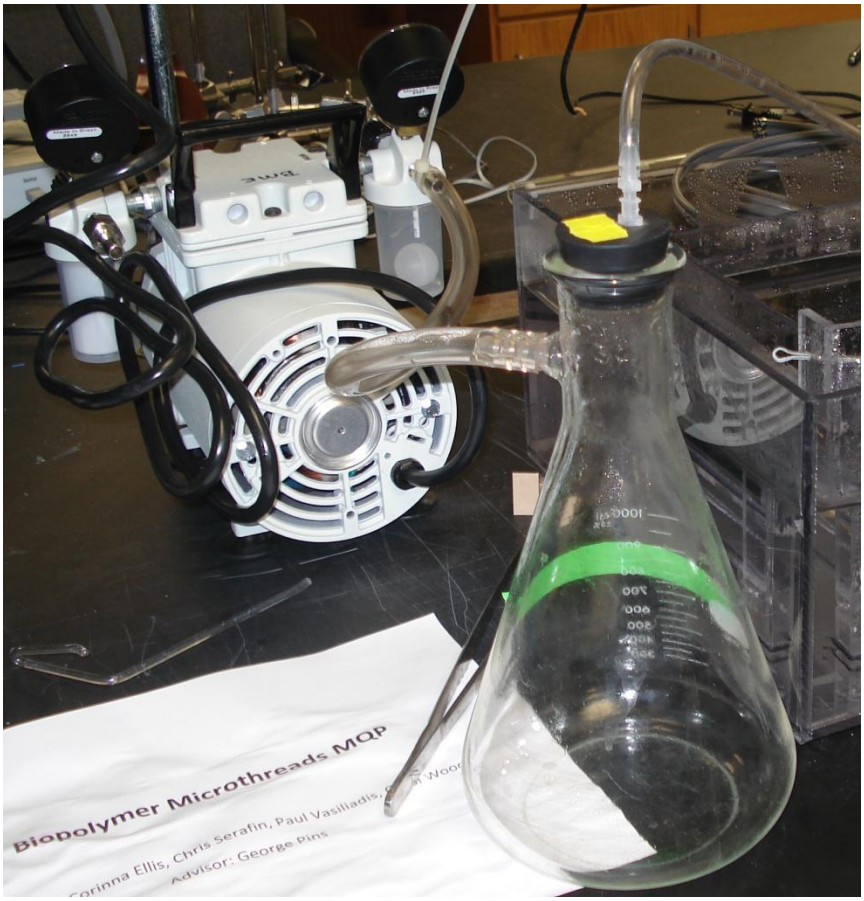


Figure 83: Drainage System

The liquid moves up the waste flask tubing with help from a waste flask stopper, which helps to create a vacuum for aspiration, and into a waste flask which collects the liquid for disposal. With this setup, it took 2 minutes and 18 seconds for one liter of solution to be properly aspirated. The design team felt that this was satisfactory and a significant improvement over the currently used drainage system.

One concern was that the force from draining the liquid might cause movement of the microthreads. Using a 1/4" outer diameter vinyl tubing, subsequent aspiration tests were performed with the inner bath filled buffer solution, using anchored collagen microthreads, which can be seen in Supplemental Video 5. The results showed that while aspirating the buffer solution caused some movement in the microthreads, there was no damage done to the threads. In addition, they remained

attached to the anchor points and so the design team concluded that aspiration was a safe method for draining the inner bath.

6.10.2 Filling of the Hopper System

For filling of the inner bath system, the design team decided on a hopper system consisting of two carboys and tubing, seen in Figure 84 below and in Supplemental Video 6.

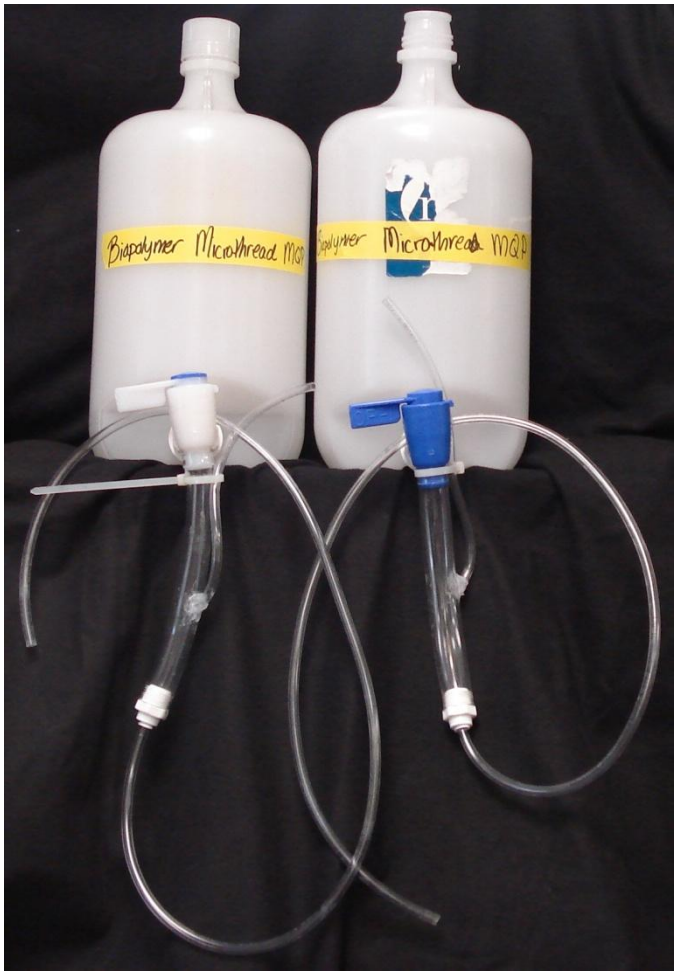


Figure 84: Fill System

Carboy #1 was needed to hold Fiber Incubation Buffer and carboy #2 was needed to hold deionized water. It was decided that the Fiber Formation Buffer could be added by hand before the extrusion process, eliminating the need for a third carboy. Originally, the project team planned to order 2 five liter high density polyethylene (HDPE) carboys (part number 73520-548 from vwr.com) that would fit into

their budget. However, they learned that the carboys were discontinued. After speaking with the WPI BME lab manager, Lisa Wall, the team discovered that they could borrow two 4L carboys from WPI. These carboys were large enough to contain buffer solution for at least 4 runs but small enough to not use a large amount of bench top space.

In order to transport liquid from the carboys into the inner bath, a 1/2" inner diameter vinyl tube was attached to the spigot of each carboy. The vinyl tubing connected to the spigot was about 6.5" in length, at which point a 1/2" to 1/4” diameter tubing joint was used to downsize the diameter of the tubing. Teflon tape was used to ensure that this joint was water tight. This was done in order to reduce the flow rate out of the carboy so that as liquid entered the inner bath it would not damage the microthreads. The design team added air channels to the ½” vinyl tubing so that no residual liquid would remain in the tube system once the spigot in the carboy was closed. This was done by drilling a 0.201” diameter hole at the end of the 1/2" vinyl tubing about 4" from the spigot, and attaching ¼” vinyl tubing with silicone adhesive. The small diameter tubing created a Y joint and allowed for airflow through the tubing as the carboy was being drained. However, even with the spigot open fully, no leakage was present.

6.10.3 Integration of the Hopper System with the Inner Bath

Using observations from drainage and fill testing, it was concluded that the back left corner was best for an inlet channel and the front right corner, where the bath comes to an incline, was best for an outlet channel. Small tubes would be placed on the corners which the aspirator could attach to. Since these tubes would be short and the aspirator and hopper tubing would be removable, these inlet and outlet tubes would not hinder extrusion. The project team attempted to attach the bath tubes to the polycarbonate using hot glue, however the heat from the bath caused the glue to soften and the tubing to detach.

An ideal solution would involve these tubes remaining permanently fixed to the bath. When a buffer change is required, an aspirator tip which is connected to waste flask tubing would fit inside bath tube #2 and an Aspiration Pump would be turned on to remove the liquid from the bath. The water easily drains to the front right corner of the inner bath due to the 5° angle constructed within it. After drainage, the aspiration tubing is detached. For subsequent inlet flow, small diameter tubing from carboy #1 or carboy #2 would be attached to bath tube #1. The valve on the carboy would be turned on, and liquid would flow through the carboy into the bath tubing. An alternate method to a stand-alone pump would involve using vacuum nozzle fixtures, which are present in the client's laboratory.

6.11 Design Changes and Construction of Extrusion System and Control Production Parameters

Successful design of an extrusion system required a simple device that could be easily built and used within the design teams time and budget. The use of VEX® robotics (Innovation First Inc., Greenville, TX, http://www.vexrobotics.com/) equipment offered many advantages over the previously examined LabVIEW™ software. The primary advantage of VEX® is its line of standardized parts, the use of which the WPI Robotics Engineering Department has extensive knowledge of. The variety of VEX® components allowed the team to build a device that fit the needs of the project using stock parts that were designed to integrate with one another and were specifically designed for control of motion. VEX® also offered another advantage in that it employs the use of microcontroller units that can be programmed in the programming language C and do not require constant attachment to a PC. The use of EasyC Pro (Intelitek, Manchester, NH, http://www.intelitekdownloads.com/easyCPRO/) written in part by Brad Miller of WPI, was recommended by the WPI Robotics Engineering laboratory manager Joseph St. Germain as a simplified method for programming the system. EasyC Pro was convenient for the design team because it required minimal programming knowledge with its drag and drop design. Programming for the system consisted of a simple loop which repeated the program directions for as

many threads as was inputted into the code. This number could be easily adjusted to create as many threads as necessary. The runtime of motor #1 and motor #2 could also be adjusted, changing the length of the threads and the space between them, respectively. Joseph also recommended the use of a linear slide over the originally conceived extrusion train cart system. A linear slide would allow for greater accuracy and precision in a linear direction, compared to the cart system due to the nature of its design and the limitations of the VEX® cart and track system.

6.11.1 Platform Construction for Extrusion System

To provide both structure and support for the extrusion system, the design team decided to create a raised platform (Figure 85) which would sit next to the bath. This was advantageous for several reasons. First, it would allow the mechanical and electrical components to be placed away from the heat and moisture of the bath system. Second, it would stabilize the extrusion system and reduce any vibrations. Also, the raised nature of the platform would create a space underneath for storage of the microcontroller, battery, and extrusion pump, further reducing the footprint of the system.

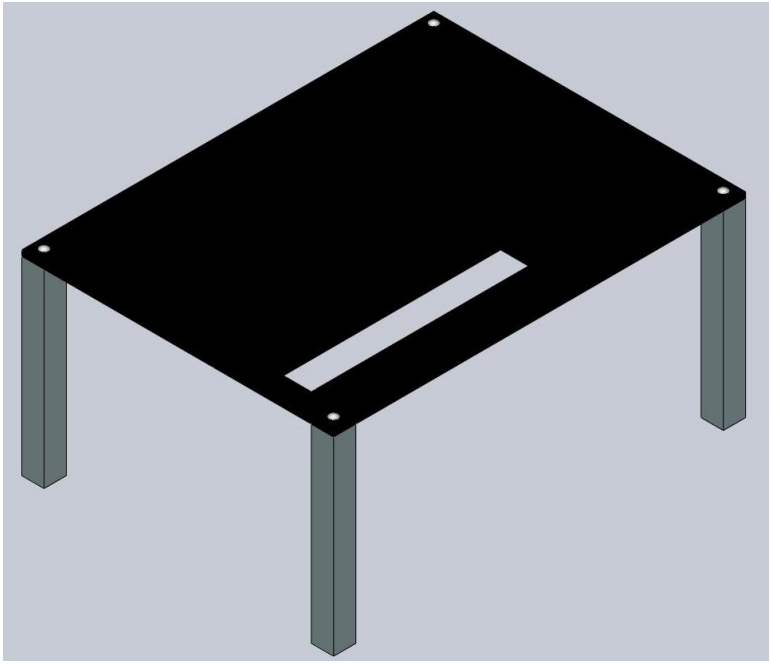


Figure 85: Model of Extrusion Platform

Initially, the design team determined the size of the platform. The platform would need to be large enough to accommodate the full range of motion in both linear slides, but also relatively compact to save laboratory bench space. To do this, the design team laid out the major components of the extrusion system on a bench-top and mimicked the full range of motion. Measurements were taken of how far each component would need to travel and how much space would need to be allotted around each component to prevent interference and allow for proper attachment. Based on this experimental set up, the design team determined the platform would need to be 14.0" x 18.5"

The platform was cut from ¼" acrylic. The design team used this material because it was stiff enough at that size to maintain a level platform, even under loads. Also, acrylic was widely available to the design team so there was no necessity to buy the material, which could have been somewhat expensive at that size. For similar reasons, 1.0" x 1.0" aluminum bar stock was used for the platform legs. The legs were initially cut to be 8.0" high. This height would allow for effective clearance of the extrusion system over the bath while maintaining a relatively low profile. One 0.221" pass through hole was drilled ½" in from each corner of the platform and each of the legs was tapped with drill holes for a #12-24 screw. Screws were purchased from Home Depot® and the platform was assembled. With the screws tightened, it was found that the platform had enough stability to withstand minor vibrations, as simulated by a member of the design team pressing down on the platform and moving it from side-to-side. Rubber feet strips, also purchased from Home Depot®, were added to the bottom of each leg to further reduce vibrations.

When the platform was first used with the bath system, there was not enough clearance between the components of the extrusion system that hung below the platform and the bath system. This was remedied by adding a second layer of rubber feet, which raised the platform just enough to eliminate any interference. Also, with the extrusion system fully in place, the client noted that some of the legs of the platform were angled slightly outward. While this was not affecting the overall success of

the extrusion system, it did make the platform seem less stable. The design team decided that some bracing might be necessary to ensure that the platform retains its proper structure. This could be accomplished with crossed aluminum brackets or by attaching more bar stock in between each pair of legs.

6.11.2 Platform Layout for Thread-to-Thread Motion

In order to properly extrude microthreads, the design team needed to create a bi-directional motor system which allowed for precise control over the movement and location of the 0.0338 in (0.86mm) inner diameter extrusion tube that would be connected to the syringe pump. Because the thread-to-thread (lateral) motion of the extrusion platform needed to be the most precise, it was the first component which was constructed and attached to the extrusion platform. The purpose of this component was to move the system from one thread to another, and so in order to increase the accuracy of the device, a set of parallel linear sliders was used to stabilize the system (linear slide #1 & linear slide #3). The linear sliders were made by VEX® Robotics and facilitated the attachment of Motor #1 to the extrusion platform. Originally, the team tried to mount and secure both linear sliders using #8 socket head screws; however, it was found that doing this created a great deal of friction and strained Motor #1 because of the difficulty involved in aligning both linear slides to be perfectly parallel. As a result, the design team decided that it would be best to mount linear slide #1 completely to the extrusion platform, but to have linear slide #2 only attached by one #8 socket head screw. This allowed for linear slide #3 to move slightly from side to side as linear slide #1 was completely extended and retracted, and reduced the amount of friction between the two linear slides.

With both linear slide #1 and linear slide #3 mounted to the extrusion platform, the team used aluminum spacer #1 to initially connect the two linear slides to one another. Aluminum was used in order to ensure structural rigidity, and the spacer was secured to both linear slide #1 and linear slide #3 by using a #29 drill bit, a #8-24 tap, and four #8 socket head screws. Aluminum spacer #1 was placed

perpendicular to linear slide #1 and linear slide #3 and fastened into place with two #8 button head screws connecting the spacer to each linear slide. Once this was complete, the design team needed to mount a gear system to linear slider #1 so that its linear motion could be controlled by a rotational stepper motor (motor #1). The two options at this point in the design were to use a gear and track system for the attachment of motor #1 to the linear slide, or to use a threaded rod and a gear system. As previously mentioned, because the side-to-side motion of the extrusion device needed to be highly accurate, a threaded rod and gear system were chosen instead of a gear and track system. By using a threaded rod instead of a track, movement could be more finely controlled. For example, to move the same distance as one revolution using a gear and track system, the threaded rod and gear system would need to rotate nine times. Because of this, linear slides #1 and #3 could be more finely controlled.

As the design team was planning how to attach the threaded rod and gear system to linear slides #1 and #3, it was decided that to reduce the overall height of the device, the threaded rod and gear system, seen in Figure 86 below, would sit underneath the extrusion platform.

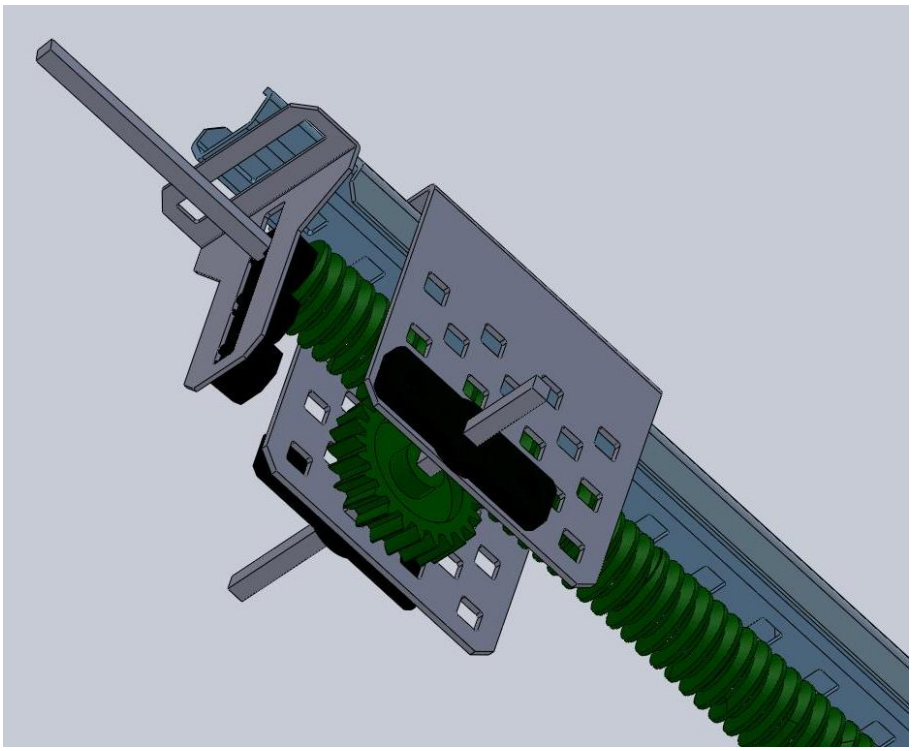


Figure 86: Gear Bracket Components

In order to do this, a 1.5” x 10” rectangle was cut into the extrusion platform using a #29 drill bit and a coping saw. The threaded rod was constructed from multiple small plastic threaded pieces which were assembled onto a square rod and was mounted underneath linear slide #1 using angle gussets and two #8 socket head screws. Mounted within the angle gussets were two Delrin bearings which held the threaded rod and allowed for it to rotate in place. It was noticed by the design team that after mounting the threaded rod there was some movement due to extra space between the threaded rod and the Delrin bearings which allowed for unwanted motion. In order to remedy this problem, a plastic spacer and washer was used in between the threaded rod and the Delrin bearings.

With the threaded rod system in place, it was necessary to attach linear slides #1 and #3 to the threaded rod so that motor #1 could extend and retract the linear slides. The design team decided that since linear slide #1 was the most secure, it would be best to attach the system at only this point. The purpose of this component (Figure 87) was to have the threaded rod, powered by motor #1, spin in

place and to have a stationary gear (gear #4) attached to linear slide #1 be moved by the turning of the threaded rod.

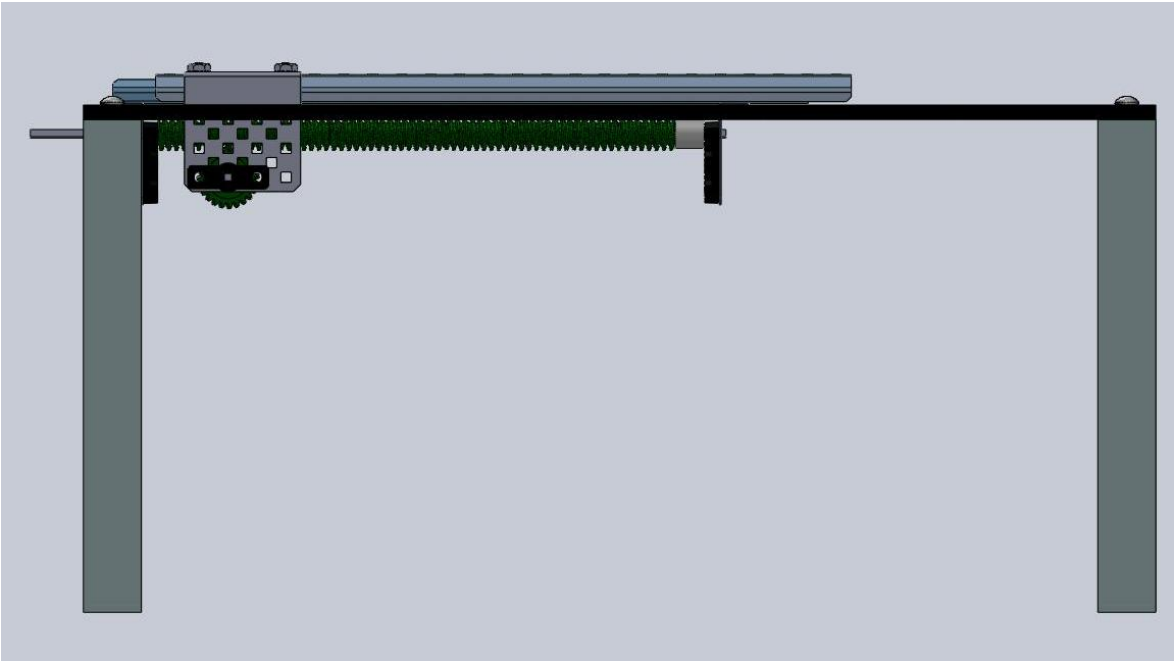


Figure 87: Underneath Mounting of Linear Slide 1

In order to do this, the design team attached a gear bracket from Vex® Robotics to the moving component of linear slide #1. Number 8 socket head screws were used to connect the gear bracket to linear slide #1, and the stationary gear was connected to the gear bracket using the same type of square rod that had been used in the assembly of the threaded rod, as well as with a #8 Keps nut and two washers. The stationary gear sat perpendicular to the threaded rod, and at all times had two teeth within the threaded rod, so that as the threaded rod was turned, the stationary gear would move the linear slide system back and forth.

Now that linear slides #1 and #3 had been attached to the threaded rod through the stationary gear system, it was necessary to mount motor #1 so that it could control the movement of the threaded rod. For increased accuracy, a large gear (gear #3) had its diameter increased with a #13 drill bit and was press fit into place on the end of the long square rod which held the threaded rod. Due to the large size of the gear, motor #1 would have to complete many revolutions in order to perform one revolution of

the threaded rod. A second gear (gear #1) was then press fit onto motor #1 and motor #1 was mounted into place on the extrusion platform. Gear #1 was smaller than Gear #3 in order to retain precise control over the threaded rod. The mounting of motor #1 was performed with the use of a #29 drill bit, a #8-32 tap, two #8 socket head screws, two #8 washers, a silicone strip, and an aluminum bracket. The purpose of the silicone strip was to reduce vibrations from the motor to the extrusion platform, and to better secure motor #1 into place. During initial testing of motor #1 and the threaded rod and linear slide system, it was noticed that in certain locations along linear slide #3 there were points of high friction. This was caused by slight variations during the creation and assembly process of the extrusion platform, and was remedied with the use of 3-in-1 oil along the slides. This helped to reduce friction and ensured there would be no stalling of motor #1.

As the design team was performing initial testing on motor #1 and linear slides #1 and #3, it was noted that as the stationary gear system reached the middle of the threaded rod, there was a tendency for the threaded rod to bend upwards, which caused skipping of the stationary gear. This meant that the linear slides could not be moved without human intervention. In order to fix this problem, a small length of wire, approximately 6" long, was secured first to the square rod which held the stationary gear in place, then up and over the threaded rod, and finally back to the other end of the square rod. This created a second point of connection between the stationary gear system and the threaded rod. Originally, there was only a force pushing upward on the threaded rod, from the stationary gear, as motor #1 turned which caused the threaded rod to bend. Now, however, the small length of wire applied a force downwards as well and did not allow the threaded rod to bend, therefore eliminating the skipping problem.

6.11.3 Platform Layout for Along Thread Motion

With motor #1 mounted and the thread-to-thread motion of the extrusion device assembled, work began on the second linear slide system. This portion of the device would allow for movement of

the extrusion head along each thread, and would be responsible for controlling the majority of the device's movement and dictating the overall geometry of the extruded threads. Through testing of motor #1 and linear slides #1 and #3, it was noted that while the threaded rod allowed for accurate movement from thread-to-thread, the speed of the movement would be too slow for this portion of the device. Because of this, a track system with a gear was used instead of a threaded rod. While this decreased the number of rotations in motor #2 needed to move the extrusion head down the bath system, it was noted that the precision of control of motor #2 that count be obtained through EasyC Pro was adequate for this purpose.

Another reason that the track and gear system was used instead of the threaded rod and gear system was that this portion of the device was required to move a linear slide which was twice as long as the linear slides used for the side-to-side movement of the extrusion head. Because of this, the torque required to move the linear slider was increased, and as a result the stepper motor needed to output this increased amount of torque without skipping. Stepper motors have increased torque at low speeds, and so by having the motor perform a lesser number of revolutions in a given time, the motor can output more torque than at a higher speed.

In order for the extrusion device to work properly, the second set of linear sliders and motors needed to be attached to the first set. The design team secured linear slider #2 perpendicular to both linear slider #1 and linear slider #3. This was done through the use of #8 button head screws instead of socket head screws to eliminate the chance of contact between this set of screws and the original #8 socket head screws which held linear slides #1 and #3 to the extrusion platform. Once attached, the team assembled the track system along the top of linear slide #2 using individual pieces of gear track made by Vex® Robotics. Each gear track piece was attached to the linear slide with two #6 button head screws.

As was previously mentioned, linear slide #2 was twice the length of linear slides #1 and #3. The reason was that this linear slide needed to move along the entire length of the inner bath system which was longer than a single linear slider could extend. As a result, the design team created one double long linear slide by attaching the top portions of two normal linear slides to one another. The linear sliders were connected end to end through the use of a strip of aluminum and four #6 button head screws placed underneath the two linear sliders. There was also a piece of gear track which helped to hold the two linear sliders together on the top. The next task was to mount Motor #2 onto linear slide #1 in order to allow it to control the movement of linear slider #2. A 4”x 3”x 3/8” platform constructed from two 3/16” pieces of polycarbonate was cut on the vertical band saw and mounted directly onto Linear Slide #1. This was done by using two #8-32 socket head screws with two #8-32 Keps nuts. One nut was placed underneath the top portion of linear slider #1 and the other was placed above the polycarbonate platform. Using this system, the platform was secured to linear slide #1 by tightening the top Keps nut while holding the bottom Keps nut in place. In addition, a small recess was made in the center of the platform in order to allow it to sit flush over a Keps nut.

Once the platform was constructed, the team mounted motor #2 in a similar fashion as Motor #1. A #29 drill bit was used to create two holes in the platform, and then a #8-32 tap set was used to thread the two holes. This allowed for the #8-32 socket head screws to be screwed directly into the platform, and eliminated the need for two #8-32 Keps nuts. Once the holes were drilled and tapped, motor #2 was secured into place with the use of an aluminum bracket, a silicone strip, and two washers. In order to minimize the height of the extrusion platform, a small sized gear was chosen for gear #2. Because the initial diameter of the opening in gear #2 was too small to fit on motor #2, a #13 drill bit was used to widen the opening, and then the gear was press-fit onto the motor.

With both motors mounted and the linear slide system connected, the design team noticed that as linear slide #2 was advanced by motor #2, there was a tendency for the extended portion of the slide

to droop downwards. In addition, as motor #2 extended linear slide #2, there were heavy vibrations caused by slight rotational movement in linear slide #2, which caused the extrusion head to wobble. In order to remedy this problem, the design team needed to better secure linear slide #2. The solution was created through the use of a 14" Topslide® drawer slide purchased from Home Depot®. The drawer slide was mounted onto linear slides #1 and #3 in a similar fashion to that of linear slide #2. Two #6 button head screws were used to secure the drawer slide so that it was perpendicular to linear slides #1 and #3, and parallel with linear slide #2.

Once the drawer slide was attached, the next step was to attach it to linear slide #2 so that both pieces moved at the same time. Due to the tight tolerances and precise manufacturing of the drawer slide, by rigidly attaching it to linear slide #2, the side-to-side motion which caused wobbling would be eliminated. This was done by using a thin strip of aluminum (aluminum spacer #2), similar to that of aluminum spacer #1. This aluminum spacer was mounted on top of linear slide #2 and the drawer slide in a perpendicular manner using 3 #6 button head screws. A #35 drill bit was used as well as a #6-32 tap set to create three holes in the aluminum spacer #2: two on the side of linear slide #2, and one on the side of the drawer slide. By tapping these three holes to be the size of #6-32 screws, the design team was able to eliminate the need for three Keps nuts while still allowing the aluminum spacer #2 to be tightly fastened to linear slide #2 and the drawer slide. With the aluminum spacer in place, the vibration and downwards droop of linear slide #2 was eliminated. Each linear slide now operated smoothly and the extrusion head no longer swayed from side-to-side.

6.11.4 Construction of Extrusion Head

Because of the height of the extrusion system, an extrusion head was necessary to extend down into the bath and keep the extrusion tubing straight and upright during the extrusion process. Furthermore, from initial testing of the extrusion system, the design team knew the extrusion head would need to be very light. With the relatively large distance that linear slide #2 extended over the

bath without support, a heavier extrusion head device would cause the linear slide to bend downwards. This height change would severely disrupt the extrusion process.

To accomplish this, the design team first tried using a metal ruler (as a model for a straight thin piece of material), but this was found to be far too heavy. Also its size was unnecessarily large. With the small diameter of the extrusion tubing, it would only require a very thin, narrow piece to support it. To satisfy this, the design team decided to use a 1.0mL Pyrex® pipette. The pipette offered several advantages. First, it was very light. Second, it was the perfect diameter to accommodate the extrusion tubing. Third, it was very straight and stiff which would keep the tubing in the desired position. Fourth, the pipette was designed to be used with laboratory and cell culture activities so the design team knew it would not cause adverse reactions with the buffer or thread solutions. Additionally, one final advantage that the design team saw during initial testing was that the pipette helped to separate the newly formed threads. It accomplished this in two ways. Because it held the end of the extrusion tubing down in the bath, adjacent newly formed threads (which float to the top if not secured properly) would not interfere with the extrusion because they were higher than the threads being extruded. Also, the pipette was able to gently push newly formed threads out the way without sticking to them as it moved along the bath.

Initially, the pipette was simply dropped down into one of the square holes in linear slide #2 and secured with tape, as can be seen below in Figure 88.

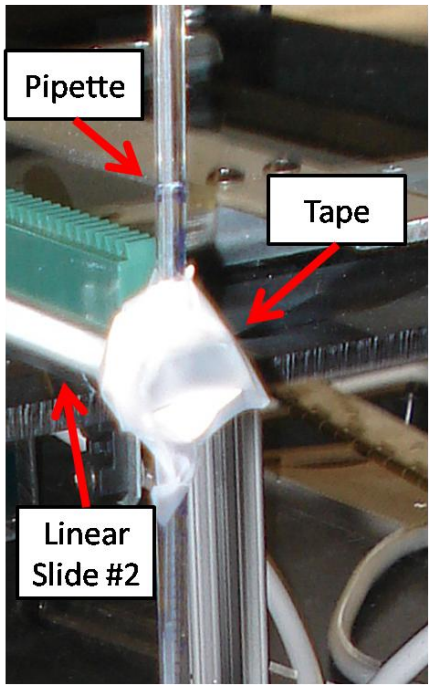


Figure 88: Tape Bracing

The diameter of the square hole was small enough to hold the pipette at a certain height but still large enough to allow the pipette to pass in and out. However, the design team found that without tape, this was not a sufficient method to keep the pipette from moving from side-to-side during extrusion, and it was essential that the pipette remain absolutely straight during extrusion to maintain the integrity of the threads and avoid overlaps. The design team decided the pipette would need a more effective system of bracing (Figure 89) to reduce this excess motion.

To accomplish this, the design team used a 2.0” x 2.0” aluminum square. 0.182” inch holes were first drilled through the top and bottom of the square with a #14 drill bit. Requiring the pipette to pass through two holes instead of one would greatly increase the stability. This square was then attached to linear slide #2 with two button head screws. Again, two screws would offer greater stability and resistance to motion in multiple directions than just one screw.

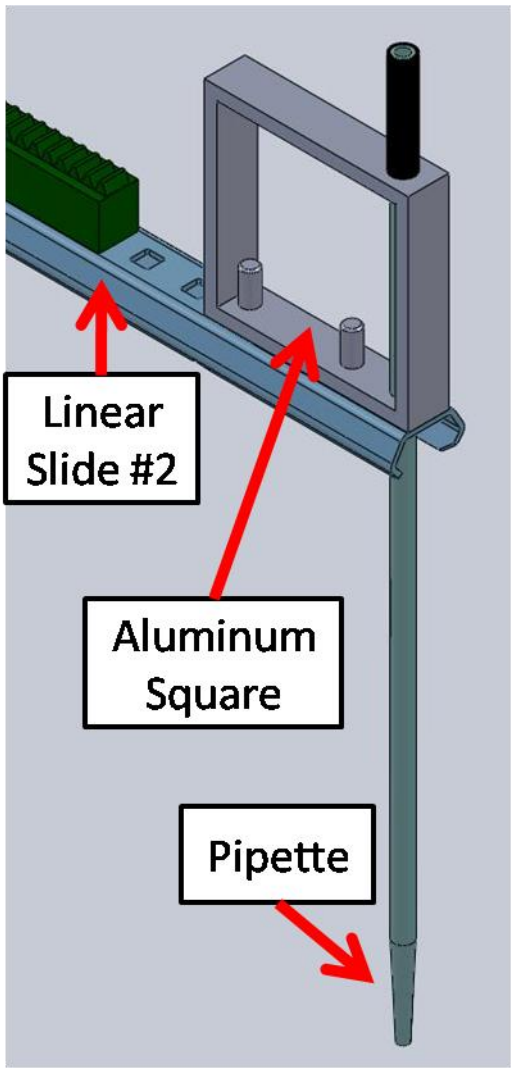


Figure 89: Model of Extrusion Head

With this aluminum bracket attached, it was found that the square hole in linear slide #2 had to be filed out slightly to allow the pipette to pass through the system. However, doing this also slightly decreased the stability of the pipette. To remedy this, the design team mounted a rounded piece of flexible silicone strip near to the hole in the bottom of the square. An adequate amount of overhang was created to slightly decrease the size of the hole and increase the stability of the pipette. However, with the soft, flexible nature of the silicone, the pipette could still pass through relatively easily.

With better side-to-side stabilization, the design team shifted its focus to achieving proper pipette height placement relative to the inner bath. Originally, the design team simply wrapped tape around the non-tapered end of the pipette. This tape was too large to pass through the holes in the aluminum square, so it kept the pipette at a constant height and made it very easy to achieve correct height placement. However, the design team felt a more permanent solution which still maintained a high degree of user friendliness was necessary. To achieve this, the design team replaced the tape with heat shrink, a type of plastic tubing typically used in electronic systems. This material is designed to permanently contract when heated, so the design team simply placed an approximately 1” long piece of heat shrink over the non-tapered end of the pipette, and used a hot air gun to shrink the tubing until it fit tightly around the pipette. This process was repeated with another layer of heat shrink to ensure that it would not pass through the holes in the aluminum square. This created a permanent fixture that would allow any user to easily place the pipette at the correct height. A picture and model of the completed extrusion system can be seen below in Figure 90 and Figure 91, respectively.

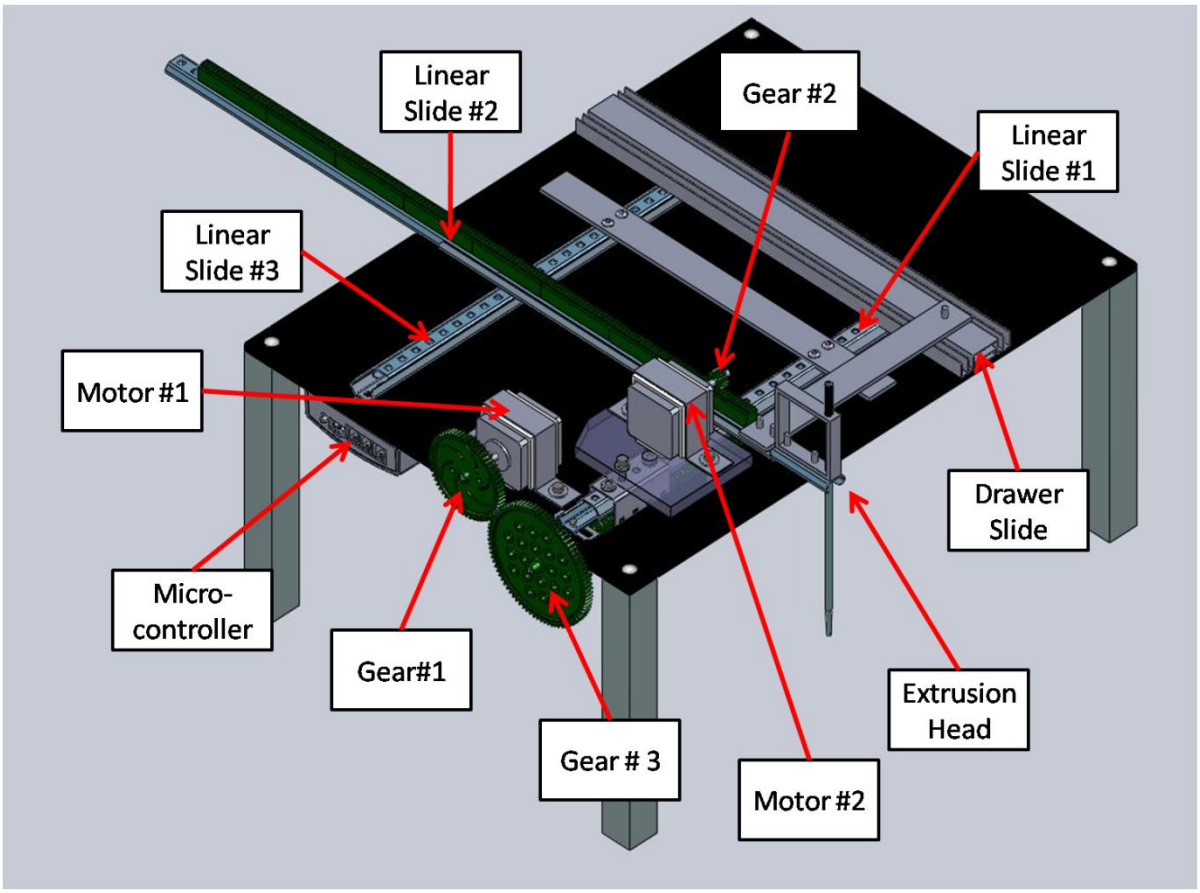


Figure 90: Model of Extrusion System

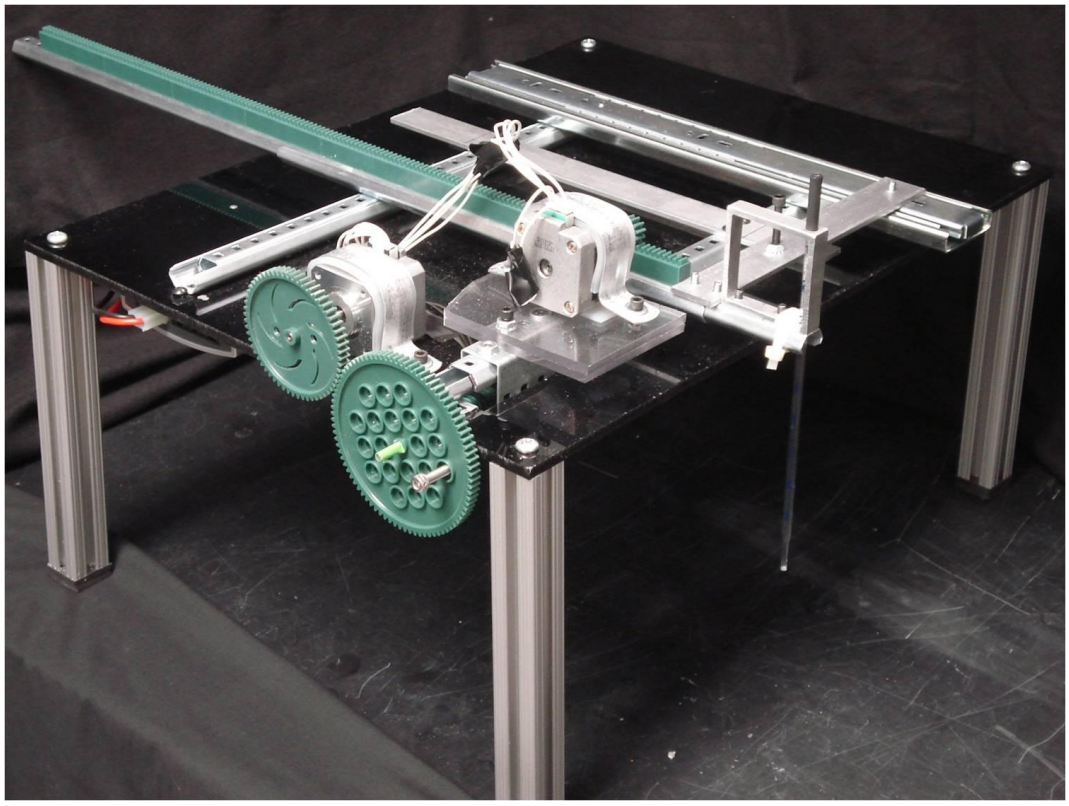


Figure 91: Picture of the Extrusion System

6.11.5 EasyC Pro Sample Code and Explanation of Code

The following code segment was the test code used by the design team for the experiments and trials of the extrusion system. Additional reprogramming information can be seen in Appendix H: Operating Instructions.

6.11.5.1 Sample Code for Control of Extrusion System

```
#include "Main.h"

void main ( void )
{
	int m1count;
	int m1dir;
	int threadcount;
	int m2count;
	int m2dir;

	m1count = 0; ;
	m1dir = 0 ;
	m2count = 0 ;
	m2dir = 0 ;
	threadcount = 1 ;
	while ( threadcount <= 15 )
	{
		SetDigitalOutput ( 11 , 0 ) ;
		Wait ( 5 ) ;
		SetDigitalOutput ( 11 , 1 ) ;
		Wait ( 5 ) ;
		m1count += 1 ;
		if ( m1count  == 5500 )
		{
			if ( threadcount < 15 )
			{
				Wait ( 1000 ) ;
				while ( m2count <= 3500 )
				{
					SetDigitalOutput ( 13 , 0 ) ;
					Wait ( 1 ) ;
					SetDigitalOutput ( 13 , 1 ) ;
					Wait ( 1 ) ;
					m2count += 1 ;
				}
				Wait ( 1000 ) ;
			}
			if ( m1dir == 0 )
			{
				m1dir = 1 ;
				SetDigitalOutput ( 12 , 1 ) ;
			}
			else if ( m1dir == 1 )
			{
```

```
                    SetDigitalOutput ( 12 , 0 ) ;
                    m1dir = 0 ;
                }
                threadcount += 1 ;
                m1count = 0 ;
                m2count = 0 ;
            }
        }
}
```

6.11.5.2 Explanation of Sample EasyC Pro Code

The code begins by defining five integer variables. Two variables (green text) are used to set motor direction m1dir and m2dir for motors #1 and #2, respectively. Another two variables (green text) are used to count each motor's iteration of the code which controls each rotation increment (blue text). A final variable (green text) is used to count which thread the system is currently extruding. After defining each variable, starting values for each variable are assigned.

The program begins with a while loop (orange text) which iterates the code once for each thread formed, in the example case the value was set to 15, meaning the program would iterate as long as the current thread count was less than or equal to 15 (yellow highlight).

The 4 lines of code following the while loop (first blue text), as previously mentioned, control the motors rotation. The number 11 within the functions call statement directs the signal to digital port 11 on the VEX® microcontroller unit, which motor #1 is connected to. The number 0 in the call statement signals the motor to activate one of two electromagnets within motor #1. Afterwards, a wait function occurs, halting the program flow for 5 milliseconds, followed by another function call this time with a value of 1, which signals the other electromagnet to activate in motor #1. Another 5 millisecond wait is implemented to create a delay between magnets when the program loops back to the beginning. It is the length of delay between activation of each electromagnet within the stepper motor that determines the motors speed. A shorter delay causes the motor to rotate more rapidly.

After the motor has incremented, the count is initiated, and an If statement occurs (light blue text) checking if the count value has reached 5500. This value represents the motor iterating 5500

times, which, through testing, was found to be the number of increments needed to move the extrusion head the length of one thread. The two 5 millisecond delays in the code mean that each motor increment takes 10 milliseconds, multiplying this by the amount of increments yields the extrusion time of a single thread, 55 seconds. If the count for motor #1 has reached 5500, a second if statement occurs that checks if the thread count is less than 15. This is needed to prevent motor #2 from running after the last thread is extruded.

If the thread count is less than 15, the program continues with a control segment similar to that which controls motor #1. These 4 lines of code (second blue text) signal digital port 12, which motor #2 is attached to. The main difference in this code segment from the previous one is the wait functions, which delay the program for 1 millisecond instead of 5. This causes a faster rotation for motor #2 despite the shorter distance of thread-to-thread motion. This speed increase is necessary because of the use of the threaded rod to move the extrusion system from thread to thread. The thread rod system requires more rotation to move the same distance as a linear system, meaning an increase in motor speed was needed to move the extrusion head this short distance each time. The count for motor #2's increments until it reaches a value of 3500, which determines the distance between threads. This value can be adjusted, with a smaller number bringing the threads closer together and a larger number spacing them farther apart.

The If statements following the code for the movement of motor #2 (olive text) check which direction motor #1 was currently set to. A value of 0 meant the extrusion was set to forward, while a value of 1 meant the motor was set to reverse. The code checks to see which direction motor #1 is set to as indicated by the variable m1dir, and signals a change in direction to digital port 12 (red text), which the directional portion of motor#1 is attached to. The code then changes the variable m1dir to represent this physical change (red text). It is by this method that the code extrudes threads in an alternating back and forth motion.

After direction change is established, the thread count increments by one, and m1count and m2count are reset to 0 for the next thread. The program loops to its beginning, and the process begins again with motor #1 now turning in the opposite direction, provided the thread count has not reached 15. The maximum thread count for this example is set to 15; however this value can be changed to create any number of threads. The code also shows two 1000 millisecond pauses between the coding for the thread-to-thread movement. This adds a 1 second pause before and after each thread is finished, ensuring attachment to the anchor is not hindered by the thread-to-thread motion of the extrusion head.

7.0 Final Design and Validation

Based on the initial verification experimentation done by the design team, a final design was developed (Figure 92 and Figure 93).

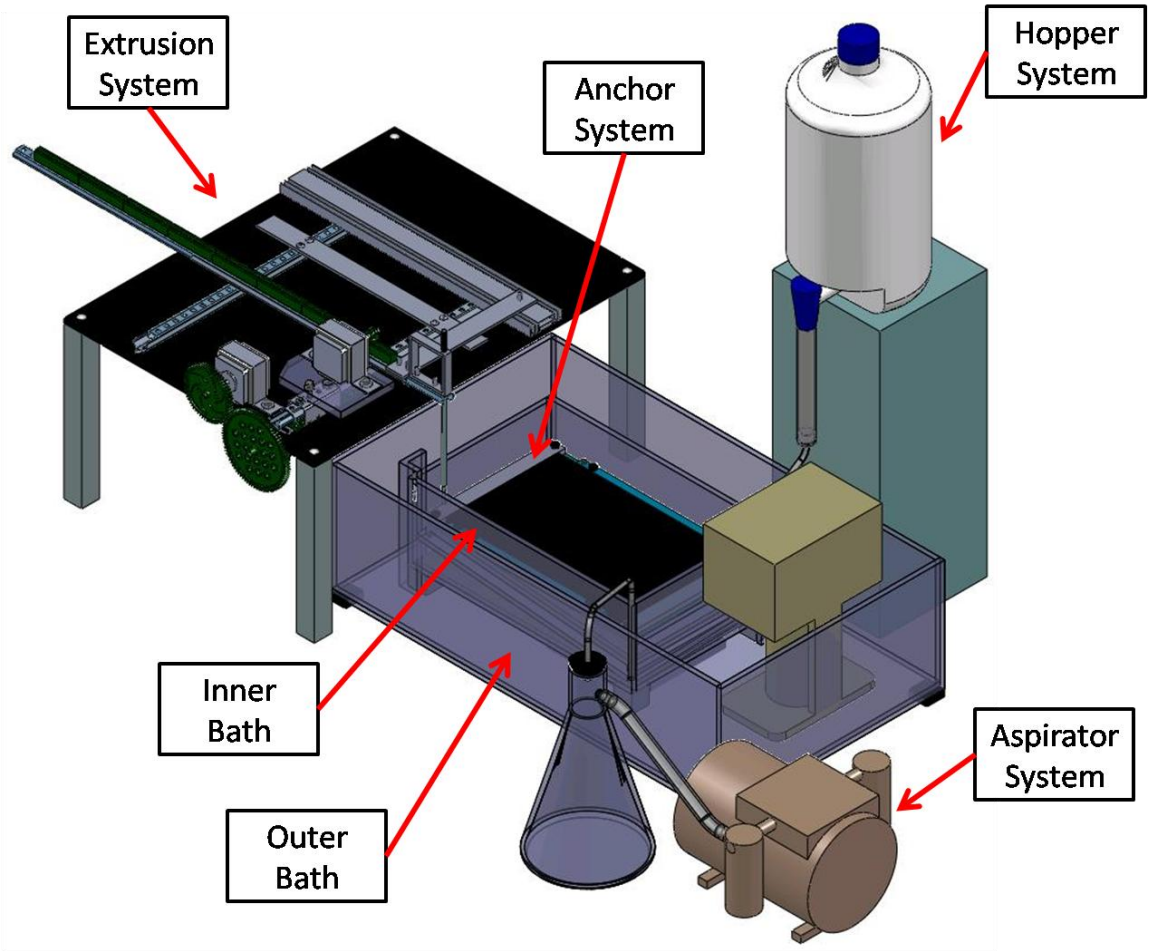


Figure 92: Model of Extrusion and Bath System

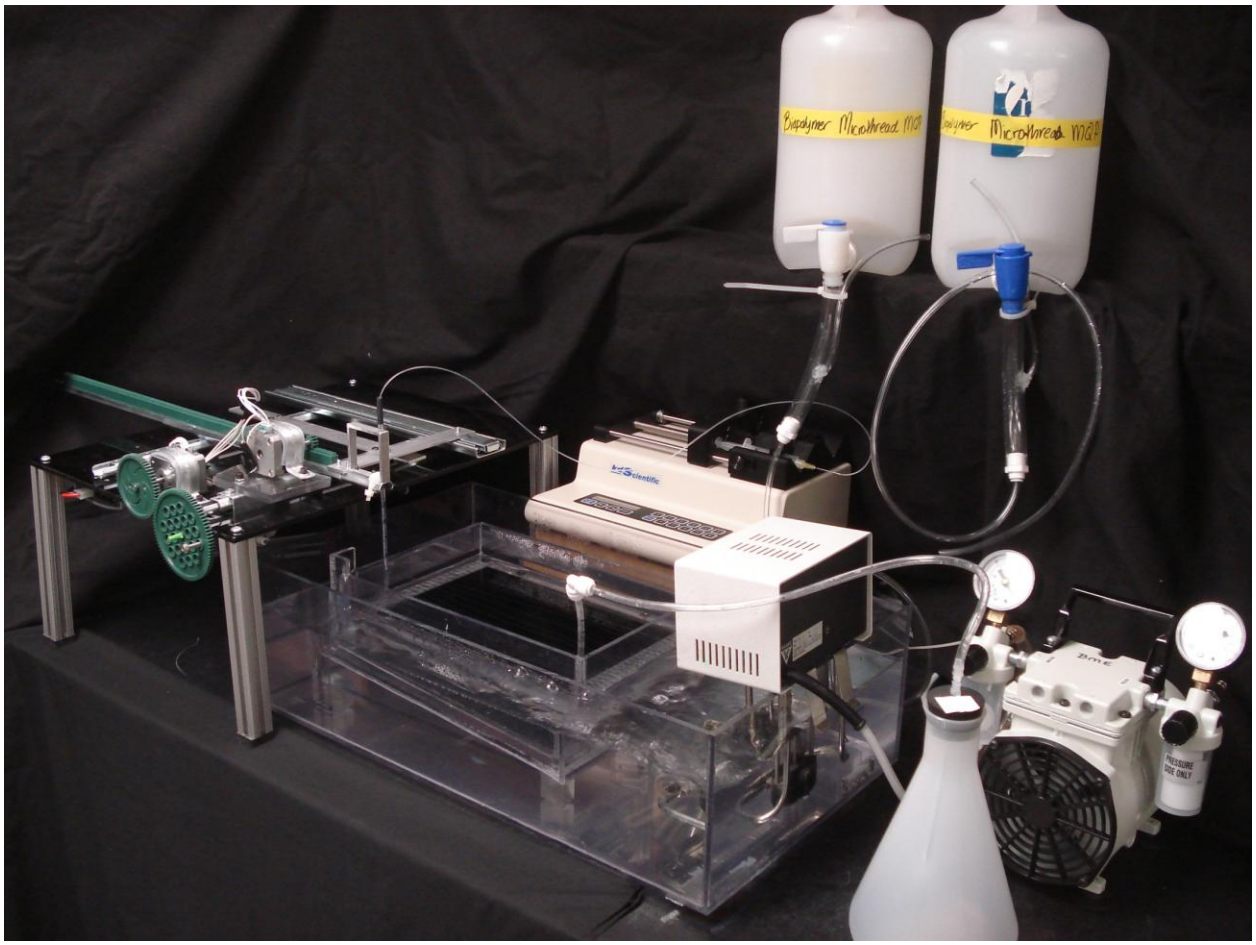


Figure 93: Picture of Extrusion and Bath System

The main components of the final design are the bath system and the extrusion system. The bath system consists of an inner bath for extrusion (which has a detachable anchor system) and an outer bath to provide heating. The extrusion system consists of a raised platform with a bi-directional linear slide apparatus that moves the extrusion tubing along each thread and from thread to thread. There are also several external components including a syringe pump, aspirator, and hopper system.

The clear polycarbonate outer bath holds approximately 12 L of water and is heated with an immersion heater running at approximately 37.1°C. With room temperature water in the outer bath initially, it takes approximately 20 minutes to reach the proper temperature. The clear polycarbonate inner bath sits within two floater wedges in the outer bath and is secured down with wedge pins. This keeps the inner bath from floating within the outer bath and also ensures proper user placement. The

inner bath has a tilted floor to allow for proper drainage, but also contains an inner wedge which provides a flat surface for the microthreads to be extruded onto.

An anchor system, consisting of an anchor block, made from black acrylic and an anchor frame, made from clear polycarbonate, sits on the inner wedge of the inner bath and is secured in place by a series of posts. This ensures proper alignment and placement of the components, but also allows the user to remove the anchor system (and the attached threads) once the extrusion process is complete. The anchor block is black to provide increased thread visibility, and the anchor frame detaches in the middle to allow for subsequent thread stretching. With the anchor system in place, 1 L of buffer solution is used to fill the inner bath.

A black acrylic extrusion platform is placed next to this bath system and aligned so that each thread overlaps the anchor points by approximately ¼" and the extrusion system runs parallel to the anchor frame. The extrusion system is built from VEX Robotics® components and consists of two perpendicular linear slides controlled by stepper motors. Motor #1 controls motion from thread to thread with gears and a threaded rod. Motor #2 controls the motion along each thread with a gear and track system. Linear slides in both directions are stabilized with parallel sliding components: either through another linear slide or a commercial drawer slide. A VEX® microcontroller is mounted underneath the extrusion platform and contains the code for the motion of the extrusion system. The code signals Motor #1 and #2 to begin running at designated times in conjunction with one another to move the extrusion head in a back and forth pattern across the width of the bath. By changing one or two variables, the system can be easily adapted for the specific needs of the user.

Approximately 5mL of rat tail collagen (10mg/mL) is needed to extrude an entire bath's worth of microthreads. To begin the process, this volume is loaded into a plastic syringe, the syringe is loaded into a syringe pump, and the extrusion tubing is attached. The tubing is inserted into the pipette on the extrusion head until it reaches a height approximately 1/16" above the anchor system (this height is

controlled by a stopper attached to the tubing so it is possible for the user to ensure correct placement each time). The syringe pump extrudes the collagen at a rate of 0.225 mL/min, and this pump must be started before the extrusion system to allow the collagen time to reach the end of the extrusion tubing. Once the collagen begins to come out the end of the extrusion tubing, the user simply turns on the VEX® microcontroller and thread formation begins.

Over the next 72 hours, as buffer changes need to take place, an aspirator is used to remove the old buffer solution. Carboys with a tubing system are then used to fill the bath again with the next solution at the correct volume. After the processes are completed, the user can lift the anchor system (and attached threads) out of the inner bath, afterwards the threads are hung to dry and stored until use (See Figure 70).

7.1 Initial Verification Test with Permanent Marker

After the design team had added the drawer slide attachment to the extrusion platform for stabilization, an experiment was performed in order to verify that the vibration and wobbling of the extrusion head had been eliminated. Due to the limited amount of collagen that the design team possessed, this experiment was performed using a permanent marker as an indicator of success. The permanent marker was first tightly secured to the pipette which normally held the tubing for the syringe pump, using electrical tape. Once the permanent marker was held in place, a sheet of 8.5" x 11" lined paper was placed underneath, and the device was turned on. As the extrusion head moved the permanent marker, a trail of ink was left behind. Using the lines created by the permanent marker, a qualitative analysis of the extrusion head could be made. As compared to the experiments performed before the addition of the drawer slide, the movement of the extrusion head was much more accurate and straight. The rigidity offered by the drawer slide stabilized the extrusion head and the design team no longer saw any vibrations in the device. A picture of the results from this experiment can be seen below in Figure 94.

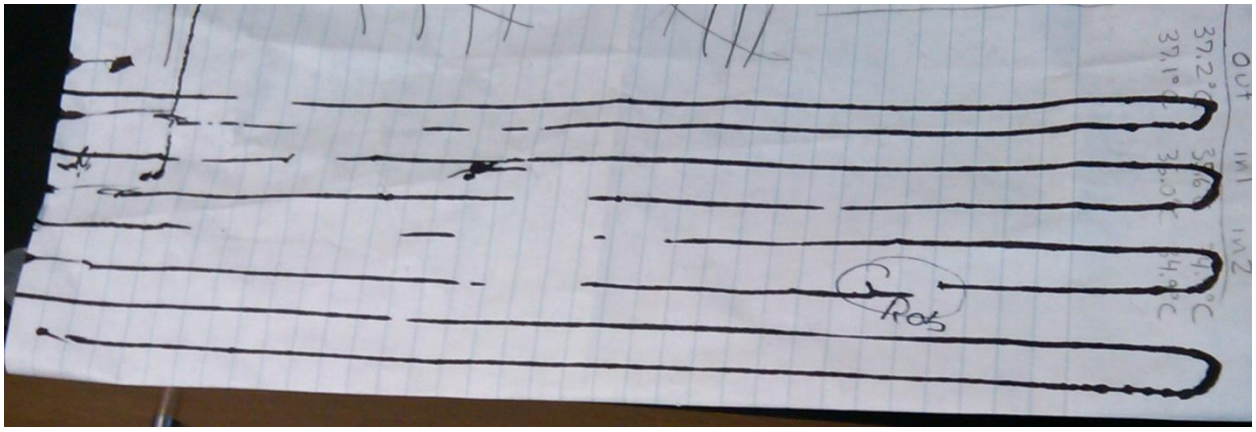


Figure 94: Marker Trial

7.2 Bench Top Trials with Bovine Collagen

In order to confirm the initial test presented above, two more trials using insoluble bovine collagen were performed. These tests were completed on the bench top and not in the bath to avoid wasting buffer solution during these preliminary trials where the design team did not intend to keep the threads after extrusion. The extrusion system was run in the same manner as before with the syringe pump and bovine collagen substituted for the marker. Production parameters such as collagen volume and syringe pump settings were identical to those used during the anchor system trials (See Section 6.5.2 Testing of the New Anchor System). After two separate trials, the threads remained straight, as can be seen in Figure 95 below and in Supplemental Video 7.

Figure 95: Benchtop Bovine Extrusion Test

Based on the successful results of these two introductory tests, the project team was able to move on to final verification using rat collagen as well as the constructed bath system.

7.3 First Trial with Rat-Tail Collagen

After verifying that the extrusion system was able to form straight, parallel lines with the bovine collagen solution, the design team decided to experiment with rat tail collagen in the bath system. Prior to the start of the experiment, the outer bath was pre-heated to 37 °C, and the first buffer solution was added to the inner bath through the use of a 1000 mL graduated cylinder. The syringe pump along with its tubing was attached to the pipette and placed over the corner of the anchor system. In addition, the Vex® Robotics microcontroller was programmed to perform a full test run of the extrusion device. The design team first made the necessary buffer solutions, following the instructions outlined in the Operations Manual in Appendix H: Operating Instructions, at the WPI Life Sciences and Bioengineering Center at Gateway Park and transported them to the WPI Goddard MQP Laboratory, along with a 50 mL

container of type I collagen from rat tails. This experiment was meant to simulate the use of the device under laboratory conditions, and would be a good first indicator of potential problems that had still not been solved.

First, the extrusion system and bath system were aligned to make sure that the threads would be extruded parallel to the anchor system. This was done by manually moving linear slides #1 and #2 and rearranging the bath system to match the motion of these slides. Once the two components were lined up, approximately 12L of tap water was added to the outer bath and the immersion heater was turned on and left for approximately 20 minutes to allow it to reach temperature. When the temperature of the bath reached a consistent 37.1°C, Fiber Formation Buffer was added to the inner bath and allowed to sit for approximately 20 minutes until it reached around 30°C. This was done in order to minimize any ill effects of extruding the threads into room temperature buffer. However, it should be noted that this is not currently done in the client's lab and threads are routinely extruded into buffer almost immediately after it is added to the dish. 3.0mL of 10mg/mL rat tail collagen was then loaded into the syringe pump at extruded at 0.225 mL/min.

During this trial, the design team had difficulty getting the threads to adhere properly to the anchor system. This had not been an issue in previous trials because they had been performed on a dry platform on the bench-top. In solution, it was found that the height of the extrusion tubing relative to the anchor points and anchor block made a significant difference in how the threads sat in the bath. From observing this initial extrusion, the design team saw that the anchor points were slightly lower than the anchor block, which meant that, over the anchor points, the extrusion tubing was too high to allow proper adherence. Because the polycarbonate on the anchor points had been scored, the thickness had decreased. However, lowering the extrusion tubing would cause it to pass too close to the anchor blocks. To solve this issue, the design team decided to raise the anchor points. Folded up Parafilm was added underneath both sides of the anchor system so that each side was closer to the

height of the anchor block. This solution was not ideal because: 1) Parafilm was only added under a small portion of the anchor sides which made the anchor system slightly unsteady, 2) this was not a very accurate system, and 3) it would not be a permanent solution. However, for the purposes of testing, the design team felt it would be sufficient to demonstrate whether or not raising the anchor systems could fix the anchor adhesion problem.

After all of the preliminary trials were completed, calipers were used to measure the height of the parafilm. Next, a set of 4in x 4in glass plates were cut to fit underneath the anchor points. Two glass plates, totaling a height of about 3/32", were stacked and used to create a level system, as seen in Figure 96 below.

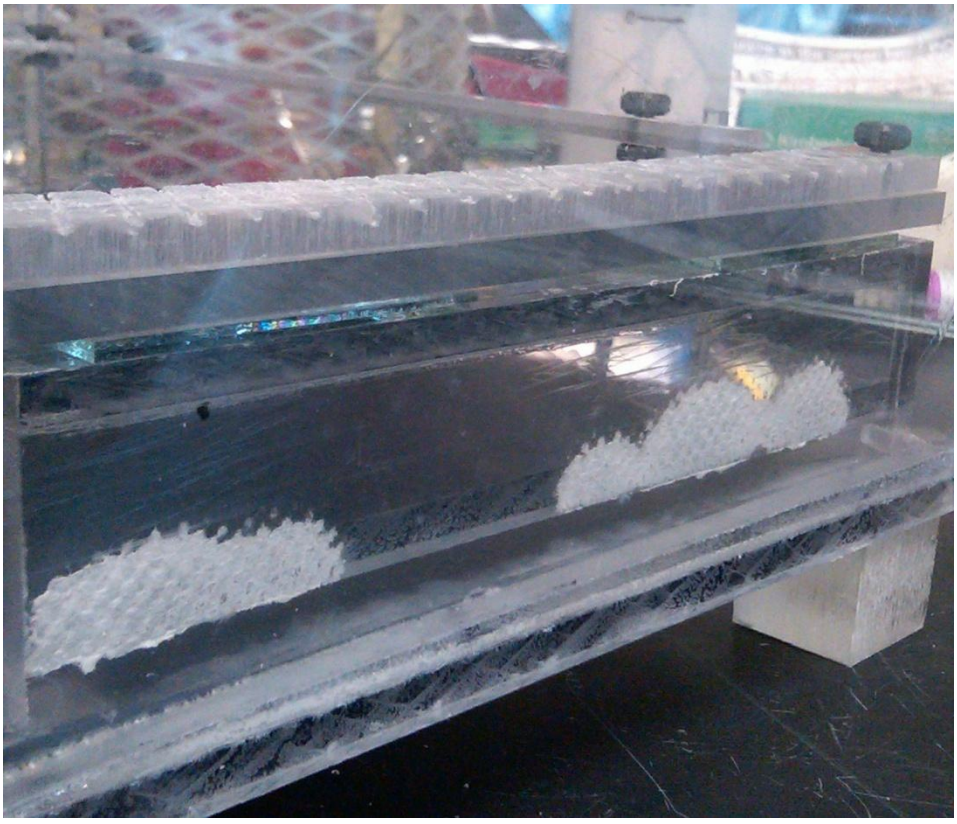


Figure 96: Glass Slides to Fix Anchor Height

The design team created a more permanent solution by ordering a 3/32" thick polycarbonate sheet from McMaster Carr® (Part Number: 8574K25) and gluing it to the bottom of the anchors for the final design using methylene chloride glue.

When the rest of the collagen was used to extrude subsequent threads, it was found that they adhered more effectively to the anchor points. Additionally, when the threads were left covered for 24 hours, the design team observed that the temperature in the inner bath had risen to the correct temperature range (36-37°C) and remained relatively constant. However, there were some other problems that the design team noticed with the extrusion. First, the threads that were extruded had a wavy quality, as seen in Figure 97, which was problematic for the integrity of the threads and also meant that the threads were longer than the distance from anchor point to anchor point.

Figure 97: Initial Wavy Threads

This meant that even though they were anchored, they could still float over a relatively large area. This caused problems with threads sticking together soon after they were formed. The design team hypothesized that there must be some sort of vibration which was causing the pipette tip to move and the threads to come out wavy. Also, while the threads were sticking to the anchor system, it was not a very effective adhesion. There was still a problem with getting one thread to finish extruding and adhere properly without being pulled along by the extrusion of the next thread. As the pipette moved from thread to thread, the newly extruded thread often was pulled along slightly and interfered with the extrusion of a new thread. The design team decided that in order to assure proper adhesion to the

anchor points, slight pauses would be added to the extrusion head as it began or completed a thread on the anchor points. This would allow a small amount of collagen to pool on the anchor points, and would help ensure that none of the threads became detached. With the Vex® Robotics microcontroller reprogrammed, the experiment was resumed and the device was allowed to complete its test run.

7.4 Second Trial with Rat-Tail Collagen

After improving the anchor adhesion, the design team then focused on how to improve the wavy nature of the threads. In order to do this, the design team felt that it was appropriate to perform a full trial of collagen extrusion with buffer changes under laboratory conditions. For this experiment, 5 mL of collagen would be extruded into the inner bath system of the device, and over the next three days the design team would perform the necessary buffer changes.

As the experiment began, the design team noticed that the first series of threads which had been extruded were wavy and too long. This was causing the threads to stick to one another, and so it was necessary to temporarily stop the experiment. The design team could see no visible motion in the extrusion system that would account for this thread pattern. Therefore, design team hypothesized that the threads were not coming out straight because the speed of Motor #2 was too slow. Because of this, too much collagen was being extruded along the length of the bath, and was causing the threads to be wavy.

As a result, the design team reprogrammed the Vex® Robotics microcontroller to increase the speed of Motor #2. Once extrusion had begun again, the design team noticed that the increase in speed of Motor #2 had significantly increased the quality of the threads. Each thread the correct length, which meant that they were held taught by the anchor points and could not interfere with further extrusion. Furthermore, the threads were now straight. A picture of the threads in solution can be seen below in Figure 98.

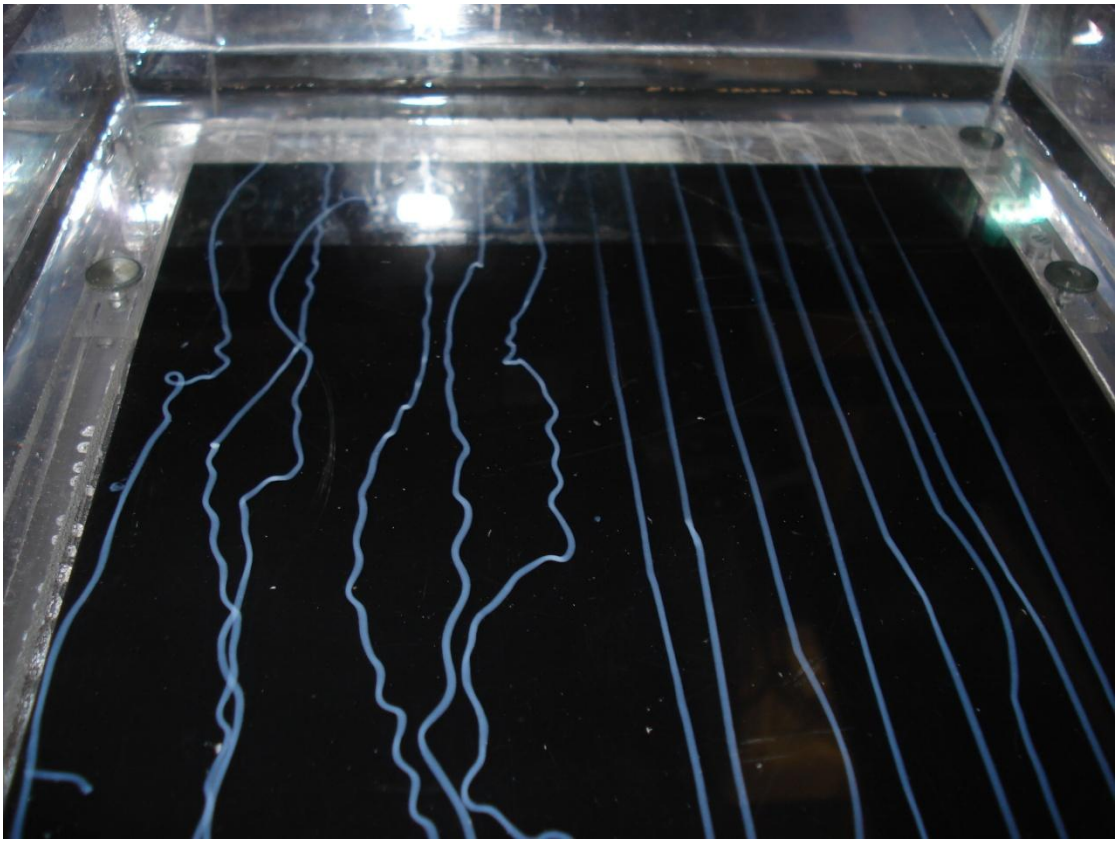


Figure 98: Bath With Straight and Wavy Threads

Once the device had finished extruding the threads, the pipette with the syringe tubing was removed from the inner bath and the acrylic covers were placed over both the inner bath and the outer bath.

Over the next three days the design team observed the threads as buffer solution changes were performed using the drainage and filling system described in Section 6.10 Construction and Testing of the Hopper System. It was noted that by the second day of the experiment, the buffer solution had equalized with the outer bath system at a temperature of 37 °C, and this was consistent throughout the rest of the thread formation process.

After performing the remaining solution changes over the next few days, the finished microthreads were evaluated under a microscope. One thread from before reprogramming (wavy) and one thread from after reprogramming (straight) were sandwiched between two large glass microscope slides seen below in Figure 99.

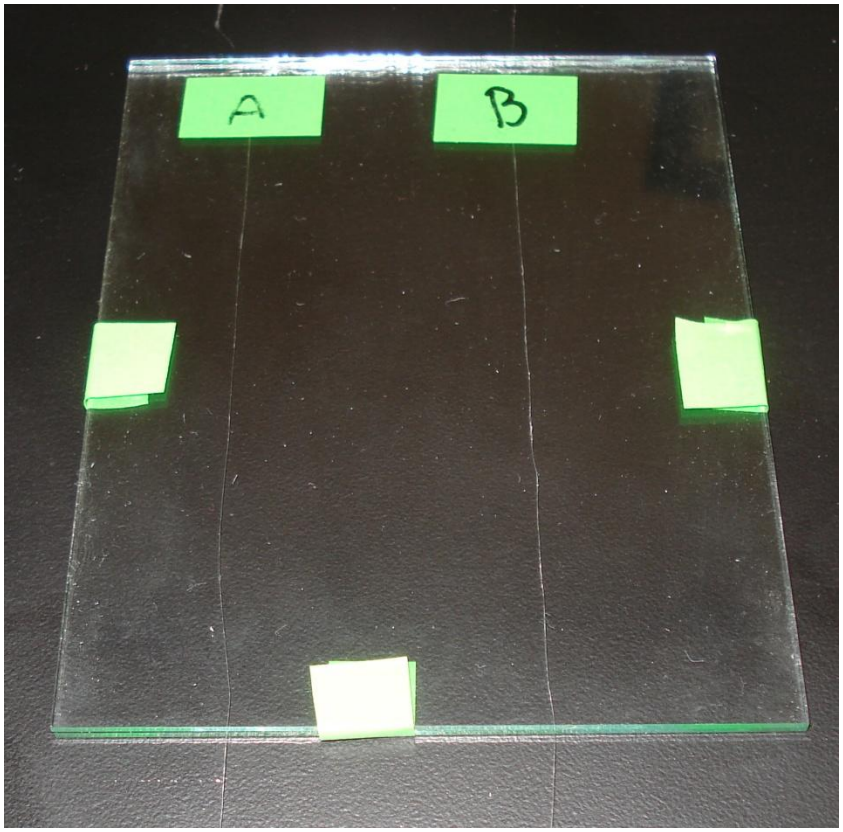


Figure 99: Microscope Set Up

Visual observations were made using an Accu-Scope inverted 30-30 microscope at 4x and 10x magnification. The design team found that both types of threads had some contaminants which were concluded to be from fibers from paper towels used to wipe the glass microscope slides. However, the overall quality of both types of threads was very high. The wavy thread showed slight bulging in several places along the length while the straight thread was uniform along its length. These observations were confirmed by taking images of the threads (Figure 100) with a Nikon Eclipse E600 microscope with a RT Color SPOT™ Diagnostic Camera (Instruments, Inc. Model #2.2.10) at 10x magnification.

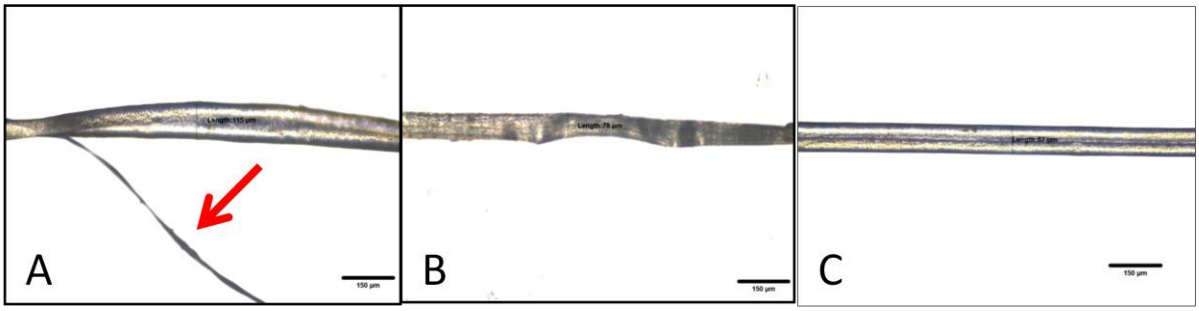


Figure 100: Microscope images showing (A) contaminants in the threads, (B) diameter bulging in wavy thread and (C) consistent diameter of straight thread

7.5 Diameter Testing

After initial qualitative testing with the microscope, the design team performed dry diameter testing in order to compare the consistency in the diameters of the microthreads extruded with the hand-drawn method, and with microthreads extruded at three different machine extrusion speeds. For this experiment, six microthreads were extruded for each of the four groups: Hand-Drawn, Machine Speed 0.496 cm/s, Machine Speed 0.617 cm/s, Machine Speed 0.816 cm/s, and hung to dry for 24 hours. Six threads per extrusion method were taped to a glass slide as seen below in Figure 101. A Nikon Eclipse E600 microscope with a RT Color SPOT™ Diagnostic Camera (Instruments, Inc. Model #2.2.1) was used to capture ten random images along each thread length at 10x magnification.

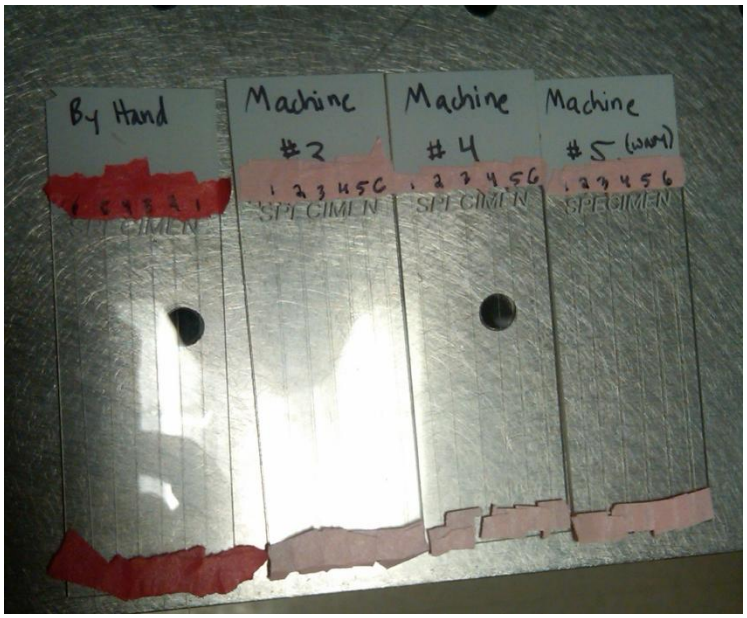


Figure 101: Slide setup for Microscopic Diameter Measurement

After each image was captured on the microscope camera, the software program SPOT™ Advanced was used to digitally measure the diameter of each thread section. An example of this can be seen in Figure 102 below. Prior to this experiment, the accuracy of the software was confirmed through the use of a calibrated eyepiece.

Figure 102: Diameter sample from machine #3

Once confirmed, ten diameter measurements were taken along the length of each of the six microthreads in each of the four groups, giving a total of 240 diameter measurements which can be

seen in Supplemental Spreadsheet 2: Diameter Testing Data. With this data, both the consistency of thread diameter between threads, as well as the consistency of thread diameter along the length of the same thread was calculated and shown below in Figure 103 and Figure 104.

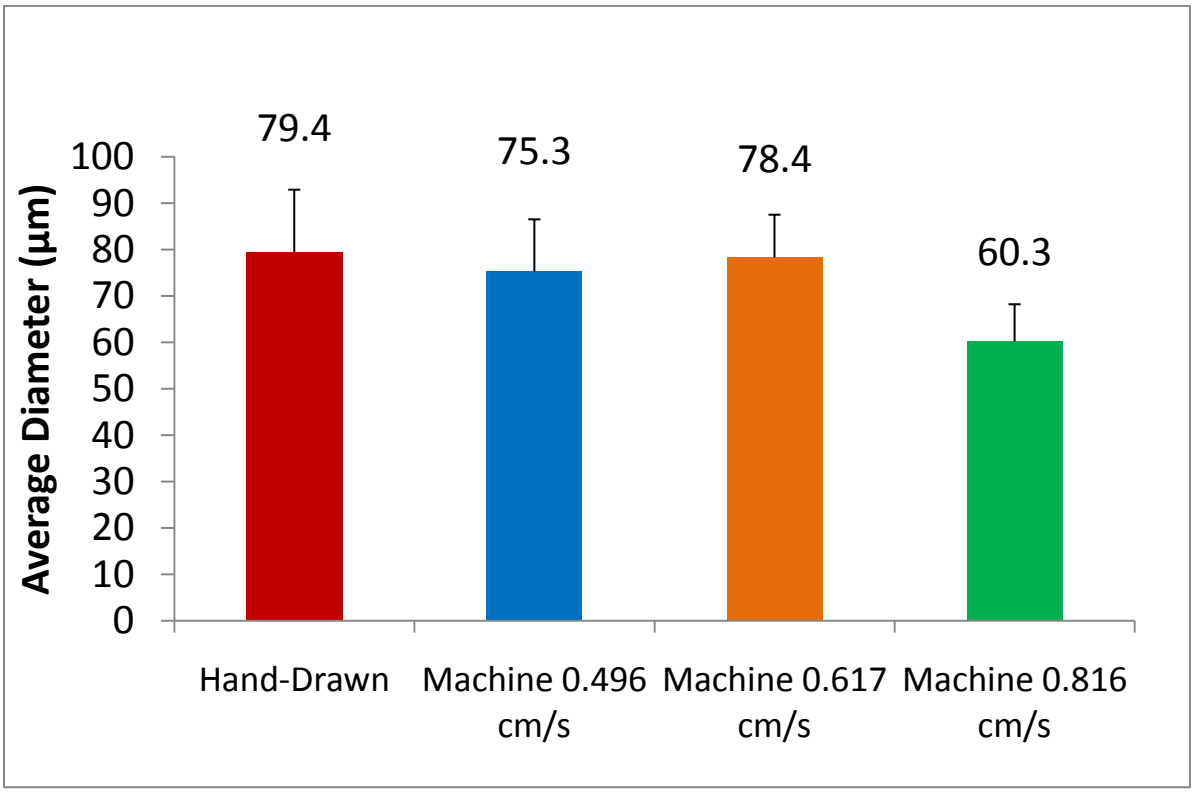


Figure 103: Thread Diameters for Different Extrusion Methods

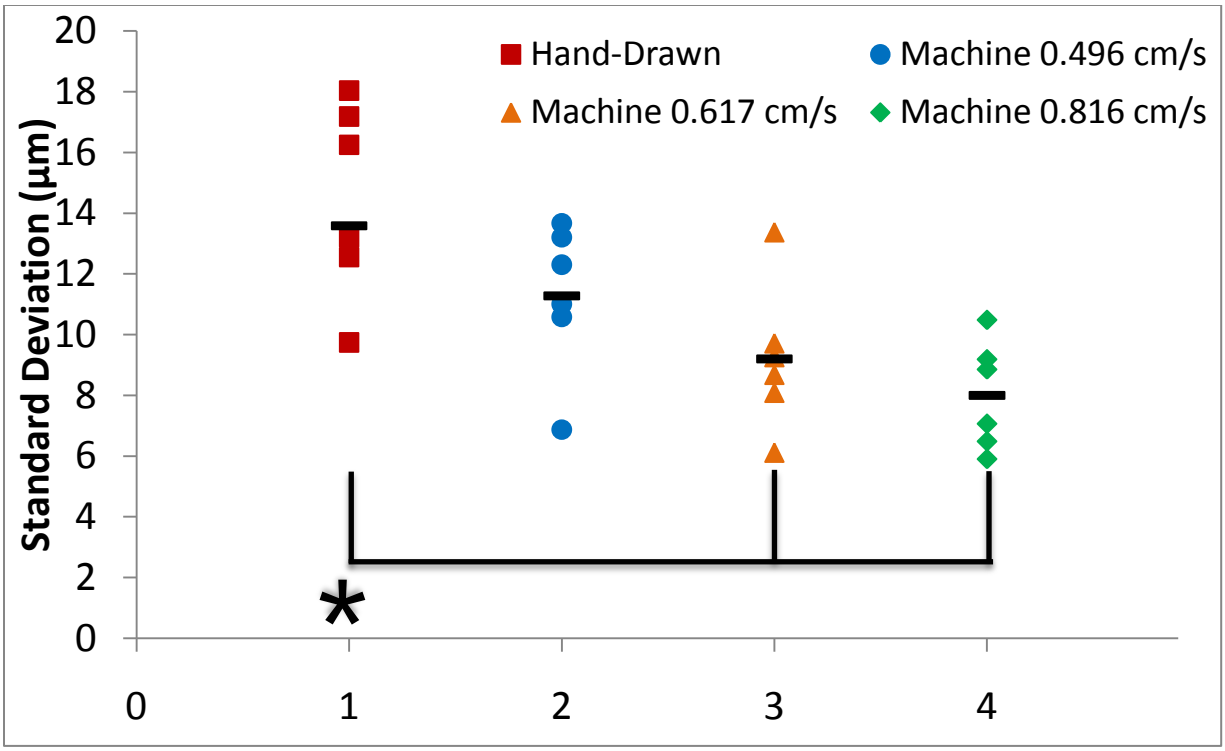


Figure 104: Consistency of Thread Diameters, * denotes statistical significance with ANOVA and Tukey test, $p < 0.05$

The overall average of diameters between microthreads, as seen in Figure 104 was calculated for each of the four groups as follows: first, the ten diameter measurements taken along the length of each microthread were averaged to give an average diameter for each microthread, and then an average was taken of the six diameter measurements for each group. In order to calculate the consistency of the diameter along the length of each microthread, the standard deviation of the ten diameter measurements for each of the six microthreads in each of the four groups was calculated and reported in Table 79. In addition, the averages of the standard deviations for each group were also calculated.

Table 79: Standard Deviation of Thread Diameters Among Test Groups

Standard Deviation (µm)	Hand-Drawn	Machine 0.496 cm/s	Machine 0.617 cm/s	Machine 0.816 cm/s
Thread 1	17.2	11.0	6.1	6.5
Thread 2	18.0	13.2	8.8	8.9
Thread 3	16.2	13.7	9.7	9.2
Thread 4	12.6	6.9	13.4	10.5
Thread 5	13.2	12.3	9.3	7.1
Thread 6	8.1	10.6	8.1	5.9
Average	13.6	11.3	9.2	8.0

The fastest extrusion speed produces the thinnest thread with the lowest standard deviation. This makes sense because the faster the extrusion head is moving, the thinner the threads will be and the less chance the threads will have to become wavy or to vary in any way. However, this pattern is not consistent across the three extrusion speeds because the slowest extrusion speed produces smaller threads with a higher standard deviation than the medium extrusion speed. This higher diameter is most likely due to the fact that the extrusion speed was too slow so the collagen material extruded in a wavy pattern rather than being pulled taught. Therefore, instead of the diameter of the thread increasing, it became slightly elongated instead. The hand-drawn extrusion method produces threads with the highest average diameter and standard deviation. This is consistent with the fact that the threads are individually extruded by hand and the extrusion speed changes with how fast or slowly the user's hand moves; thus, the threads have a very high variance in diameter along the length of the thread.

In order to determine any statistical difference between diameters and standard deviations in the hand-drawn microthreads and the machine extruded microthreads, as shown in Appendix I: Statistical Data, a single-factor analysis of variance (ANOVA) was performed using Statistical Analysis Software (SAS) and Microsoft® Excel with a p-value < 0.05 considered to be statistically significant. For the diameter testing, a p-value of 3.54×10^{-5} was calculated which confirmed that there was a statistically significant difference between the diameters of threads extruded with the hand-drawn and

different machine extrusion speeds. For the standard deviations, a p-value of 0.0013 was calculated which confirmed a statistical difference in the results, and so to determine which of the results was significantly different than the others a Tukey honest significant difference post-hoc test was performed, again using SAS. The results of the Tukey test showed that the hand-drawn microthreads had a statistically higher standard deviation than the machine extruded microthreads at the 0.617 cm/s and 0.816 cm/s extrusion speeds, and confirmed the device's ability to make microthreads with a more consistent diameter than the current hand-drawn method. The machine extruded microthreads at 0.496 cm/s were most likely not statistically more consistent than the hand-drawn threads because of the previously mentioned problem with wavy extrusion.

7.6 Mechanical Testing

Tensile testing was performed on all four experimental groups (as explained in Appendix H: Operating Instructions) using an Instron ElectroPuls E1000 device. Full experimental materials and methods can be found in the Operations Manual in Appendix H: Operating Instructions. Briefly, 18-20 thread samples from each experimental group were attached to velum paper test frames and rehydrated, and pulled to breakage at a 50% strain rate (10mm/min) with load and extension measurements taken every 100 ms and every 0.025 N. Supplemental Video 8: Tensile Test shows an example of a typical tensile test run. Hydrated diameter measurements (see Supplemental Spreadsheet 2: Diameter Testing Data) were taken of each thread before testing and used to calculate the ultimate tensile strength and strain to failure of each sample. Three diameter measurements were taken for each sample and then averaged to increase the accuracy of these measurements. These average diameters were used to calculate the stress-strain data found below. Figure 105 shows a representative curve for each extrusion method, while Table 80 shows color corresponding respective UTS and strain to failure.

See Supplemental Spreadsheet 3: Mechanical Testing Data for all of the raw data and Appendix J: Mechanical Testing Graphs for graphs from each thread and extrusion speed.

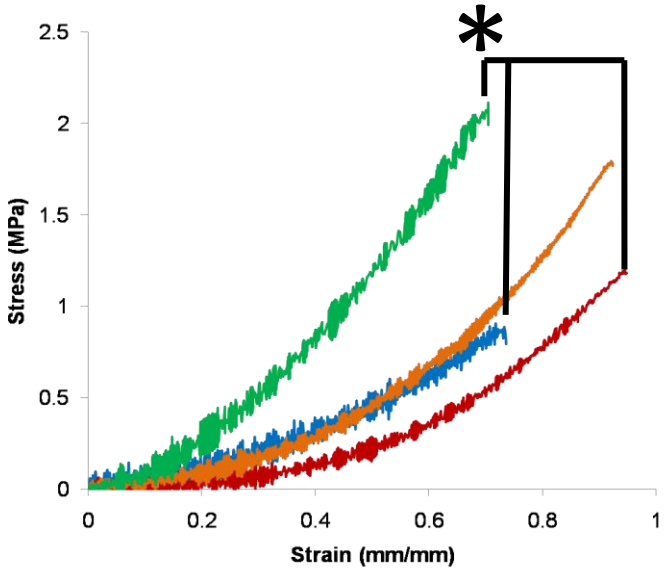


Figure 105: Representative Stress-Strain Curves, * denotes statistical significance with ANOVA and Tukey test, $p < 0.05$

Table 80: Representative UTS Values

Experimental Groups	UTS (MPa)	Strain to Failure (mm/mm)
Hand-drawn	0.93 ± 0.37	0.70 ± 0.24
Machine 0.496 cm/s	1.22 ± 0.36	0.87 ± 0.21
Machine 0.617 cm/s	1.63 ± 0.37	0.84 ± 0.16
Machine 0.816 cm/s	1.93 ± 0.97	0.80 ± 0.20
ANOVA p-value	0.0001	0.1507

After acquisition of the tensile testing data, the design team observed that hand-drawn threads had a lower average UTS and strain to failure. Additionally, as the speed of machine extrusion increased,

the UTS also increased and the strain to failure decreased (while still remaining greater than the average strain to failure of the hand-drawn threads). Statistical analyses, similar to that performed with the diameter variance data, were performed to confirm these observations. Results from the two ANOVA tests confirmed a statistical difference between the UTSs of the four groups but not between the strain to failure data, therefore only UTS data was examined further. Results of the Tukey test showed that threads extruded at 0.816 cm/s had statistically higher UTS than both the 0.496 cm/s extrusion speed and the hand-drawn threads. This result seems to be consistent with early predictions since 0.496 cm/s is the devices slowest extrusion speed and matches the speed of a hand-drawn extrusion most closely. It is therefore not surprising that the machine's slowest setting differs statistically, along with hand-drawn, from the fastest machine extrusion speed. These higher UTSs are most likely the result of the threads extruded at those speeds being thinner. It has been postulated [Dunn, 1993] that thinner threads have fewer imperfections due to their lower volume. A thicker thread would have more room for imperfections to occur. The number of imperfections is aggregated when bundling is performed and a graft made from many more thin threads will have much fewer imperfections, and therefore better mechanical properties, than a graft composed of fewer thicker threads.

8.0 Discussion

8.1 Project Discussion

The purpose of this project was to design an automated system which facilitates the production and post-production of biopolymer microthreads for regenerative therapies. The completed device was able to meet the objectives and functions outlined in the design process. The main functions to be accomplished included producing threads, anchor threads, facilitate solution changes, facilitate physical post-production modification, and control production parameters. Each of these parameters was met with one or more components of the final design.

Production of threads was accomplished through the design of the bi-directional extrusion system. By designing the extrusion device to work with the preexisting tools used for thread extrusion, such as the extrusion pump, the device has the potential to create threads of various biomaterials and configurations. Also, extrusion system allows for an increased production rate because more threads can be produced in a smaller amount of time. The VEX robotics microcontroller allow it to be completely standalone and easily reprogrammable. Part of this reprogramming was the ability to extrude threads at three different extrusion speeds, which the current production system does not have the ability to perform. Diameter measurements confirmed the ability of the extrusion system to create thinner microthreads than the current system. Additionally these thinner threads were shown to have statistically higher UTSs than hand-drawn threads with statistically smaller standard deviations.

Anchoring threads was accomplished through the integrated, removable anchor system. The ability to anchor threads securely throughout the entire formation process and being able to easily remove all threads simultaneously are both major advantages that the anchor system allows. Furthermore, the anchor system allows threads to be easily moved into post-production procedures and serves as a drying and stretching rack. All these features were confirmed through qualitative design testing and significantly decrease the time, manual labor, and thread handling necessary for production and post-production modifications.

Facilitating solution changes was accomplished through the fill and drain system, as well as the design of the inner bath. Using aspiration with the tilted bottom bath tailored to minimize thread disruption helps to preventing thread loss through handling. The hopper system serves as an effective method for holding solution for multiple runs and can fill the inner bath without disturbing the threads. The simplified method for changing solutions also allows for the addition of solution based crosslinking or sterilizing agents.

Facilitating physical post-production modifications was accomplished with both the anchor and extrusion systems. The anchor system features a removable middle portion, which allows the two anchored ends to be mounted onto a stretching rack and pulled apart. The extrusion system can be reprogrammed to extrude threads closer together in separate groups, assisting in bundling. With the threads bundled together during extrusion, it becomes much easier to accomplish twisting later on.

Control of production parameters was achieved through the extrusion pump's programming and the speed settings on the extrusion pump, as well as the bath system's heater. As previously mentioned, thread diameter was shown to be affected by the speed of the extrusion system, where a faster setting produced thinner threads. An adjustment to the extrusion pumps extrusion settings can also be made to further adjust thread diameter. Testing verified the heater and bath system's ability perform consistent, long term temperature control and the heater can also be adjusted to fit the user's needs.

8.2 Impact Analysis

Because this device will be used by the client and possibly by others in the future, it is necessary to perform an analysis of the political, social, and economical ramifications of the device.

8.2.1 Economics

Because the use of collagen microthreads is currently limited to research in animal models or *in vitro*, there is little economic impact that will be caused by this device in the foreseeable future. While collagen microthreads have the potential to solve problems such as ligament repair, currently there are no human clinical trials. This is due to both the high costs associated with clinical trials, and the limited research data which is currently available. Before this device can have any significant economic impact, much more research must be performed with collagen microthreads. If in the future collagen microthreads were to be used in humans, then our device would have some economic impact because a controlled source of collagen would have to be found and used for mass production. This source would

most likely be from a controlled bovine source which is closed to the environment and costs such as food, nutrients, and living quarters would become part of the economic impact of any collagen based treatments.

8.2.2 Environmental Impact

As collagen scaffold production methods, such as this project's device, become more efficient and scaled up to meet the clinical demand, the demand for collagen sources, such as bovine, porcine, and marine animals, will increase. The increase in demand of these sources will most likely lead to an increase in source populations, which will require a number of resources such as land, energy for facilities, food, and increased labor force to maintain the higher source population. These living sources also produce waste which must be managed. The increase in energy needed and waste produced may have a significant impact on the environment. Land or water must be allotted as living space for the increased numbers, which may involve clearing trees and dredging, severely impacting the area's natural environment. Food sources also require additional land allocation, leading to the same issues. Electrical energy for running facilities requires an increased use of limited natural fuel resources. Finally, increased waste production from sources must be properly disposed of which requires additional energy and manpower. This increase in source population can most likely be properly regulated and environmentally beneficial actions could be taken, such as the use of renewable energy sources or converting waste from sources into fertilizer. Through proper management the impact of an increased collagen source population can have minimal effects on the environment, while still meeting the growing medical demands.

8.2.3 Societal Influence

This product has a very large potential to effect society. For example, when collagen threads are able to help repair damaged ligaments and tendons, the quality of life of the average person will be greatly improved. A dependable cure for their injury will also create a happier, less stressed patient. In

addition, since the device makes it easier to create and modify biopolymer microthreads, it will help increase the productivity and efficiency of laboratory research.

8.2.4 Political Ramifications

This device currently has minimal political ramifications. Microthread technology is very much in the research and initial development stages so there is little effect on the commercial or industrial market. Use of this device will hopefully increase the efficiency and production of biopolymer microthreads, but mostly in a research and development setting. Though there are research laboratories in various countries who would find this device useful, the impact on the global market as a whole would be relatively small. Until microthread technology becomes more commercialized or the device is adapted and developed to create threads on a commercial scale, there would be little effect on the international market.

8.2.5 Ethical Concerns

There are minimal ethical concerns with this device. It is designed to create materials that could later be used on tissue regeneration technologies that improve the quality of life for patients with ACL injuries. The only ethical concern that could be raised is with the materials that are used in this device. For instance, most collagen is derived from rat or bovine sources. Some potential users may feel uncomfortable using materials from animal sources, even when that material was obtained in an ethical manner.

8.2.6 Health and Safety Issues

This product has the potential to greatly improve the health of patients with damaged ligaments and tendons by creating threads that have the ability to act as fibers for injuries such as a torn ACL. This is important because the overall goal of this project is to develop a product that will improve a patient's quality of life. After initial development, extensive tests will be needed to prove the products safety,

reliability, and reproducibility. Once these tests are completed and the product is approved by the FDA, it will be considered safe for the majority of the population. However, there may be an allergic reaction associated with this, so each patient would need to be individually tested before coming in contact with the collagen to ensure that they will not have an adverse reaction to it. In addition, the collagen would need to be tested first to make sure that no other prior condition within the animal would affect the resulting threads. It would be ideal to find a reproducible, consistent collagen source that could be used in a commercial setting.

8.2.7 Manufacturability

The device created by the design team was meant to be as straightforward and easy to use and reproduce as possible. While much experimentation was performed before a final design was created, if given the opportunity, the device could be reconstructed for less than $600 by using the instructions, descriptions, and pictures found in this paper. The experiments could also be repeated, no complex machinery was required for construction, and all the materials are readily available. The biggest problem facing large scale collagen-microthread production is finding an acceptable source of collagen with little variation between the physical and mechanical properties over time. Currently, the collagen used by the client is harvested from rats, but in order to scale up production this source would most likely have to come from a bovine source. A problem with this is that herds would have to be kept in closed conditions in order to minimize the effects of the environment on the animals, which would raise costs. Until a more cost effective solution for this problem arises, the large scale up of collagen microthread production will be difficult.

8.2.8 Sustainability

This device is mostly constructed from plastics such as polycarbonate and acrylic. These materials require large amounts of energy to produce, and so, in themselves, are not very sustainable. If the project were geared towards renewable energy, other less energy intensive materials could be used.

However, the device itself uses very little power when operational and its use is not harmful to the environment. Because of this, our device is somewhat sustainable, and has no major negative impact on the biological or ecological world.

9.0 Conclusion and Recommendations

The design team went through the design process and was able to successfully design, built, and test a final device with four main components: an anchor system, a bath system, an extrusion system, and a drain and fill system. Through qualitative observations, diameter measurements, and mechanical testing, the design team was able to prove that their multifunctional semi-automated extrusion system vastly improved not only the quality of collagen microthreads, but the efficiency and ease of use of the production process. The final device was able to successfully produce microthreads at three different extrusion speeds and also easily facilitates bundling, stretching, and crosslinking. The finished product cost less than $600, had a smaller footprint than the current system, and will be implemented in the client's lab immediately. However, even with the multiple advantages of this system, there is always future work that can be done.

9.1 Scale-Up

The most important future endeavor is the scale up of the final device. Currently, it is designed to produce a minimum of fifteen threads. However, the device can be scaled up with a larger bath system to produce more threads at a time, saving the client and the user even more time and money. Ideally, the device would also be able to be converted from a laboratory setup to a commercial scale.

9.2 Use with Other Materials

The automated extrusion device was tested using collagen. However, it is designed to facilitate the production and post production of both collagen and fibrin threads. As previously mentioned, these

methods are similar with fibrin only requiring one buffer change and no heating. The hopper and bath systems easily facilitate these production modifications and satisfy these objectives. As mentioned in Section 3.2.2.1, microthreads have also been made out of other natural materials such as silk, alginate, and chitin. Eventually, the final device could be modified to facilitate production and post production of these types of threads as well.

9.3 Use for Other Applications

The purpose of this project was to design a device to produce microthreads for use in uniaxial load bearing applications. However, microthreads can be used for several other applications as well. For example, as mentioned in Section 3.2.2, fibrin microthreads have demonstrated the ability to serve as a suture-like delivery vehicle to support hMSC proliferation, survival, and differentiation ability as a part of therapies to regenerate cardiac tissue. In addition, microthreads have also been used as wound dressings [Murphy, 2008]. Another microthread cell technology involved the design of a co-culture system which used collagen microthreads to support the growth of human umbilical vein endothelial cells and human dermal fibroblasts for therapies that may potentially support angiogenesis [Carey, 2009a]. Threads produced with the final design would be viable for multiple applications, including those stated above, because their overall quality has been aptly demonstrated.

9.4 Automated Drain and Fill System

In order to make the device fully automated, the drain and fill system would need to be modified. To accomplish this, a system would need to be put in place which automatically turns on and off after the appropriate amount of buffer solution was added or removed from the bath. One careful design consideration for this system would be the need to permanently attach the drain and fill tubing to the inner bath without interfering with the path of the extrusion system as it moves up and down the inner bath. This would require careful placement of the tubing in the far corners of the inner bath.

9.4 Stretching Mechanism

In order to achieve maximum uniformity and strength, each microthread can be stretched after production. Stretching is important because as each microthread is stretched, the small fibers which compose the microthread are pulled taught and become better aligned with one another. This gives each microthread a higher strength, and a more uniform nature. Because of this, a stretching mechanism would need to be created which integrates with the current Anchor System and allows for the stretching of a complete batch of microthreads.

The stretching mechanism would be composed of three main parts, the first being two moveable platforms constructed out of ½" thick polycarbonate. The second component will either be a stainless steel or aluminum threaded rod which connects the two rectangular pieces of polycarbonate, and the final component will be two guide rods which are also composed either out of stainless steel or aluminum.

For assembly, the two polycarbonate pieces will each have three holes drilled into them lengthwise. In the center will be a threaded hole where the threaded rod will be attached. Along the two outer sides of each polycarbonate platform will be a hole where the guide rods will be inserted. Located at the four corners of the stretching device will be four posts which will allow the Anchor System to be easily mounted. Once assembled, as the threaded rod is turned by-hand, the polycarbonate pieces will either move closer or further apart, and the guide rods will act as stabilizers to stop any side to side movement. With the Anchor System mounted on the stretching mechanism, the batch of microthreads can be stretched to a uniform length. However, due to lack of time, this portion of the project could not be completed.

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Glossary

1) Acrylic→a synthetic glassy thermoplastic
2) Allografts→a tissue or organ transplanted from a donor of the same species but different genetic makeup
3) Alternative Designs → a design that satisfies particular functions and specifications
4) ANOVA (one way)→a statistical test which determines if there is a difference between experiemental groups
5) Aspirate→ to remove liquid by suction
6) Autografts→a biological part such as a tissue or organ that is reimplanted in the same individual that it originated from
7) Bandsaw→ an endless saw consisting of a toothed metal band that is driven around two wheels
8) Bovine Collagen→insoluable collagen that comes from a Bovine source, less reliable and useful in comparison to rat tail collagen
9) Braiding→ a processing method that involves combining fibers in a braided fashion to enhance strength and stability
10) Bundling→ a processing method that improves strength and stability by grouping threads together
11) Carboy→a large plastic container with a narrow neck used for storing liquid
12) Cell Culture/Seeding→ attaching cells to microthreads to bring them to an affected area, allowing them to grow and differentiate on different scaffolding until the cells can form their own extracellular matrix
13) Cell→ the basic structural and functional unit of all organisms
14) Client→ the individual who wants the design conceived, provides initial client statement, project, description and expectation of deliverables
15) CNC Machine→ a Computer Numerically Controlled (CNC) device for machining that is programmed and controlled by computer for precision
16) Collagen→ a fibrous protein found within the body that can be used to make microthreads
17) Conceptual Design → a combination of the winning alternative designs
18) Constraint→ a limiting factor of the design
19) Coping Saw→ a hand saw with a thin blade
20) Crosslinking→ a processing method that involves joining molecules by covalent bonds, an important procedure used to strengthen and stabilize fibers
21) Decision Matrix→ a spreadsheet where each row contains a design objective/constraint and each column contains an alternative design which is ranked according to how well it satisfies each objective/constraint
22) Designer→ developer of a product using design specifications which satisfy both the client and the user
23) Drill Press→ an electric drill that is pressed into the work with a hand lever
24) Dry Jet Wet Spinning→ a form of wet spinning that involves using a jet of air to stretch the material before it enters the fiber formation buffer
25) EasyC Pro→programming software for VEX
26) Engineering Design→ thoughtful development of objects that perform desired functions within given limits
27) Extrusion→ to form or shape by forcing through an opening
28) Fibrin→ a fibrous protein involved in blood clotting that can be used to make microthreads
29) Function→ a design parameter that describes what the device must do
30) Gantt Chart→ a chart or list that maps design activities against a time line and the specific designer who will complete the task

31) Hacksaw→ saw that can be used by hand for cutting metal or plastic
32) Heat Shrink →a tube which undergoes a controlled shrinkage in diameter when heated
33) Immersion Heater → a device that is submerged into a liquid and converts electrical energy to heat in order to raise the temperature of the liquid
34) Indented Objectives List→ a clustered hierarchal list of what the device must have or what it must be
35) LabVIEW ®→ an acronym for Laboratory Virtual Instrumentation Engineering Workbench, a program used for instrument control
36) Laser Cutter→ a machine that uses a laser to cut materials in a precise manner, can be used with acrylic but not with polycarbonate
37) Ligament→a fibrous tissue in the body that connects one bone to another
38) Metric→ a numerical ranking system which allows designers to quantitatively asses how well alternative designs satisfy design objectives and constraints
39) Microthread→a specific form of scaffold morphology
40) Morphological Chart→ a table which organizes the various design functions along with the ways that these functions can be achieved
41) Need→ something necessary for the success of the device
42) Objective→a design parameter that describes what the device must have or what it must be
43) Pairwise Comparison Chart→ a matrix or chart used to rank design objectives
44) Parafilm→a flexible film used for sealing
45) Pasture Pipette→long, thin droppers used to transfer small quantities of liquid
46) Rat Tail Collagen→ collagen from the tail of a rat, a reliable source for making biopolymer microthreads, currently used by client
47) Regulatory Signals→molecules that are used to stimulate specific physiological responses or prompt desired interactions to accur between implanted materials and the surrounding biological environment
48) Scaffolds→a temporary frame used as a support, has the ability to act as a delivery vehicle
49) Silk→ a biological material made from insect larvae that can be used to make microthreads
50) Specifications → statements which outline the properties and attributes that the design must contain
51) Strain to Failure→ the strain at the moment of rupture or breakage
52) Sterilization→ using chemicals or energy to eliminate the possibility of bacterial contamination of microthreads
53) Surface Modification→ a processing method that involves changing the exterior of the threads through physiochemical means or surface coatings
54) Tendon→a fibrous tissue in the body that can withstand tension and connects muscle to bone
55) Tensile Test→a test used to examine mechanical strength; a test piece is gripped at either end by an apparatus apparatus in a testing machine which slowly exerts an axial pull which pulls the sample until it breaks; ultimate tensile strength and strain to failure were obtained from this test
56) The Extracellular Matrix→ connective tissues and fibers that are not part of a cell but provide structural support for it
57) Tissue Engineering→an interdisciplinary field that applies the principles and methods of bioengineering, material science, and life sciences toward the assembly of biologic substitutes that will restore, maintain, and improve tissue functions following damage either by disease or traumatic process
58) Tukey Test→a statistical test of honest significant difference which is generally performed after an ANOVA and determines how experimental groups differ

59) Twisting→ a processing method that improves strength and stability by twisting groups of microthreads together
60) Uniaxial Load→an application of force in which a weight is placed only along a single axis
61) Ultimate Tensile Strength→the stress at moment of rupture or breakage
62) User→ person who will actually be using the final device
63) Vex Robotic Design System®→ an introductory robotic construction kit
64) Want→ an aspect of the design that is desired
65) Wet Spinning→extruding the thread material through a spinneret into a buffer solution

Level 1 Objectives	Versatile	Accurate	Precision or Reproducibility of Results	User Friendly	Automated	Able to be Scaled Up	**Design Team Totals**
Versatile		1.0	0.0	1.0	0.0	0.5	2.5
Accurate	0.0		0.0	0.0	0.0	1.0	1.0
Precision or Reproducibility of Results	1.0	1.0		1.0	0.5	1.0	4.5
User Friendly	0.0	1.0	0.0		0.0	1.0	2.0
Automated	1.0	1.0	0.5	1.0		1.0	4.5
Able to be Scaled Up	0.5	0.0	0.0	0.0	0.0		0.5

Versatile Level 2	Production	Post-Production	**Design Team Totals**
Production		0.5	0.5
Post-Production	0.5		0.5

Production Level 3	Sterile Threads	Different Materials	Different Diameters	Formation Patterns	Temperature	Anchored Threads	Thread Drying	Buffer Changes	Uniformity and Precision of Thread Stretching	Seeding Cells in Threads	**Design Team Totals**
Sterile Threads		0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	1.0	2.0
Different Materials	1.0		1.0	1.0	0.0	0.0	0.5	0.0	0.5	1.0	5.0
Different Diameters	1.0	0.0		1.0	0.0	0.0	0.0	0.0	1.0	0.0	3.0
Formation Patterns	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0
Temperature	1.0	1.0	1.0	1.0		0.5	0.5	0.5	1.0	1.0	7.5
Anchored Threads	1.0	1.0	1.0	1.0	0.5		0.5	0.5	1.0	1.0	7.5
Thread Drying	1.0	0.5	1.0	1.0	0.5	0.5		0.5	0.0	1.0	6.0
Buffer Changes	1.0	1.0	1.0	1.0	0.5	0.5	0.5		1.0	1.0	7.5
Uniformity and Precision of Thread Stretching	1.0	0.5	0.0	1.0	0.0	0.0	1.0	0.0		1.0	4.5
Seeding Cells in Threads	0.0	0.0	1.0	1.0	0.0	0.0	0.0	0.0	0.0		2.0

Post-Production Level 3	Uniformity and Precision of Thread Stretching	Sterilize	Seed Threads	Crosslink	Bundle	Braid	Twist	Surface Modification	**Design Team Totals**
Uniformity and Precision of Thread Stretching		1.0	1.0	0.0	0.0	1.0	0.0	0.0	3.0
Sterilize	0.0		1.0	0.5	0.0	1.0	0.5	0.5	3.5
Seed Threads	0.0	0.0		0.0	0.0	1.0	0.0	0.0	1.0
Crosslink	1.0	0.5	1.0		0.5	1.0	1.0	1.0	6.0
Bundle	1.0	1.0	1.0	0.5		1.0	0.5	1.0	6.0
Braid	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0
Twist	1.0	0.5	1.0	0.0	0.5	1.0		1.0	5.0
Surface Modification	1.0	0.5	1.0	0.0	0.0	1.0	0.0		3.5

Precision or Reproducibility of Results Level 2	Thread Diameter	Thread Length	Mechanical Properties	Specified Parameters	**Design Team Totals**
Thread Diameter		1.0	0.0	0.0	1.0
Thread Length	0.0		0.0	0.0	0.0
Mechanical Properties	1.0	1.0		0.0	2.0
Specified Parameters	1.0	1.0	1.0		3.0

User Friendly Level 2	Easy to Use	Easy to Clean	Efficient Turnaround-from batch to batch	Easily Maintained	Reliability	**Design Team Totals**
Easy to Use		1.0	0.5	0.0	0.0	1.5
Easy to Clean	0.0		0.0	0.0	0.0	0.0
Efficient Turnaround	0.5	1.0		1.0	0.5	3.0
Easily Maintained	1.0	1.0	0.0		0.0	2.0
Reliability	1.0	1.0	0.5	1.0		3.5

Automated Level 2	Minimize Human Contribution	High Production Rate	**Design Team Totals**
Minimize Human Contribution		1.0	1.0
High Production Rate	0.0		0.0

Level 1 Objectives	Versatile	Accurate	Precision or Reproducibility of Results	User Friendly	Automated	Able to be Scaled Up	**Client Totals**
Versatile		1.0	0.5	1.0	1.0	1.0	4.5
Accurate	0.0		0.0	0.0	0.0	0.0	0.0
Precision or Reproducibility of Results	0.5	1.0		1.0	1.0	1.0	4.5
User Friendly	0.0	1.0	0.0		0.5	1.0	2.5
Automated	0.0	1.0	0.0	0.5		1.0	2.5
Able to be Scaled Up	0.0	1.0	0.0	0.0	0.0		1.0

Client

Versatile Level 2	Production	Post-Production	**Client/User Totals**
Production		1.0	1.0
Post-Production	0.0		0.0

Production Level 3	Sterile Threads	Different Materials	Different Diameters	Formation Patterns	Temperature	Anchored Threads	Thread Drying	Buffer Changes	Uniformity and Precision of Thread Stretching	Seeding Cells in Threads	**Client Totals**
Sterile Threads		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Different Materials	1.0		1.0	1.0	0.0	0.0	0.0	0.0	0.0	1.0	4.0
Different Diameters	1.0	0.0		1.0	0.0	0.0	0.0	0.0	0.0	1.0	3.0
Formation Patterns	1.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0	1.0	2.0
Temperature	1.0	1.0	1.0	1.0		0.5	0.5	0.5	0.5	1.0	7.0
Anchored Threads	1.0	1.0	1.0	1.0	0.5		0.5	0.5	0.5	1.0	7.0
Thread Drying	1.0	1.0	1.0	1.0	0.5	0.5		0.5	0.5	1.0	7.0
Buffer Changes	1.0	1.0	1.0	1.0	0.5	0.5	0.5		0.5	1.0	7.0
Uniformity and Precision of Thread Stretching	1.0	1.0	1.0	1.0	0.5	0.5	0.5	0.5		1.0	7.0
Seeding Cells in Threads	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		1.0

Post-Production Level 3	Uniformity and Precision of Thread Stretching	Sterilize	Seed Threads	Crosslink	Bundle	Braid	Twist	Surface Modification	**Client Totals**
Uniformity and Precision of Thread Stretching		1.0	1.0	0.0	0.5	1.0	0.0	1.0	4.5
Sterilize	0.0		1.0	0.0	0.0	0.5	0.0	1.0	2.5
Seed Threads	0.0	0.0		0.0	0.0	1.0	0.0	1.0	2.0
Crosslink	1.0	1.0	1.0		0.0	1.0	1.0	1.0	6.0
Bundle	0.5	1.0	1.0	1.0		1.0	1.0	1.0	6.5
Braid	0.0	0.5	0.0	0.0	0.0		0.0	0.0	0.5
Twist	1.0	1.0	1.0	0.0	0.0	1.0		1.0	5.0
Surface Modification	0.0	0.0	0.0	0.0	0.0	1.0	0.0		1.0

Precision or Reproducibility of Results Level 2	Thread Diameter	Thread Length	Mechanical Properties	Specified Parameters	**Client Totals**
Thread Diameter		1.0	0.5	1.0	2.5
Thread Length	0.0		0.0	1.0	1.0
Mechanical Properties	0.5	1.0		1.0	2.5
Specified Parameters	0.0	0.0	0.0		0.0

User Friendly Level 2	Easy to Use	Easy to Clean	Efficient Turnaround-from batch to batch	Easily Maintained	Reliability	**Client Totals**
Easy to Use		0.5	0.5	1.0	0.5	2.5
Easy to Clean	0.5		0.5	0.5	0.0	1.5
Efficient Turnaround	0.5	0.5		1.0	0.0	2.0
Easily Maintained	0.0	0.5	0.0		0.5	1.0
Reliability	0.5	1.0	1.0	0.5		3.0

Automated Level 2	Minimize Human Contribution	High Production Rate	**Client Totals**
Minimize Human Contribution		0.5	0.5
High Production Rate	0.5		0.5

Level 1 Objectives	Versatile	Accurate	Precision or Reproducibility of Results	User Friendly	Automated	Able to be Scaled Up	**User Totals**
Versatile		1.0	0.5	1.0	1.0	1.0	4.5
Accurate	0.0		0.0	0.0	0.0	0.0	0.0
Precision or Reproducibility of Results	0.5	1.0		1.0	1.0	1.0	4.5
User Friendly	0.0	1.0	0.0		0.5	1.0	2.5
Automated	0.0	1.0	0.0	0.5		0.5	2.0
Able to be Scaled Up	0.0	1.0	0.0	0.0	0.5		1.5

User

Versatile Level 2	Production	Post-Production	**User Totals**
Production		1.0	1.0
Post-Production	0.0		0.0

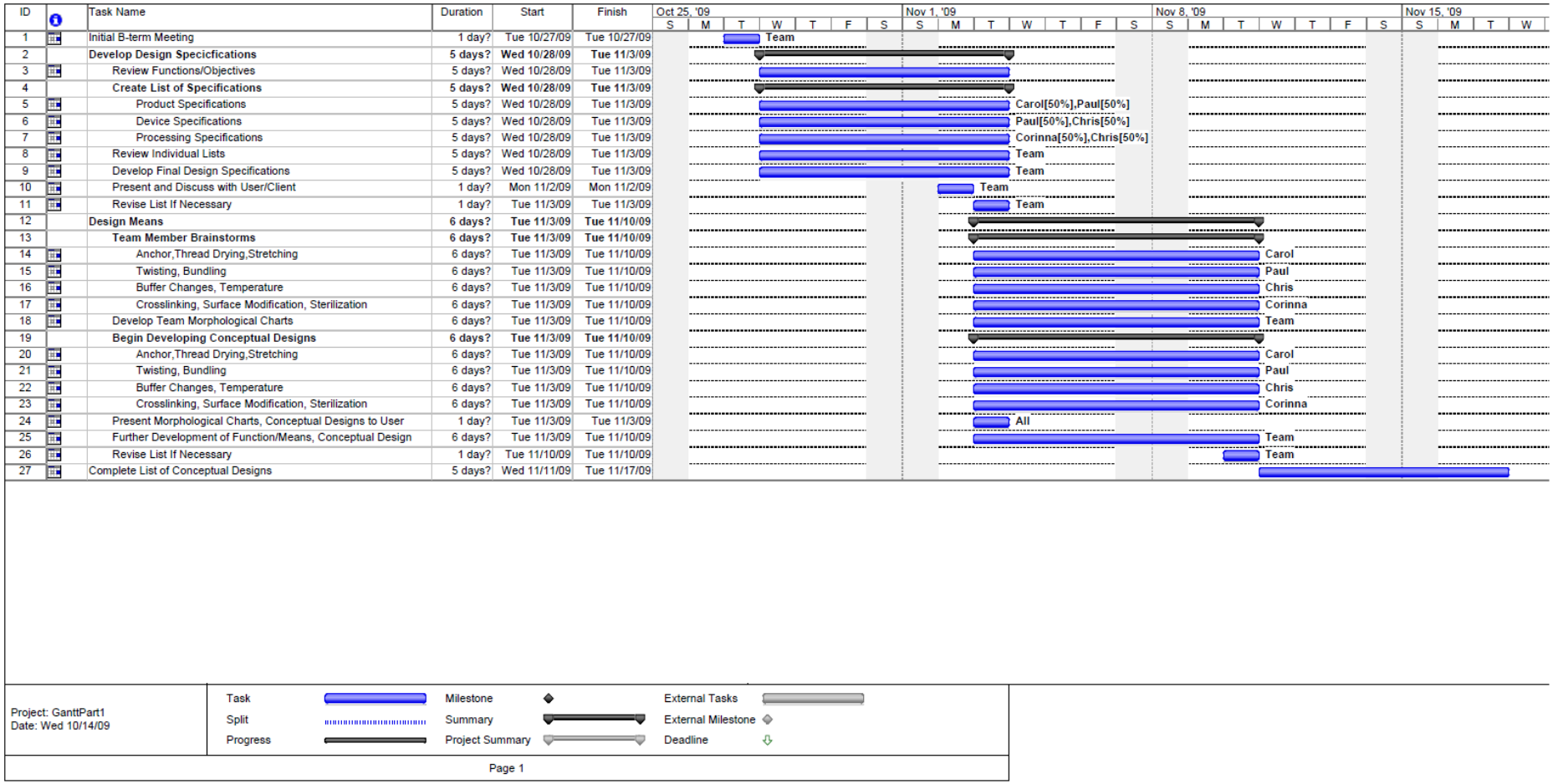
Production Level 3	Sterile Threads	Different Materials	Different Diameters	Formation Patterns	Temperature	Anchored Threads	Thread Drying	Buffer Changes	Uniformity and Precision of Thread Stretching	Seeding Cells in Threads	**User Totals**
Sterile Threads		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Different Materials	1.0		1.0	1.0	0.0	0.0	0.0	0.0	0.0	1.0	4.0
Different Diameters	1.0	0.0		0.5	0.0	0.0	0.0	0.0	0.0	1.0	2.5
Formation Patterns	1.0	0.0	0.5		0.0	0.0	0.0	0.0	0.0	0.5	2.0
Temperature	1.0	1.0	1.0	1.0		0.5	0.5	0.5	0.5	1.0	7.0
Anchored Threads	1.0	1.0	1.0	1.0	0.5		0.5	0.5	0.5	1.0	7.0
Thread Drying	1.0	1.0	1.0	1.0	0.5	0.5		0.5	0.5	1.0	7.0
Buffer Changes	1.0	1.0	1.0	1.0	0.5	0.5	0.5		0.5	1.0	7.0
Uniformity and Precision of Thread Stretching	1.0	1.0	1.0	1.0	0.5	0.5	0.5	0.5		1.0	7.0
Seeding Cells in Threads	1.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0		1.5

Post-Production Level 3	Uniformity and Precision of Thread Stretching	Sterilize	Seed Threads	Crosslink	Bundle	Braid	Twist	Surface Modification	**User Totals**
Uniformity and Precision of Thread Stretching		1.0	1.0	0.0	0.5	1.0	0.0	0.0	3.5
Sterilize	0.0		1.0	0.0	0.0	1.0	0.5	0.0	2.5
Seed Threads	0.0	0.0		0.0	0.0	1.0	0.0	0.0	1.0
Crosslink	1.0	1.0	1.0		0.0	1.0	1.0	0.5	5.5
Bundle	0.5	1.0	1.0	1.0		1.0	1.0	1.0	6.5
Braid	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0
Twist	1.0	0.5	1.0	0.0	0.0	1.0		0.0	3.5
Surface Modification	1.0	1.0	1.0	0.5	0.0	1.0	1.0		5.5

Precision or Reproducibility of Results Level 2	Thread Diameter	Thread Length	Mechanical Properties	Specified Parameters	**User Totals**
Thread Diameter		1.0	0.5	1.0	2.5
Thread Length	0.0		0.0	1.0	1.0
Mechanical Properties	0.5	1.0		1.0	2.5
Specified Parameters	0.0	0.0	0.0		0.0

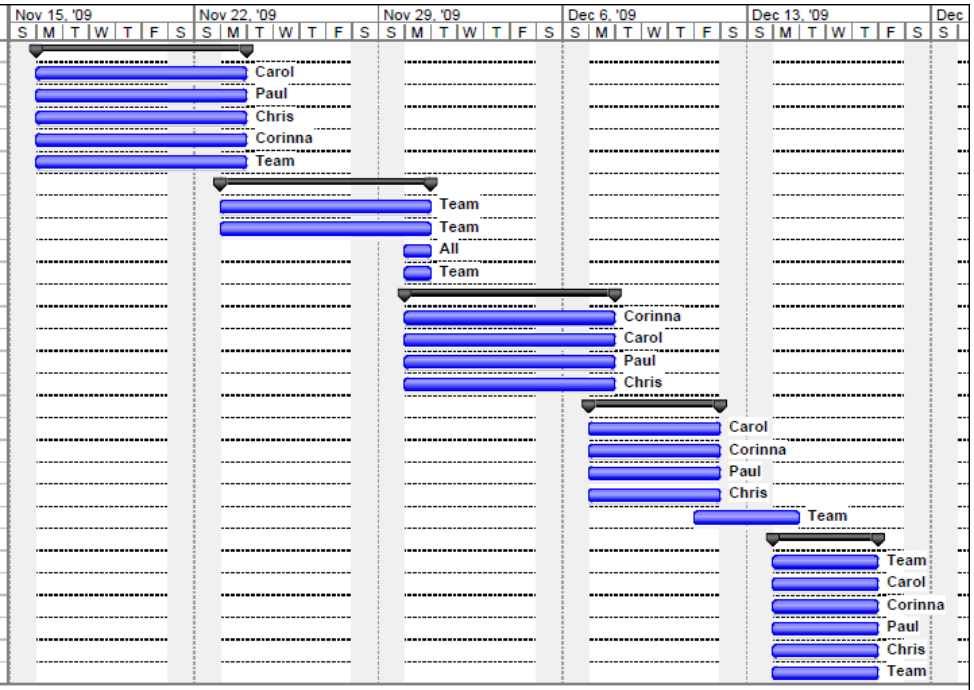
User Friendly Level 2	Easy to Use	Easy to Clean	Efficient Turnaround-from batch to batch	Easily Maintained	Reliability	**User Totals**
Easy to Use		0.5	0.5	0.5	0.5	2.0
Easy to Clean	0.5		0.5	0.5	0.0	1.5
Efficient Turnaround	0.5	0.5		1.0	0.0	2.0
Easily Maintained	0.5	0.5	0.0		0.5	1.5
Reliability	0.5	1.0	1.0	0.5		3.0

Automated Level 2	Minimize Human Contribution	High Production Rate	**User Totals**
Minimize Human Contribution		0.5	0.5
High Production Rate	0.5		0.5



ID	Task Name	Duration	Start	Finish
1	Initial B-term Meeting	1 day?	Tue 10/27/09	Tue 10/27/09
2	**Develop Design Specicfications**	**5 days?**	**Wed 10/28/09**	**Tue 11/3/09**
3	Review Functions/Objectives	5 days?	Wed 10/28/09	Tue 11/3/09
4	**Create List of Specifications**	**5 days?**	**Wed 10/28/09**	**Tue 11/3/09**
5	Product Specifications	5 days?	Wed 10/28/09	Tue 11/3/09
6	Device Specifications	5 days?	Wed 10/28/09	Tue 11/3/09
7	Processing Specifications	5 days?	Wed 10/28/09	Tue 11/3/09
8	Review Individual Lists	5 days?	Wed 10/28/09	Tue 11/3/09
9	Develop Final Design Specifications	5 days?	Wed 10/28/09	Tue 11/3/09
10	Present and Discuss with User/Client	1 day?	Mon 11/2/09	Mon 11/2/09
11	Revise List If Necessary	1 day?	Tue 11/3/09	Tue 11/3/09
12	**Design Means**	**6 days?**	**Tue 11/3/09**	**Tue 11/10/09**
13	**Team Member Brainstorms**	**6 days?**	**Tue 11/3/09**	**Tue 11/10/09**
14	Anchor,Thread Drying,Stretching	6 days?	Tue 11/3/09	Tue 11/10/09
15	Twisting, Bundling	6 days?	Tue 11/3/09	Tue 11/10/09
16	Buffer Changes, Temperature	6 days?	Tue 11/3/09	Tue 11/10/09
17	Crosslinking, Surface Modification, Sterilization	6 days?	Tue 11/3/09	Tue 11/10/09
18	Develop Team Morphological Charts	6 days?	Tue 11/3/09	Tue 11/10/09
19	**Begin Developing Conceptual Designs**	**6 days?**	**Tue 11/3/09**	**Tue 11/10/09**
20	Anchor,Thread Drying,Stretching	6 days?	Tue 11/3/09	Tue 11/10/09
21	Twisting, Bundling	6 days?	Tue 11/3/09	Tue 11/10/09
22	Buffer Changes, Temperature	6 days?	Tue 11/3/09	Tue 11/10/09
23	Crosslinking, Surface Modification, Sterilization	6 days?	Tue 11/3/09	Tue 11/10/09
24	Present Morphological Charts, Conceptual Designs to User	1 day?	Tue 11/3/09	Tue 11/3/09
25	Further Development of Function/Means, Conceptual Design	6 days?	Tue 11/3/09	Tue 11/10/09
26	Revise List If Necessary	1 day?	Tue 11/10/09	Tue 11/10/09
27	Complete List of Conceptual Designs	5 days?	Wed 11/11/09	Tue 11/17/09

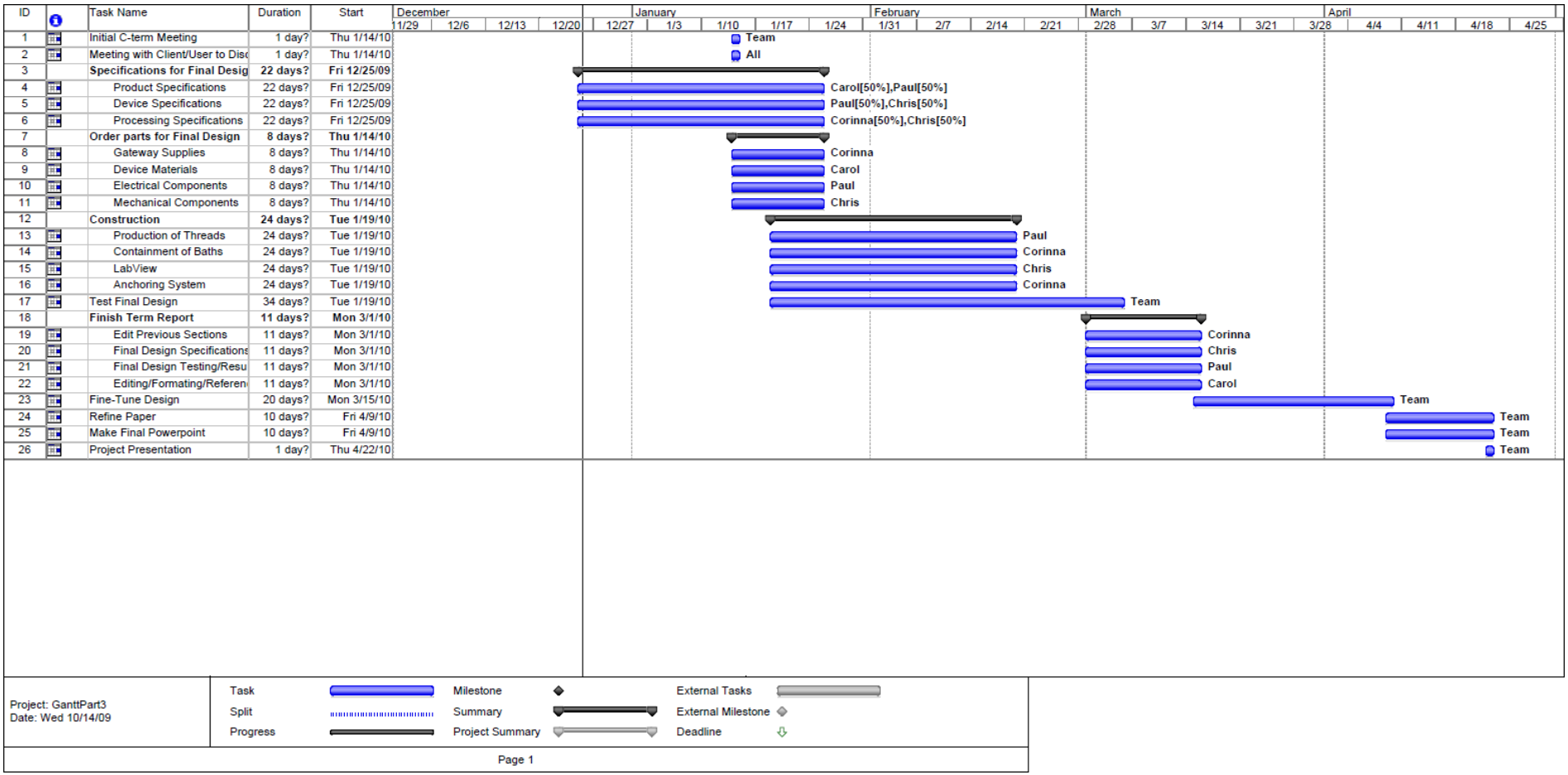
ID	Task Name	Duration	Start	Finish	Predecessors	Resource Names
1	**Metrics For Decision Matrix**	**6 days?**	**Mon 11/16/09**	**Mon 11/23/09**		
2	Versatile: Production	6 days?	Mon 11/16/09	Mon 11/23/09		Carol
3	Versatile: Post-Production	6 days?	Mon 11/16/09	Mon 11/23/09		Paul
4	Accuracy, Precision, Scale	6 days?	Mon 11/16/09	Mon 11/23/09		Chris
5	User Friendly, Automated	6 days?	Mon 11/16/09	Mon 11/23/09		Corinna
6	Decision Matrix	6 days?	Mon 11/16/09	Mon 11/23/09		Team
7	**Develop Preliminary/Alternati**	**6 days?**	**Mon 11/23/09**	**Mon 11/30/09**		
8	Review Top Conceptual D	6 days?	Mon 11/23/09	Mon 11/30/09		Team
9	Develop Preliminary/Altern	6 days?	Mon 11/23/09	Mon 11/30/09		Team
10	Present and Discuss with (	1 day?	Mon 11/30/09	Mon 11/30/09		All
11	Revise List If Necessary	1 day?	Mon 11/30/09	Mon 11/30/09		Team
12	**Order Materials For Testing**	**6 days?**	**Mon 11/30/09**	**Mon 12/7/09**		
13	Gateway Supplies	6 days?	Mon 11/30/09	Mon 12/7/09		Corinna
14	Device Materials	6 days?	Mon 11/30/09	Mon 12/7/09		Carol
15	Electrical Components	6 days?	Mon 11/30/09	Mon 12/7/09		Paul
16	Mechanical Components	6 days?	Mon 11/30/09	Mon 12/7/09		Chris
17	**Evaluate/Test Top Designs**	**5 days?**	**Mon 12/7/09**	**Fri 12/11/09**		
18	Design 1	5 days?	Mon 12/7/09	Fri 12/11/09		Carol
19	Design 2	5 days?	Mon 12/7/09	Fri 12/11/09		Corinna
20	Design 3	5 days?	Mon 12/7/09	Fri 12/11/09		Paul
21	Design 4	5 days?	Mon 12/7/09	Fri 12/11/09		Chris
22	Analyze Results/Preliminary Da	2 days?	Fri 12/11/09	Mon 12/14/09		Team
23	**Finish Term Report**	**4 days?**	**Mon 12/14/09**	**Thu 12/17/09**		
24	Edit Previous Sections	4 days?	Mon 12/14/09	Thu 12/17/09		Team
25	Design 1 Write Up	4 days?	Mon 12/14/09	Thu 12/17/09		Carol
26	Design 2 Write Up	4 days?	Mon 12/14/09	Thu 12/17/09		Corinna
27	Design 3 Write Up	4 days?	Mon 12/14/09	Thu 12/17/09		Paul
28	Design 4 Write Up	4 days?	Mon 12/14/09	Thu 12/17/09		Chris
29	Editing/Formating	4 days?	Mon 12/14/09	Thu 12/17/09		Team



Project: GanttPart2
Date: Wed 10/14/09

Task
Split
Progress
Milestone
Summary
Project Summary
External Tasks
External Milestone
Deadline

Page 1


ID
Task Name
Duration
Start
December
January
February
March
April
11/29
12/6
12/13
12/20
12/27
1/3
1/10
1/17
1/24
1/31
2/7
2/14
2/21
2/28
3/7
3/14
3/21
3/28
4/4
4/11
4/18
4/25
1 Initial C-term Meeting 1 day? Thu 1/14/10
2 Meeting with Client/User to Disc 1 day? Thu 1/14/10
3 Specifications for Final Desig 22 days? Fri 12/25/09
4 Product Specifications 22 days? Fri 12/25/09
5 Device Specifications 22 days? Fri 12/25/09
6 Processing Specifications 22 days? Fri 12/25/09
7 Order parts for Final Design 8 days? Thu 1/14/10
8 Gateway Supplies 8 days? Thu 1/14/10
9 Device Materials 8 days? Thu 1/14/10
10 Electrical Components 8 days? Thu 1/14/10
11 Mechanical Components 8 days? Thu 1/14/10
12 Construction 24 days? Tue 1/19/10
13 Production of Threads 24 days? Tue 1/19/10
14 Containment of Baths 24 days? Tue 1/19/10
15 LabView 24 days? Tue 1/19/10
16 Anchoring System 24 days? Tue 1/19/10
17 Test Final Design 34 days? Tue 1/19/10
18 Finish Term Report 11 days? Mon 3/1/10
19 Edit Previous Sections 11 days? Mon 3/1/10
20 Final Design Specifications 11 days? Mon 3/1/10
21 Final Design Testing/Resu 11 days? Mon 3/1/10
22 Editing/Formating/Referen 11 days? Mon 3/1/10
23 Fine-Tune Design 20 days? Mon 3/15/10
24 Refine Paper 10 days? Fri 4/9/10
25 Make Final Powerpoint 10 days? Fri 4/9/10
26 Project Presentation 1 day? Thu 4/22/10
Team
All
Carol[50%],Paul[50%]
Paul[50%],Chris[50%]
Corinna[50%],Chris[50%]
Corinna
Carol
Paul
Chris
Paul
Corinna
Chris
Corinna
Team
Corinna
Chris
Paul
Carol
Team
Team
Team
Team
Project: GanttPart3
Date: Wed 10/14/09
Task
Split
Progress
Milestone
Summary
Project Summary
External Tasks
External Milestone
Deadline
Page 1

Appendix C: Metrics

Production

Objective: Produce Sterile Threads 0.03 | **0.0042**
Units: Rating the ability of a design to produce sterile threads during initial production
1) too large or difficult to fit into sterile environment
2) would fit into sterile environment

Objective: Produce Threads of Various Materials 0.1 | **0.0140**
Units: Rating the number of materials that a design can make threads out of
1) only collagen or fibrin
2) collagen and fibrin
3) collagen, fibrin, and other materials

Objective: Produce Threads of Various Diameters 0.07 | **0.0098**
Units: Rating the range of thread diameters that can be produced
1) only one diameter
2) range of 20microns
3) range of 40 microns
4) range of 60 microns

Objective: Produce Threads in Various Formation Patterns 0.04 | **0.0056**
Units: Rating the ability of a design to produce threads in different formation patterns
1) only one pattern
2) multiple patterns, but requires human contribution
3) multiple patterns by itself

Objective: Produce Threads at Various Temperatures 0.15 | **0.0210**
Units: Rating the ability of a design to produce threads at different temperatures
1) only one temperature
2) two distinct temperatures
3) range of temperatures

Objective: Anchor Threads 0.15 | **0.0210**
Units: Rating the effectiveness of the design to anchor threads throughout production and post-production processes
1) cannot carry threads through any processes
2) can carry threads through some processes
3) can carry threads through all processes

Objective: Facilitate Thread Drying 0.14 | **0.0196**
Units: Rating the ability of a design to facilitate thread drying (under their own weight)
1) does not allow threads to be dried
2) does allow threads to be dried

Objective: Facilitate Buffer Changes 0.15 | **0.0210**
Units: Rating the ability of a design to facilitate buffer changes
1) must move threads in order to drain or fill
2) allows for both draining and filling without moving threads

Objective: Uniformity and Precision of Thread Stretching During Production 0.13 | **0.0182**
Units: Rating the ability of a design to uniformly and precisely stretch threads during production
1) stretched threads are visibly non-uniform
2) stretched threads are visibly similar, testing reveals some inconsistency
3) stretched threads are visibly similar, no inconsistency in testing

Objective: Seeding Cells in Threads During Production 0.05 | **0.0070**
Units: Rating the ability of a design to allow cell seeding in threads during production
1) does not allow for cell seeding during production
2) does allow for cell seeding during production

Post-Production : Self Contained in Our Device (not what it allows for)

Objective: Post Production Uniformity and Precision of Thread Stretching 0.13 | **0.0117**
Units: Rating the ability of a design to uniformly and precisely stretch threads after production, drying, and rehydration
1) stretched threads are visibly non-uniform
2) stretched threads are visibly similar, testing reveals some inconsistency
3) stretched threads are visibly similar, no inconsistency in testing

Objective: Post Production Sterilization of Threads 0.11 | **0.0099**
Units: Rating the ability of a design to sterilize threads after production
1) could not sterilize threads
2) could sterilize threads

Objective: Seed Threads with Cells Post-Production 0.06 | **0.0054**
Units: Rating the ability of a design to seed threads with cells after production
1) could not perform necessary seeding functions
2) could perform some seeding functions
3) could perform all seeding functions

Crosslink Threads 0.19 | **0.0170**
Units: Rating the ability of the design to perform chemical and energy based crosslinking
1) Does not perform crosslinking
2) Performs either chemical or energy based crosslinking
3) Performs both chemical and energy based crosslinking

Bundle Threads 0.20 | **0.0179**
Units: Rating the ability of the design to bundle threads
1) Does not bundle threads
2) Bundles threads

Braid Threads 0.03 | **0.0027**
Units: Rate the ability of the design to braid threads
1) Does not braid threads
2) Braids threads

Twist Threads 0.15 | **0.0135**
Units: Rate the ability of the design to twist threads
1) Does not twist threads
2) Twists threads

Surface Modify Threads 0.12 | **0.0108**
Units: Rate the ability of the design to modify the surface of threads
1) Does not surface modify threads
2) Does surface modify threads

Accuracy: How close to defined standard 0.06 | **0.06**
Units: Rate the ability of the design to produce threads on the micrometer range
1) Never
2) Sometimes
3) Always

Precision or Reproducibility of Thread Diameter 0.30 | **0.08**
Units: Rate the ability of the design to produce threads of consistent diameter
1) Not consistent at all
2) Acceptable range of diameters +/- 10microns
3) Above and beyond, within +/- 5 microns

Precision or Reproducibility of Thread Length 0.17 | **0.04**
Units: Rate the ability of the design to produce threads of consistent length
1) Not consistent at all
2) Acceptable range of length, +/- ½ inch
3) Within +/- 1/4inch 4-all exactly the same

Precision or Reproducibility of Thread Mechanical Properties 0.33 | **0.09**
Units: Rate the ability of the design to produce threads of consistent Mechanical Properties
1) Worse than current system
2) Same as current system
3) Better than current system

Objective: End Product matches Specified Parameters 0.20 | **0.05**
Units: Rating the ability of the output value to match the input value
1) no correlation between input and output
2) consistent correlation between input and output
3) input matches output consistently

Objective: Easy to Use 0.20 | **0.03**
Units: Rating how difficult it is to perform the functions of the device

1) difficult to use even after experience
2) easy to use with some experience
3) easy to use without experience

Objective: Easy to Clean 0.13 | **0.02**
Units: Rating the amount ofcomponent breakdown that is required to clean a specific component

1) difficult to clean regardless
2) requires complete disassembly
3) requires removal of certain components
4) can be cleaned as is

Objective: Efficient Turnaround – from batch to batch 0.22 | **0.04**
Units: Rating the amount of time it takes to being a new batch assuming machine is already set up and ready to go (not including set up or cleaning)

1) must wait until previous batch is completely done
2) can begin new batch in between buffer changes of previous (requires moving threads)
3) continuous production

Objective: Easily Maintained 0.17 | **0.03**
Units: Rating how difficult the device is to fix when it breaks

1) requires highly trained personnel
2) can be troubleshooted by anyone with a guide
3) anyone could fix it without prior knowledge

Objective: Reliability 0.28 | **0.05**
Units: Rating how often the sytem breaks

1) every time it's used
2) days-weeks
3) weeks-months
4) no foreseeable problems

Objective: Minimize Human Contribution 0.56 | **0.12**
Units: Rating the amount of time a person must spend with the device while it is in use

1) person must work with device 100% of the time
2) person must check in often
3) person must check in infrequently
4) person does not have to monitor at all

Note→ For Anchoring:
Units: Rating the number of steps that a person must do in order to anchor the threads

1) two steps
2) one step

Objective: High Production Rate 0.44 | **0.08**

Units: Rating the ability of the design to produce threads that are viable for use

1) worse than current system
2) same as current system
3) better than current system

Objective: Able to be Scaled Up 0.10 | **0.10**

Units: Rating the ability of a design to be converted from a smaller scale to a larger scale

1) Cannot be scaled up
2) Requires effort to be scaled up
3) Easily scaled up

Appendix D: Old Decision Matrix

Produce Threads

	Weight %	Attribute	**Bi-directional motor head**	Weighted	Normalized	**Conveyor Belt**	Weighted	Normalized	**Manual Guide**	Weighted	Normalized	**Extrusion Train**	Weighted	Normalized
C		Time Limit (A-D Term)	Y			Y			Y			Y		
C		Money (624 USD)	Y			Y			Y			Y		
C		Must fit on lab bench	Y			Y			Y			Y		
C		Limited Expertise of Design Team	Y			Y			Y			Y		
C		Safe for User	Y			Y			Y			Y		
C		Made of Non-reactive Materials	Y			Y			Y			Y		
		Production												
O	0.42	Sterile Threads	2.0	0.8	0.4	1.0	0.4	0.2	2.0	0.8	0.4	2.0	0.8	0.4
O	1.40	Thread Materials	2.0	2.8	0.9	2.0	2.8	0.9	2.0	2.8	0.9	2.0	2.8	0.9
O	0.98	Thread Diameter	4.0	3.9	1.0	4.0	3.9	1.0	4.0	3.9	1.0	4.0	3.9	1.0
O	0.56	Formation Pattern	3.0	1.7	0.6	3.0	1.7	0.6	2.0	1.1	0.4	2.0	1.1	0.4
O	2.10	Facilitates Buffer Changes	2.0	4.2	2.1	2.0	4.2	2.1	2.0	4.2	2.1	2.0	4.2	2.1
O	0.70	Seeding Cells in Threads	2.0	1.4	0.7	2.0	1.4	0.7	2.0	1.4	0.7	2.0	1.4	0.7
O	6.00	**Accuracy: How close to defined standard**	2.0	12.0	6.0	2.0	12.0	6.0	2.0	12.0	6.0	2.0	12.0	6.0
		Precision and Reproducibility of Results												
O	8.00	Thread Diameter	3.0	24.0	8.0	2.0	16.0	5.3	2.0	16.0	5.3	3.0	24.0	8.0
O	4.00	Thread Length	3.0	12.0	3.0	2.0	8.0	2.0	2.0	8.0	2.0	3.0	12.0	3.0
O	5.00	End Product matches Specified Parameters	3.0	15.0	5.0	3.0	15.0	5.0	2.0	10.0	3.3	3.0	15.0	5.0
		User Friendly												
O	3.00	Easy to Use	2.0	6.0	2.0	2.0	6.0	2.0	2.0	6.0	2.0	3.0	9.0	3.0
O	2.00	Easy to Clean	3.0	6.0	1.5	3.0	6.0	1.5	4.0	8.0	2.0	3.0	6.0	1.5
O	4.00	Efficient Turnaround –from batch to batch	3.0	12.0	4.0	3.0	12.0	4.0	3.0	12.0	4.0	3.0	12.0	4.0
O	3.00	Easily Maintained	1.0	3.0	1.0	1.0	3.0	1.0	3.0	9.0	3.0	2.0	6.0	2.0
O	5.00	Reliability	3.0	15.0	3.8	2.0	10.0	2.5	4.0	20.0	5.0	3.0	15.0	3.8
		Automated												
O	12.00	Minimize Human Contribution	4.0	48.0	12.0	4.0	48.0	12.0	1.0	12.0	3.0	3.0	36.0	9.0
O	8.00	High Production rate	3.0	24.0	8.0	3.0	24.0	8.0	3.0	24.0	8.0	3.0	24.0	8.0
O	10.00	**Able to be Scaled Up**	2.0	20.0	6.7	2.0	20.0	6.7	3.0	30.0	10.0	3.0	30.0	10.0
		TOTALS	47.0	211.8	66.6	43.0	194.4	61.5	45.0	181.3	59.2	48.0	215.3	68.8

	Weight %	Attribute	**Molding**	Weighted	Normalized	**Cookie Cutter**	Weighted	Normalized	**Rolling**	Weighted	Normalized
C		Time Limit (A-D Term)	Y			Y			Y		
C		Money (624 USD)	Y			Y			Y		
C		Must fit on lab bench	Y			Y			Y		
C		Limited Expertise of Design Team	Y			Y			Y		
C		Safe for User	Y			Y			Y		
C		Made of Non-reactive Materials	Y			Y			Y		
		Production									
O	0.42	Sterile Threads	2.0	0.8	0.4	2.0	0.8	0.4	2.0	0.8	0.4
O	1.40	Thread Materials	1.0	1.4	0.5	2.0	2.8	0.9	2.0	2.8	0.9
O	0.98	Thread Diameter	4.0	3.9	1.0	4.0	3.9	1.0	4.0	3.9	1.0
O	0.56	Formation Pattern	2.0	1.1	0.4	2.0	1.1	0.4	2.0	1.1	0.4
O	2.10	Facilitates Buffer Changes	1.0	2.1	1.1	2.0	4.2	2.1	2.0	4.2	2.1
O	0.70	Seeding Cells in Threads	2.0	1.4	0.7	1.0	0.7	0.4	2.0	1.4	0.7
O	6.00	**Accuracy: How close to defined standard**	2.0	12.0	6.0	1.0	6.0	3.0	1.0	6.0	3.0
		Precision and Reproducibility of Results									
O	8.00	Thread Diameter	3.0	24.0	8.0	1.0	8.0	2.7	1.0	8.0	2.7
O	4.00	Thread Length	3.0	12.0	3.0	3.0	12.0	3.0	2.0	8.0	2.0
O	5.00	End Product matches Specified Parameters	3.0	15.0	5.0	1.0	5.0	1.7	1.0	5.0	1.7
		User Friendly									
O	3.00	Easy to Use	2.0	6.0	2.0	1.0	3.0	1.0	1.0	3.0	1.0
O	2.00	Easy to Clean	2.0	4.0	1.0	4.0	8.0	2.0	4.0	8.0	2.0
O	4.00	Efficient Turnaround –from batch to batch	1.0	4.0	1.3	3.0	12.0	4.0	3.0	12.0	4.0
O	3.00	Easily Maintained	1.0	3.0	1.0	1.0	3.0	1.0	3.0	9.0	3.0
O	5.00	Reliability	3.0	15.0	3.8	3.0	15.0	3.8	4.0	20.0	5.0
		Automated									
O	12.00	Minimize Human Contribution	4.0	48.0	12.0	1.0	12.0	3.0	1.0	12.0	3.0
O	8.00	High Production rate	3.0	24.0	8.0	2.0	16.0	5.3	1.0	8.0	2.7
O	10.00	**Able to be Scaled Up**	3.0	30.0	10.0	3.0	30.0	10.0	2.0	20.0	6.7
		TOTALS	42.0	207.8	65.1	37.0	143.6	45.6	38.0	133.3	42.2

Anchor Threads

	Weight %	Attribute	Mesh Screen	Weighted	Normalized	Platform with Hole	Weighted	Normalized	Clamps	Weighted	Normalized	Roughened Surface	Weighted	Normalized
C		Time Limit (A-D Term)	Y			Y			Y			Y		
C		Money (624 USD)	Y			Y			Y			Y		
C		Must fit on lab bench	Y			Y			Y			Y		
C		Limited Expertise of Design Team	Y			Y			Y			Y		
C		Safe for User	Y			Y			Y			Y		
C		Made of Non-reactive Materials	Y			Y			Y			Y		
		Production												
O	0.42	Sterile Threads	2.0	0.8	0.4	2.0	0.8	0.4	2.0	0.8	0.4	2.0	0.8	0.4
O	0.98	Thread Diameter	4.0	3.9	1.0	4.0	3.9	1.0	4.0	3.9	1.0	4.0	3.9	1.0
O	0.56	Formation Pattern	3.0	1.7	0.6	3.0	1.7	0.6	2.0	1.1	0.4	2.0	1.1	0.4
O	1.96	Facilitates Thread Drying	1.0	2.0	1.0	1.0	2.0	1.0	2.0	3.9	2.0	2.0	3.9	2.0
O	2.10	Facilitates Buffer Changes	2.0	4.2	2.1	2.0	4.2	2.1	2.0	4.2	2.1	2.0	4.2	2.1
O	1.82	Uniformity and Precision of Thread Stretching	1.0	1.8	0.6	1.0	1.8	0.6	1.0	1.8	0.6	2.0	3.6	1.2
		Post-Production												
O	1.17	Uniformity and Precision of Thread Stretching	1.0	1.2	0.4	1.0	1.2	0.4	1.0	1.2	0.4	2.0	2.3	0.8
O	0.99	Sterilize Threads	2.0	2.0	1.0	2.0	2.0	1.0	2.0	2.0	1.0	2.0	2.0	1.0
O	0.54	Seed Threads with Cells	2.0	1.1	0.4	2.0	1.1	0.4	2.0	1.1	0.4	2.0	1.1	0.4
O	1.70	Crosslink Threads	3.0	5.1	1.7	3.0	5.1	1.7	3.0	5.1	1.7	3.0	5.1	1.7
O	1.79	Bundle Threads	1.0	1.8	0.9	1.0	1.8	0.9	2.0	3.6	1.8	2.0	3.6	1.8
O	1.35	Twist Threads	1.0	1.4	0.7	1.0	1.4	0.7	2.0	2.7	1.4	2.0	2.7	1.4
O	1.08	Surface Modify Threads	1.0	1.1	0.5	1.0	1.1	0.5	2.0	2.2	1.1	2.0	2.2	1.1
		User Friendly												
O	3.00	Easy to Use	3.0	9.0	3.0	3.0	9.0	3.0	1.0	3.0	1.0	3.0	9.0	3.0
O	2.00	Easy to Clean	4.0	8.0	2.0	4.0	8.0	2.0	3.0	6.0	1.5	4.0	8.0	2.0
O	3.00	Easily Maintained	3.0	9.0	3.0	3.0	9.0	3.0	2.0	6.0	2.0	3.0	9.0	3.0
O	5.00	Reliability	4.0	20.0	5.0	4.0	20.0	5.0	4.0	20.0	5.0	4.0	20.0	5.0
		Automated												
O	12.00	Minimize Human Contribution	2.0	24.0	12.0	2.0	24.0	12.0	1.0	12.0	6.0	2.0	24.0	12.0
O	10	**Able to be Scaled Up**	3.0	30.0	10.0	3.0	30.0	10.0	3.0	30.0	10.0	3.0	30.0	10.0
		TOTALS	43.0	128.0	46.2	43.0	128.0	46.2	41.0	110.6	39.6	48.0	136.6	50.1

	Weight %	Attribute	Fill Holes	Weighted	Normalized	Grooved Ends	Weighted	Normalized	Pegs	Weighted	Normalized	Combs	Weighted	Normalized
C		Time Limit (A-D Term)	Y			Y			Y			Y		
C		Money (624 USD)	Y			Y			Y			Y		
C		Must fit on lab bench	Y			Y			Y			Y		
C		Limited Expertise of Design Team	Y			Y			Y			Y		
C		Safe for User	Y			Y			Y			Y		
C		Made of Non-reactive Materials	Y			Y			Y			Y		
		Production												
O	0.42	Sterile Threads	2.0	0.8	0.4	2.0	0.8	0.4	2.0	0.8	0.4	2.0	0.8	0.4
O	0.98	Thread Diameter	4.0	3.9	1.0	4.0	3.9	1.0	4.0	3.9	1.0	4.0	3.9	1.0
O	0.56	Formation Pattern	2.0	1.1	0.4	2.0	1.1	0.4	2.0	1.1	0.4	2.0	1.1	0.4
O	1.96	Facilitates Thread Drying	2.0	3.9	2.0	2.0	3.9	2.0	2.0	3.9	2.0	2.0	3.9	2.0
O	2.10	Facilitates Buffer Changes	2.0	4.2	2.1	2.0	4.2	2.1	2.0	4.2	2.1	2.0	4.2	2.1
O	1.82	Uniformity and Precision of Thread Stretching	2.0	3.6	1.2	2.0	3.6	1.2	2.0	3.6	1.2	2.0	3.6	1.2
		Post-Production												
O	1.17	Uniformity and Precision of Thread Stretching	2.0	2.3	0.8	2.0	2.3	0.8	2.0	2.3	0.8	2.0	2.3	0.8
O	0.99	Sterilize Threads	2.0	2.0	1.0	2.0	2.0	1.0	2.0	2.0	1.0	2.0	2.0	1.0
O	0.54	Seed Threads with Cells	2.0	1.1	0.4	2.0	1.1	0.4	2.0	1.1	0.4	2.0	1.1	0.4
O	1.70	Crosslink Threads	3.0	5.1	1.7	3.0	5.1	1.7	3.0	5.1	1.7	3.0	5.1	1.7
O	1.79	Bundle Threads	2.0	3.6	1.8	2.0	3.6	1.8	2.0	3.6	1.8	2.0	3.6	1.8
O	1.35	Twist Threads	2.0	2.7	1.4	2.0	2.7	1.4	2.0	2.7	1.4	2.0	2.7	1.4
O	1.08	Surface Modify Threads	2.0	2.2	1.1	2.0	2.2	1.1	2.0	2.2	1.1	2.0	2.2	1.1
		User Friendly												
O	3.00	Easy to Use	2.0	6.0	2.0	2.0	6.0	2.0	2.0	6.0	2.0	2.0	6.0	2.0
O	2.00	Easy to Clean	4.0	8.0	2.0	4.0	8.0	2.0	4.0	8.0	2.0	4.0	8.0	2.0
O	3.00	Easily Maintained	2.0	6.0	2.0	2.0	6.0	2.0	2.0	6.0	2.0	2.0	6.0	2.0
O	5.00	Reliability	4.0	20.0	5.0	4.0	20.0	5.0	4.0	20.0	5.0	4.0	20.0	5.0
		Automated												
O	12.00	Minimize Human Contribution	2.0	24.0	12.0	2.0	24.0	12.0	2.0	24.0	12.0	2.0	24.0	12.0
O	10	**Able to be Scaled Up**	3.0	30.0	10.0	3.0	30.0	10.0	3.0	30.0	10.0	2.0	20.0	6.7
		TOTALS	46.0	130.6	48.1	46.0	130.6	48.1	46.0	130.6	48.1	45.0	120.6	44.8

	Weight %	Attribute	**Anchor and Platform**	Weighted	Normalized	**Grooved Platform**	Weighted	Normalized
C		Time Limit (A-D Term)	Y			Y		
C		Money (624 USD)	Y			Y		
C		Must fit on lab bench	Y			Y		
C		Limited Expertise of Design Team	Y			Y		
C		Safe for User	Y			Y		
C		Made of Non-reactive Materials	Y			Y		
		Production						
O	0.42	Sterile Threads	2.0	0.8	0.4	2.0	0.8	0.4
O	0.98	Thread Diameter	4.0	3.9	1.0	1.0	1.0	0.2
O	0.56	Formation Pattern	3.0	1.7	0.6	1.0	0.6	0.2
O	1.96	Facilitates Thread Drying	2.0	3.9	2.0	1.0	2.0	1.0
O	2.10	Facilitates Buffer Changes	2.0	4.2	2.1	2.0	4.2	2.1
O	1.82	Uniformity and Precision of Thread Stretching	2.0	3.6	1.2	1.0	1.8	0.6
		Post-Production						
O	1.17	Uniformity and Precision of Thread Stretching	2.0	2.3	0.8	1.0	1.2	0.4
O	0.99	Sterilize Threads	2.0	2.0	1.0	2.0	2.0	1.0
O	0.54	Seed Threads with Cells	2.0	1.1	0.4	2.0	1.1	0.4
O	1.70	Crosslink Threads	3.0	5.1	1.7	3.0	5.1	1.7
O	1.79	Bundle Threads	2.0	3.6	1.8	1.0	1.8	0.9
O	1.35	Twist Threads	2.0	2.7	1.4	1.0	1.4	0.7
O	1.08	Surface Modify Threads	2.0	2.2	1.1	1.0	1.1	0.5
		User Friendly						
O	3.00	Easy to Use	3.0	9.0	3.0	3.0	9.0	3.0
O	2.00	Easy to Clean	4.0	8.0	2.0	4.0	8.0	2.0
O	3.00	Easily Maintained	2.0	6.0	2.0	1.0	3.0	1.0
O	5.00	Reliability	3.0	15.0	3.8	4.0	20.0	5.0
		Automated						
O	12.00	Minimize Human Contribution	2.0	24.0	12.0	2.0	24.0	12.0
O	10	**Able to be Scaled Up**	2.0	20.0	6.7	3.0	30.0	10.0
		TOTALS	46.0	119.1	44.7	36.0	117.9	43.1

Facilitate Buffer Changes

	Weight %	Attribute	**Hopper System**	Weighted	Normalized	**Tilted Floor**	Weighted	Normalized	**Aspirate**	Weighted	Normalized	**Tipped Bath**	Weighted	Normalized
C		Time Limit (A-D Term)	Y			Y			Y			Y		
C		Money (624 USD)	Y			Y			Y			Y		
C		Must fit on lab bench	Y			Y			Y			Y		
C		Limited Expertise of Design Team	Y			Y			Y			Y		
C		Safe for User	Y			Y			Y			Y		
C		Made of Non-reactive Materials	Y			Y			Y			Y		
O		**Production**												
O	0.42	Sterile Threads	2.0	0.8	0.4	2.0	0.8	0.4	2.0	0.8	0.4	2.0	0.8	0.4
		User Friendly												
O	3.00	Easy to Use	3.0	9.0	3.0	3.0	9.0	3.0	2.0	6.0	2.0	2.0	6.0	2.0
O	2.00	Easy to Clean	4.0	8.0	2.0	3.0	6.0	1.5	3.0	6.0	1.5	4.0	8.0	2.0
O	3.00	Easily Maintained	2.0	6.0	2.0	3.0	9.0	3.0	1.0	3.0	1.0	3.0	9.0	3.0
O	5.00	Reliability	4.0	20.0	5.0	3.0	15.0	3.8	3.0	15.0	3.8	3.0	15.0	3.8
		Automated												
O	12.00	Minimize Human Contribution	4.0	48.0	12.0	4.0	48.0	12.0	1.0	12.0	3.0	1.0	12.0	3.0
O	8.00	High Production rate	3.0	24.0	8.0	3.0	24.0	8.0	3.0	24.0	8.0	3.0	24.0	8.0
O	10.00	**Able to be Scaled Up**	3.0	30.0	10.0	3.0	30.0	10.0	3.0	30.0	10.0	2.0	20.0	6.7
		TOTALS	25.0	145.8	42.4	24.0	141.8	41.7	18.0	96.8	29.7	20.0	94.8	28.8

Facilitate Physical Post-Production Modification

	Weight %	Attribute	Tilting Blocks	Weighted	Normalized	Sliding Anchors	Weighted	Normalized	Stacking Anchors	Weighted	Normalized	Jellyroll	Weighted	Normalized
C		Time Limit (A-D Term)	Y			Y			Y			Y		
C		Money (624 USD)	Y			Y			Y			Y		
C		Must fit on lab bench	Y			Y			Y			Y		
C		Limited Expertise of Design Team	Y			Y			Y			Y		
C		Safe for User	Y			Y			Y			Y		
C		Made of Non-reactive Materials	Y			Y			Y			Y		
O		**Post-Production**												
O	1.17	Uniformity and Precision of Thread Stretching	2.0	2.3	0.8	2.0	2.3	0.8	1.0	1.2	0.4	2.0	2.3	0.8
O	0.99	Sterilize Threads	2.0	2.0	1.0	2.0	2.0	1.0	2.0	2.0	1.0	2.0	2.0	1.0
O	0.54	Seed Threads with Cells	2.0	1.1	0.4	2.0	1.1	0.4	2.0	1.1	0.4	2.0	1.1	0.4
O	1.70	Crosslink Threads	3.0	5.1	1.7	3.0	5.1	1.7	3.0	5.1	1.7	3.0	5.1	1.7
O	1.08	Surface Modify Threads	2.0	2.2	0.5	2.0	2.2	0.5	2.0	2.2	0.5	2.0	2.2	0.5
		User Friendly												
O	3.00	Easy to Use	2.0	6.0	2.0	3.0	9.0	3.0	1.0	3.0	1.0	2.0	6.0	2.0
O	2.00	Easy to Clean	4.0	8.0	2.0	3.0	6.0	1.5	3.0	6.0	1.5	4.0	8.0	2.0
O	3.00	Easily Maintained	3.0	9.0	3.0	3.0	9.0	3.0	3.0	9.0	3.0	3.0	9.0	3.0
O	5.00	Reliability	4.0	20.0	5.0	4.0	20.0	5.0	4.0	20.0	5.0	4.0	20.0	5.0
		Automated												
O	12.00	Minimize Human Contribution	1.0	12.0	3.0	1.0	12.0	3.0	1.0	12.0	3.0	1.0	12.0	3.0
O	8.00	High Production rate	3.0	24.0	8.0	3.0	24.0	8.0	3.0	24.0	8.0	3.0	24.0	8.0
O	10.00	**Able to be Scaled Up**	2.0	20.0	6.7	3.0	30.0	10.0	3.0	30.0	10.0	3.0	30.0	10.0
		TOTALS	30.0	111.7	34.0	31.0	122.7	37.9	28.0	115.5	35.5	31.0	121.7	37.4

	Weight %	Attribute	**Thread Plate Sweeper**	Weighted	Normalized	**Twisting Caps**	Weighted	Normalized	**Crank Mechanism**	Weighted	Normalized	**Vertical Weights**	Weighted	Normalized
C		Time Limit (A-D Term)	Y			Y			Y			Y		
C		Money (624 USD)	Y			Y			Y			Y		
C		Must fit on lab bench	Y			Y			Y			Y		
C		Limited Expertise of Design Team	Y			Y			Y			Y		
C		Safe for User	Y			Y			Y			Y		
C		Made of Non-reactive Materials	Y			Y			Y			Y		
O		**Post-Production**												
O	1.17	Uniformity and Precision of Thread Stretching	1.0	1.2	0.4	2.0	2.3	0.8	2.0	2.3	0.8	2.0	2.3	0.8
O	0.99	Sterilize Threads	2.0	2.0	1.0	2.0	2.0	1.0	2.0	2.0	1.0	2.0	2.0	1.0
O	0.54	Seed Threads with Cells	2.0	1.1	0.4	2.0	1.1	0.4	2.0	1.1	0.4	1.0	0.5	0.2
O	1.70	Crosslink Threads	3.0	5.1	1.7	3.0	5.1	1.7	3.0	5.1	1.7	2.0	3.4	1.1
O	1.08	Surface Modify Threads	2.0	2.2	0.5	2.0	2.2	0.5	2.0	2.2	0.5	2.0	2.2	0.5
		User Friendly												
O	3.00	Easy to Use	2.0	6.0	2.0	3.0	9.0	3.0	3.0	9.0	3.0	3.0	9.0	3.0
O	2.00	Easy to Clean	4.0	8.0	2.0	4.0	8.0	2.0	4.0	8.0	2.0	4.0	8.0	2.0
O	3.00	Easily Maintained	3.0	9.0	3.0	3.0	9.0	3.0	2.0	6.0	2.0	2.0	6.0	2.0
O	5.00	Reliability	4.0	20.0	5.0	4.0	20.0	5.0	3.0	15.0	3.8	3.0	15.0	3.8
		Automated												
O	12.00	Minimize Human Contribution	1.0	12.0	3.0	1.0	12.0	3.0	1.0	12.0	3.0	1.0	12.0	3.0
O	8.00	High Production rate	2.0	16.0	5.3	3.0	24.0	8.0	3.0	24.0	8.0	3.0	24.0	8.0
O	10.00	**Able to be Scaled Up**	3.0	30.0	10.0	3.0	30.0	10.0	3.0	30.0	10.0	3.0	30.0	10.0
		TOTALS	29.0	112.5	34.3	32.0	124.7	38.4	30.0	116.7	36.1	28.0	114.4	35.4

Control Production Parameters

	Weight %	Attribute	LabVIEW	Weighted	Normalized	Manual	Weighted	Normalized	Heat Solutions	Weighted	Normalized
C		Time Limit (A-D Term)	Y			Y			Y		
C		Money (624 USD)	Y			Y			Y		
C		Must fit on lab bench	Y			Y			Y		
C		Limited Expertise of Design Team	Y			Y			Y		
C		Safe for User	Y			Y			Y		
C		Made of Non-reactive Materials	Y			Y			Y		
O		**Production**									
O	0.98	Thread Diameter	4.0	3.9	1.0	4.0	3.9	1.0			
O	0.56	Formation Pattern	3.0	1.7	0.6	2.0	1.1	0.4			
O	2.10	Temperature	3.0	6.3	2.1	3.0	6.3	2.1			
O	1.82	Uniformity and Precision of Thread Stretching	3.0	5.5	1.8	3.0	5.5	1.8			
O		**Post-Production**									
O	1.17	Uniformity and Precision of Thread Stretching	3.0	3.5	1.2	3.0	3.5	1.2			
O	6.00	**Accuracy: How close to defined standard**	2.0	12.0	4.0	2.0	12.0	4.0	2.0	12.0	4.0
		Precision and Reproducibility of Results									
O	8.00	Thread Diameter	3.0	24.0	8.0	2.0	16.0	5.3			
O	4.00	Thread Length	3.0	12.0	3.0	3.0	12.0	3.0			
O	5.00	End Product matches Specified Parameters	3.0	15.0	5.0	1.0	5.0	1.7			
		User Friendly									
O	3.00	Easy to Use	3.0	9.0	3.0	1.0	3.0	1.0	3.0	9.0	3.0
O	3.00	Easily Maintained	1.0	3.0	1.0	3.0	9.0	3.0	3.0	9.0	3.0
O	5.00	Reliability	4.0	20.0	5.0	4.0	20.0	5.0	4.0	20.0	5.0
		Automated									
O	12.00	Minimize Human Contribution	4.0	48.0	12.0	1.0	12.0	3.0	3.0	36.0	9.0
O	10	**Able to be Scaled Up**	3.0	30.0	10.0	3.0	30.0	10.0	3.0	30.0	10.0
		TOTALS	42.0	193.9	57.6	35.0	139.3	42.4	18.0	116.0	34.0

	Weight %	Attribute	Heated Bath	Weighted	Normalized	Oven	Weighted	Normalized	Heated Room	Hot Plate
C		Time Limit (A-D Term)	Y			Y			N	Y
C		Money (624 USD)	Y			Y			N	Y
C		Must fit on lab bench	Y			Y			N	Y
C		Limited Expertise of Design Team	Y			Y			Y	Y
C		Safe for User	Y			Y			Y	N
C		Made of Non-reactive Materials	Y			Y			Y	Y
O		**Production**								
O	0.98	Thread Diameter								
O	0.56	Formation Pattern								
O	2.10	Temperature								
O	1.82	Uniformity and Precision of Thread Stretching								
O		**Post-Production**								
O	1.17	Uniformity and Precision of Thread Stretching								
O	6.00	**Accuracy: How close to defined standard**	3.0	18.0	6.0	3.0	18.0	6.0		
		Precision and Reproducibility of Results								
O	8.00	Thread Diameter								
O	4.00	Thread Length								
O	5.00	End Product matches Specified Parameters								
		User Friendly								
O	3.00	Easy to Use	3.0	9.0	3.0	3.0	9.0	3.0		
O	3.00	Easily Maintained	2.0	6.0	2.0	1.0	3.0	1.0		
O	5.00	Reliability	3.0	15.0	3.8	3.0	15.0	3.8		
		Automated								
O	12.00	Minimize Human Contribution	4.0	48.0	12.0	4.0	48.0	12.0		
O	10	**Able to be Scaled Up**	3.0	30.0	10.0	3.0	30.0	10.0		
		TOTALS	18.0	126.0	36.8	17.0	123.0	35.8		

Appendix E: New Decision Matrix

Produce Threads

	Weight %	Attribute	**Bi-directional motor head**	Normalized	Weighted Sum	**Conveyor Belt**	Normalized	Weighted Sum	**Manual Guide**	Normalized	Weighted Sum	**Extrusion Train**	Normalized	Weighted Sum
C		Time Limit (A-D Term)	Y			Y			Y			Y		
C		Money (624 USD)	Y			Y			Y			Y		
C		Must fit on lab bench	Y			Y			Y			Y		
C		Limited Expertise of Design Team	Y			Y			Y			Y		
C		Safe for User	Y			Y			Y			Y		
C		Made of Non-reactive Materials	Y			Y			Y			Y		
	14.00	**Production**		**5.7**	**79.3**		**5.2**	**72.3**		**5.3**	**74.7**		**5.3**	**74.7**
O		Sterile Threads	2.0	1.0		1.0	0.5		2.0	1.0		2.0	1.0	
O		Thread Materials	2.0	0.7		2.0	0.7		2.0	0.7		2.0	0.7	
O		Thread Diameter	4.0	1.0		4.0	1.0		4.0	1.0		4.0	1.0	
O		Formation Pattern	3.0	1.0		3.0	1.0		2.0	0.7		2.0	0.7	
O		Facilitates Buffer Changes	2.0	1.0		2.0	1.0		2.0	1.0		2.0	1.0	
O		Seeding Cells in Threads	2.0	1.0		2.0	1.0		2.0	1.0		2.0	1.0	
O	**6.00**	**Accuracy: How close to defined standard**	2.0	**0.7**	**4.0**	2.0	**0.7**	**4.0**	2.0	**0.7**	**4.0**	2.0	**0.7**	**4.0**
	26.00	**Precision and Reproducibility of Results**		**3.0**	**78.0**		**2.3**	**60.7**		**2.0**	**52.0**		**3.0**	**78.0**
O		Thread Diameter	3.0	1.0		2.0	0.7		2.0	0.7		3.0	1.0	
O		Thread Length	3.0	1.0		2.0	0.7		2.0	0.7		3.0	1.0	
O		End Product matches Specified Parameters	3.0	1.0		3.0	1.0		2.0	0.7		3.0	1.0	
	16.00	**User Friendly**		**4.1**	**65.3**		**4.8**	**76.0**		**4.7**	**74.7**		**4.8**	**76.0**
O		Easy to Use	2.0	0.7		2.0	0.7		2.0	0.7		3.0	1.0	
O		Easy to Clean	3.0	0.8		3.0	0.8		4.0	1.0		3.0	0.8	
O		Efficient Turnaround –from batch to batch	3.0	1.0		3.0	1.0		3.0	1.0		3.0	1.0	
O		Easily Maintained	1.0	0.3		1.0	0.3		3.0	1.0		2.0	0.7	
O		Reliability	3.0	1.3		2.0	2.0		4.0	1.0		3.0	1.3	
	19.00	**Automated**		**2.0**	**38.0**		**2.0**	**38.0**		**1.3**	**23.8**		**1.8**	**33.3**
O		Minimize Human Contribution	4.0	1.0		4.0	1.0		1.0	0.3		3.0	0.8	
O		High Production rate	3.0	1.0		3.0	1.0		3.0	1.0		3.0	1.0	
O	**10.00**	**Able to be Scaled Up**	2.0	**0.7**	**6.7**	2.0	**0.7**	**6.7**	3.0	**1.0**	**10.0**	3.0	**1.0**	**10.0**
		TOTALS	47.0		271.3	43.0		257.7	45.0		239.1	48.0		275.9

	Weight %	Attribute	Molding	Normalized	Weighted Sum	Cookie Cutter	Normalized	Weighted Sum	Rolling	Normalized	Weighted Sum
C		Time Limit (A-D Term)	Y			Y			Y		
C		Money (624 USD)	Y			Y			Y		
C		Must fit on lab bench	Y			Y			Y		
C		Limited Expertise of Design Team	Y			Y			Y		
C		Safe for User	Y			Y			Y		
C		Made of Non-reactive Materials	Y			Y			Y		
	14.00	**Production**		**4.5**	**63.0**		**4.8**	**67.7**		**5.3**	**74.7**
O		Sterile Threads	2.0	1.0		2.0	1.0		2.0	1.0	
O		Thread Materials	1.0	0.3		2.0	0.7		2.0	0.7	
O		Thread Diameter	4.0	1.0		4.0	1.0		4.0	1.0	
O		Formation Pattern	2.0	0.7		2.0	0.7		2.0	0.7	
O		Facilitates Buffer Changes	1.0	0.5		2.0	1.0		2.0	1.0	
O		Seeding Cells in Threads	2.0	1.0		1.0	0.5		2.0	1.0	
O	**6.00**	**Accuracy: How close to defined standard**	2.0	**0.7**	**4.0**	1.0	**0.3**	**2.0**	1.0	**0.3**	**2.0**
	26.00	**Precision and Reproducibility of Results**		**3.0**	**78.0**		**1.7**	**43.3**		**1.3**	**34.7**
O		Thread Diameter	3.0	1.0		1.0	0.3		1.0	0.3	
O		Thread Length	3.0	1.0		3.0	1.0		2.0	0.7	
O		End Product matches Specified Parameters	3.0	1.0		1.0	0.3		1.0	0.3	
	16.00	**User Friendly**		**3.2**	**50.7**		**4.0**	**64.0**		**4.3**	**69.3**
O		Easy to Use	2.0	0.7		1.0	0.3		1.0	0.3	
O		Easy to Clean	2.0	0.5		4.0	1.0		4.0	1.0	
O		Efficient Turnaround –from batch to batch	1.0	0.3		3.0	1.0		3.0	1.0	
O		Easily Maintained	1.0	0.3		1.0	0.3		3.0	1.0	
O		Reliability	3.0	1.3		3.0	1.3		4.0	1.0	
	19.00	**Automated**		**2.0**	**38.0**		**0.9**	**17.4**		**0.6**	**11.1**
O		Minimize Human Contribution	4.0	1.0		1.0	0.3		1.0	0.3	
O		High Production rate	3.0	1.0		2.0	0.7		1.0	0.3	
O	**10.00**	**Able to be Scaled Up**	3.0	**1.0**	**10.0**	3.0	**1.0**	**10.0**	2.0	**0.7**	**6.7**
		TOTALS	42.0		243.7	37.0		204.4	38.0		198.4

Anchor Threads

	Weight %	Attribute	**Mesh Screen**	Normalized	Weighted Sum	**Platform with Hole**	Normalized	Weighted Sum	**Clamps**	Normalized	Weighted Sum	**Roughened Surface**	Normalized	Weighted Sum
C		Time Limit (A-D Term)	Y			Y			Y			Y		
C		Money (624 USD)	Y			Y			Y			Y		
C		Must fit on lab bench	Y			Y			Y			Y		
C		Limited Expertise of Design Team	Y			Y			Y			Y		
C		Safe for User	Y			Y			Y			Y		
C		Made of Non-reactive Materials	Y			Y			Y			Y		
	14.00	**Production**		**4.9**	**68.8**		**4.9**	**68.8**		**5.0**	**70.0**		**5.5**	**77.0**
O		Sterile Threads	2.0	1.0		2.0	1.0		2.0	1.0		2.0	1.0	
O		Thread Diameter	4.0	1.3		4.0	1.3		4.0	1.3		4.0	1.3	
O		Formation Pattern	3.0	0.8		3.0	0.8		2.0	0.5		2.0	0.5	
O		Facilitates Thread Drying	1.0	0.3		1.0	0.3		2.0	0.7		2.0	0.7	
O		Facilitates Buffer Changes	2.0	1.0		2.0	1.0		2.0	1.0		2.0	1.0	
O		Uniformity and Precision of Thread Stretching	1.0	0.5		1.0	0.5		1.0	0.5		2.0	1.0	
	26.00	**Post-Production**		**4.5**	**117.0**		**4.5**	**117.0**		**6.0**	**156.0**		**6.3**	**164.7**
O		Uniformity and Precision of Thread Stretching	1.0	0.3		1.0	0.3		1.0	0.3		2.0	0.7	
O		Sterilize Threads	2.0	1.0		2.0	1.0		2.0	1.0		2.0	1.0	
O		Seed Threads with Cells	2.0	0.7		2.0	0.7		2.0	0.7		2.0	0.7	
O		Crosslink Threads	3.0	1.0		3.0	1.0		3.0	1.0		3.0	1.0	
O		Bundle Threads	1.0	0.5		1.0	0.5		2.0	1.0		2.0	1.0	
O		Twist Threads	1.0	0.5		1.0	0.5		2.0	1.0		2.0	1.0	
O		Surface Modify Threads	1.0	0.5		1.0	0.5		2.0	1.0		2.0	1.0	
	16.00	**User Friendly**		**4.0**	**64.0**		**4.0**	**64.0**		**2.8**	**44.0**		**4.0**	**64.0**
O		Easy to Use	3.0	1.0		3.0	1.0		1.0	0.3		3.0	1.0	
O		Easy to Clean	4.0	1.0		4.0	1.0		3.0	0.8		4.0	1.0	
O		Easily Maintained	3.0	1.0		3.0	1.0		2.0	0.7		3.0	1.0	
O		Reliability	4.0	1.0		4.0	1.0		4.0	1.0		4.0	1.0	
	19.00	**Automated**		**1.7**	**31.7**		**1.7**	**31.7**		**1.3**	**25.3**		**1.7**	**31.7**
O		Minimize Human Contribution	2.0	0.7		2.0	0.7		1.0	0.3		2.0	0.7	
O	**10**	**Able to be Scaled Up**	3.0	**1.0**	**10.0**	3.0	**1.0**	**10.0**	3.0	**1.0**	**10.0**	3.0	**1.0**	**10.0**
		TOTALS	43.0		275.5	43.0		275.5	41.0		289.3	48.0		331.3

	Weight %	Attribute	Fill Holes	Normalized	Weighted Sum	Grooved Ends	Normalized	Weighted Sum	Pegs	Normalized	Weighted Sum	Combs	Normalized	Weighted Sum
C		Time Limit (A-D Term)	Y			Y			Y			Y		
C		Money (624 USD)	Y			Y			Y			Y		
C		Must fit on lab bench	Y			Y			Y			Y		
C		Limited Expertise of Design Team	Y			Y			Y			Y		
C		Safe for User	Y			Y			Y			Y		
C		Made of Non-reactive Materials	Y			Y			Y			Y		
	14.00	**Production**		5.5	77.0		5.5	77.0		5.5	77.0		5.5	77.0
O		Sterile Threads	2.0	1.0		2.0	1.0		2.0	1.0		2.0	1.0	
O		Thread Diameter	4.0	1.3		4.0	1.3		4.0	1.3		4.0	1.3	
O		Formation Pattern	2.0	0.5		2.0	0.5		2.0	0.5		2.0	0.5	
O		Facilitates Thread Drying	2.0	0.7		2.0	0.7		2.0	0.7		2.0	0.7	
O		Facilitates Buffer Changes	2.0	1.0		2.0	1.0		2.0	1.0		2.0	1.0	
O		Uniformity and Precision of Thread Stretching	2.0	1.0		2.0	1.0		2.0	1.0		2.0	1.0	
	26.00	**Post-Production**		6.3	164.7		6.3	164.7		6.3	164.7		6.3	164.7
O		Uniformity and Precision of Thread Stretching	2.0	0.7		2.0	0.7		2.0	0.7		2.0	0.7	
O		Sterilize Threads	2.0	1.0		2.0	1.0		2.0	1.0		2.0	1.0	
O		Seed Threads with Cells	2.0	0.7		2.0	0.7		2.0	0.7		2.0	0.7	
O		Crosslink Threads	3.0	1.0		3.0	1.0		3.0	1.0		3.0	1.0	
O		Bundle Threads	2.0	1.0		2.0	1.0		2.0	1.0		2.0	1.0	
O		Twist Threads	2.0	1.0		2.0	1.0		2.0	1.0		2.0	1.0	
O		Surface Modify Threads	2.0	1.0		2.0	1.0		2.0	1.0		2.0	1.0	
	16.00	**User Friendly**		3.3	53.3		3.3	53.3		3.3	53.3		3.3	53.3
O		Easy to Use	2.0	0.7		2.0	0.7		2.0	0.7		2.0	0.7	
O		Easy to Clean	4.0	1.0		4.0	1.0		4.0	1.0		4.0	1.0	
O		Easily Maintained	2.0	0.7		2.0	0.7		2.0	0.7		2.0	0.7	
O		Reliability	4.0	1.0		4.0	1.0		4.0	1.0		4.0	1.0	
	19.00	**Automated**		1.7	31.7		1.7	31.7		1.7	31.7		1.7	31.7
O		Minimize Human Contribution	2.0	0.7		2.0	0.7		2.0	0.7		2.0	0.7	
O	**10**	**Able to be Scaled Up**	3.0	**1.0**	**10.0**	3.0	**1.0**	**10.0**	3.0	**1.0**	**10.0**	2.0	**0.7**	**6.7**
		TOTALS	46.0		320.7	46.0		320.7	46.0		320.7	45.0		317.3

	Weight %	Attribute	**Anchor and Platform**	Normalized	Weighted Sum	**Grooved Platform**	Normalized	Weighted Sum
C		Time Limit (A-D Term)	Y			Y		
C		Money (624 USD)	Y			Y		
C		Must fit on lab bench	Y			Y		
C		Limited Expertise of Design Team	Y			Y		
C		Safe for User	Y			Y		
C		Made of Non-reactive Materials	Y			Y		
	14.00	**Production**		**5.8**	**80.5**		**3.4**	**47.8**
O		Sterile Threads	2.0	1.0		2.0	1.0	
O		Thread Diameter	4.0	1.3		1.0	0.3	
O		Formation Pattern	3.0	0.8		1.0	0.3	
O		Facilitates Thread Drying	2.0	0.7		1.0	0.3	
O		Facilitates Buffer Changes	2.0	1.0		2.0	1.0	
O		Uniformity and Precision of Thread Stretching	2.0	1.0		1.0	0.5	
	26.00	**Post-Production**		**6.3**	**164.7**		**4.5**	**117.0**
O		Uniformity and Precision of Thread Stretching	2.0	0.7		1.0	0.3	
O		Sterilize Threads	2.0	1.0		2.0	1.0	
O		Seed Threads with Cells	2.0	0.7		2.0	0.7	
O		Crosslink Threads	3.0	1.0		3.0	1.0	
O		Bundle Threads	2.0	1.0		1.0	0.5	
O		Twist Threads	2.0	1.0		1.0	0.5	
O		Surface Modify Threads	2.0	1.0		1.0	0.5	
	16.00	**User Friendly**		**3.4**	**54.7**		**3.3**	**53.3**
O		Easy to Use	3.0	1.0		3.0	1.0	
O		Easy to Clean	4.0	1.0		4.0	1.0	
O		Easily Maintained	2.0	0.7		1.0	0.3	
O		Reliability	3.0	0.8		4.0	1.0	
	19.00	**Automated**		**1.4**	**26.9**		**1.7**	**31.7**
O		Minimize Human Contribution	2.0	0.7		2.0	0.7	
O	**10**	**Able to be Scaled Up**	2.0	**0.7**	**6.7**	3.0	**1.0**	**10.0**
		TOTALS	46.0		321.4	36.0		243.8

Facilitate Buffer Changes

	Weight %	Attribute	**Hopper System**	Normalized	Weighted Sum	**Tilted Floor**	Normalized	Weighted Sum	**Aspirate**	Normalized	Weighted Sum	**Tipped Bath**	Normalized	Weighted Sum
C		Time Limit (A-D Term)	Y			Y			Y			Y		
C		Money (624 USD)	Y			Y			Y			Y		
C		Must fit on lab bench	Y			Y			Y			Y		
C		Limited Expertise of Design Team	Y			Y			Y			Y		
C		Safe for User	Y			Y			Y			Y		
C		Made of Non-reactive Materials	Y			Y			Y			Y		
O	**14.00**	**Production**		**1.0**	**14.0**		**1.0**	**14.0**		**1.0**	**14.0**		**1.0**	**14.0**
O		Sterile Threads	2.0	1.0		2.0	1.0		2.0	1.0		2.0	1.0	
	16.00	**User Friendly**		**3.7**	**58.7**		**3.5**	**56.0**		**2.5**	**40.0**		**3.4**	**54.7**
O		Easy to Use	3.0	1.0		3.0	1.0		2.0	0.7		2.0	0.7	
O		Easy to Clean	4.0	1.0		3.0	0.8		3.0	0.8		4.0	1.0	
O		Easily Maintained	2.0	0.7		3.0	1.0		1.0	0.3		3.0	1.0	
O		Reliability	4.0	1.0		3.0	0.8		3.0	0.8		3.0	0.8	
	19.00	**Automated**		**2.0**	**38.0**		**2.0**	**38.0**		**1.3**	**23.8**		**1.3**	**23.8**
O		Minimize Human Contribution	4.0	1.0		4.0	1.0		1.0	0.3		1.0	0.3	
O		High Production rate	3.0	1.0		3.0	1.0		3.0	1.0		3.0	1.0	
O	**10.00**	**Able to be Scaled Up**	3.0	**1.0**	**10.0**	3.0	**1.0**	**10.0**	3.0	**1.0**	**10.0**	2.0	**0.7**	**6.7**
		TOTALS	25.0		120.7	24.0		118.0	18.0		87.8	20.0		99.1

Facilitate Physical Post-Production Modifications

	Weight %	Attribute	**Tilting Blocks**	Normalized	Weighted Sum	**Sliding Anchors**	Normalized	Weighted Sum	**Stacking Anchors**	Normalized	Weighted Sum	**Jellyroll**	Normalized	Weighted Sum
C		Time Limit (A-D Term)	Y			Y			Y			Y		
C		Money (624 USD)	Y			Y			Y			Y		
C		Must fit on lab bench	Y			Y			Y			Y		
C		Limited Expertise of Design Team	Y			Y			Y			Y		
C		Safe for User	Y			Y			Y			Y		
C		Made of Non-reactive Materials	Y			Y			Y			Y		
O	**26.00**	**Post-Production**		**4.3**	**112.7**		**4.3**	**112.7**		**4.0**	**104.0**		**4.3**	**112.7**
O		Uniformity and Precision of Thread Stretching	2.0	0.7		2.0	0.7		1.0	0.3		2.0	0.7	
O		Sterilize Threads	2.0	1.0		2.0	1.0		2.0	1.0		2.0	1.0	
O		Seed Threads with Cells	2.0	0.7		2.0	0.7		2.0	0.7		2.0	0.7	
O		Crosslink Threads	3.0	1.0		3.0	1.0		3.0	1.0		3.0	1.0	
O		Reliability	4.0	1.0		4.0	1.0		4.0	1.0		4.0	1.0	
	16.00	**User Friendly**		**3.7**	**58.7**		**3.8**	**60.0**		**3.1**	**49.3**		**3.7**	**58.7**
O	3.00	Easy to Use	2.0	0.7		3.0	1.0		1.0	0.3		2.0	0.7	
O	2.00	Easy to Clean	4.0	1.0		3.0	0.8		3.0	0.8		4.0	1.0	
O	3.00	Easily Maintained	3.0	1.0		3.0	1.0		3.0	1.0		3.0	1.0	
O	5.00	Reliability	4.0	1.0		4.0	1.0		4.0	1.0		4.0	1.0	
	19.00	**Automated**		**1.3**	**23.8**		**1.3**	**23.8**		**1.3**	**23.8**		**1.3**	**23.8**
O		Minimize Human Contribution	1.0	0.3		1.0	0.3		1.0	0.3		1.0	0.3	
O		High Production rate	3.0	1.0		3.0	1.0		3.0	1.0		3.0	1.0	
O	**10.00**	**Able to be Scaled Up**	2.0	**0.7**	**6.7**	3.0	**1.0**	**10.0**	3.0	**1.0**	**10.0**	3.0	**1.0**	**10.0**
		TOTALS	19.0		201.8	20.0		206.4	19.0		187.1	20.0		205.1

	Weight %	Attribute	**Thread Plate Sweeper**	Normalized	Weighted Sum	**Twisting Caps**	Normalized	Weighted Sum	**Crank Mechanism**	Normalized	Weighted Sum	**Vertical Weights**	Normalized	Weighted Sum
C		Time Limit (A-D Term)	Y			Y			Y			Y		
C		Money (624 USD)	Y			Y			Y			Y		
C		Must fit on lab bench	Y			Y			Y			Y		
C		Limited Expertise of Design Team	Y			Y			Y			Y		
C		Safe for User	Y			Y			Y			Y		
C		Made of Non-reactive Materials	Y			Y			Y			Y		
O	**26.00**	**Post-Production**		**4.0**	**104.0**		**4.3**	**112.7**		**4.1**	**106.2**		**3.4**	**88.8**
O		Uniformity and Precision of Thread Stretching	1.0	0.3		2.0	0.7		2.0	0.7		2.0	0.7	
O		Sterilize Threads	2.0	1.0		2.0	1.0		2.0	1.0		2.0	1.0	
O		Seed Threads with Cells	2.0	0.7		2.0	0.7		2.0	0.7		1.0	0.3	
O		Crosslink Threads	3.0	1.0		3.0	1.0		3.0	1.0		2.0	0.7	
O		Reliability	4.0	1.0		4.0	1.0		3.0	0.8		3.0	0.8	
	16.00	**User Friendly**		**3.7**	**58.7**		**4.0**	**64.0**		**3.4**	**54.7**		**3.4**	**54.7**
O	3.00	Easy to Use	2.0	0.7		3.0	1.0		3.0	1.0		3.0	1.0	
O	2.00	Easy to Clean	4.0	1.0		4.0	1.0		4.0	1.0		4.0	1.0	
O	3.00	Easily Maintained	3.0	1.0		3.0	1.0		2.0	0.7		2.0	0.7	
O	5.00	Reliability	4.0	1.0		4.0	1.0		3.0	0.8		3.0	0.8	
	19.00	**Automated**		**0.9**	**17.4**		**1.3**	**23.8**		**1.3**	**23.8**		**1.3**	**23.8**
O		Minimize Human Contribution	1.0	0.3		1.0	0.3		1.0	0.3		1.0	0.3	
O		High Production rate	2.0	0.7		3.0	1.0		3.0	1.0		3.0	1.0	
O	**10.00**	**Able to be Scaled Up**	3.0	**1.0**	**10.0**	3.0	**1.0**	**10.0**	3.0	**1.0**	**10.0**	3.0	**1.0**	**10.0**
		TOTALS	31.0		190.1	20.0		210.4	19.0		194.6	17.0		177.3

Control Production Parameters

	Weight %	Attribute	LabVIEW	Normalized	Weighted Sum	Manual	Normalized	Weighted Sum	Heat Solutions	Normalized	Weighted Sum
C		Time Limit (A-D Term)	Y			Y			Y		
C		Money (624 USD)	Y			Y			Y		
C		Must fit on lab bench	Y			Y			Y		
C		Limited Expertise of Design Team	Y			Y			Y		
C		Safe for User	Y			Y			Y		
C		Made of Non-reactive Materials	Y			Y			Y		
O	**14.00**	**Production**		**2.8**	**38.5**		**2.5**	**35.0**			
O		Thread Diameter	4.0	1.0		4.0	1.0				
O		Formation Pattern	3.0	0.8		2.0	0.5				
O		Temperature	3.0	1.0		3.0	1.0				
O	**26.00**	**Post-Production**		**1.0**	**26.0**		**1.0**	**26.0**			
O		Uniformity and Precision of Thread Stretching	3.0	1.0		3.0	1.0				
O	**6.00**	**Accuracy: How close to defined standard**	2.0	**0.7**	**4.0**	2.0	**0.7**	**4.0**		**0.7**	**4.0**
	26.00	**Precision and Reproducibility of Results**		**3.0**	**78.0**		**2.0**	**52.0**			
O		Thread Diameter		1.0		2.0	0.7				
O		Thread Length	3.0	1.0		3.0	1.0				
O		End Product matches Specified Parameters	3.0	1.0		1.0	0.3				
	16.00	**User Friendly**		**2.3**	**37.3**		**2.3**	**37.3**		**2.7**	**42.7**
O		Easy to Use	3.0	1.0		1.0	0.3		3.0	1.0	
O		Easily Maintained	1.0	0.3		3.0	1.0		2.0	0.7	
O		Reliability	4.0	1.0		4.0	1.0		4.0	1.0	
	19.00	**Automated**		**1.0**	**19.0**		**0.3**	**4.8**		**0.8**	**14.3**
O		Minimize Human Contribution	4.0	1.0		1.0	0.3		3.0	0.8	
O	**10**	**Able to be Scaled Up**	3.0	**1.0**	**10.0**	3.0	**1.0**	**10.0**	3.0	**1.0**	**10.0**
		TOTALS	36.0		212.8	30.0		169.1	17.0		70.9

	Weight %	Attribute	Heated Bath	Normalized	Weighted Sum	Incubator	Normalized	Weighted Sum	Heated Room	Hot Plate
C		Time Limit (A-D Term)	Y			Y			N	Y
C		Money (624 USD)	Y			Y			N	Y
C		Must fit on lab bench	Y			Y			N	Y
C		Limited Expertise of Design Team	Y			Y			Y	Y
C		Safe for User	Y			Y			Y	N
C		Made of Non-reactive Materials	Y			Y			Y	Y
O	14.00	**Production**								
O		Thread Diameter								
O		Formation Pattern								
O		Temperature								
O	26.00	**Post-Production**								
O		Uniformity and Precision of Thread Stretching								
O	6.00	**Accuracy: How close to defined standard**	3.0	1.0	6.0	3.0	1.0	6.0		
	26.00	**Precision and Reproducibility of Results**								
O		Thread Diameter								
O		Thread Length								
O		End Product matches Specified Parameters								
	16.00	**User Friendly**		2.4	38.7		2.1	33.3		
O		Easy to Use	3.0	1.0		3.0	1.0			
O		Easily Maintained	2.0	0.7		1.0	0.3			
O		Reliability	3.0	0.8		3.0	0.8			
	19.00	**Automated**		1.0	19.0		1.0	19.0		
O		Minimize Human Contribution	4.0	1.0		4.0	1.0			
O	10	**Able to be Scaled Up**	3.0	1.0	10.0	3.0	1.0	10.0		
		TOTALS	18.0		73.7	17.0		68.3		

Appendix F: Parts List

Component	Quantity	Parts Name	Description
Outer Bath	1	Outer Bath Bottom	self-explanatory
	2	Outer Bath Long Side	self-explanatory
	2	Outer Bath Short Side	self-explanatory
	2	Floater Wedges	pieces which keep the inner bath from floating
	2	Wedge Pins	pins which fit into the outer bath wedges and keep
	5	Outer Bath Feet	pieces of rubber stuck on the bottom
	1	Outer Bath Lid	self-explanatory
Inner Bath	1	Inner Bath Bottom	self-explanatory (flat bottom)
	2	Inner Bath Long Side	self-explanatory
	2	Inner Bath Short Side	self-explanatory
	1	Inner Bath Incline	tilted bottom of inner bath
	1	Inner Bath Mini-Incline	thin piece at one end which allows water to drain into one of the corners
	1	Inner Wedge	inner wedge which allows threads to be made on a flat surface, could also be broken down into Top, Bottom, and Sides
	2	Block Posts	Small two posts in the center area which the Anchor Block (big black piece described later) sits on
	2	Anchor Posts	Longer posts which the Anchor System sits on
	1	Inner Bath Lid	self-explanatory
Anchor System	1	Anchor Block	large black rectangle which sits in the middle
	2	Anchor Points	rectangular pieces which are roughened and attach to the threads
	2	Anchor Sides	two C shaped pieces which the Anchor Points attach to, sit on either end of the anchor system, would attach to stretch mechanism
	2	Anchor Brackets	two bars which are screwed into the two sides of the anchor system, can be removed to allow for stretching
	4	Bracket Posts	Glued into the two Anchor Sides, Anchor Brackets slide over these when they are attached, prevent the Anchor System from squishing in either direction
	8	Nylatron Screws	used to attached all anchor system parts

Hopper System	1	Carboy #1	holds Fiber Formation Buffer (buffer #1)
	1	Carboy #2	holds Fiber Incubation Buffer (buffer #2)
	2	Large Diameter Y Tubing	attaches to carboy spigot, has small tubing vent hole (hence the "Y")
	2	Small Diameter Tubing	goes from large tubing into the bath
	2	Tubing Joint	connects large diameter Y tubing to small diameter tubing
	1	Aspiration Pump	pumps air for aspiration system
	1	Waste Flask	where waste solution is pumped (currently we've been using a 1L Erlenmeyer flask
	1	Waste Flask Tubing	tubing for the waste flask which runs both from the bath to the flask and from the flask to the pump
	1	Waste Flask Stopper	sits in the top of the waste flask, helps to create a vacuum for aspiration
Extrusion System	1	Extrusion Platform	large piece of black acrylic where all the extusion equipment resides
	4	Platform Legs	the legs of the platform
	4	Platform Leg Screws	screws which attach the extrusion platform to the platform legs, #12
	1	Motor #1	drives the motion from thread to thread, sits directly on the platform
	1	Medium-Large Gear (Gear #1)	attached to motor #1
	1	Linear Slide #1	main control of motion from thread to thread, uses the threaded rod system, also where motor #2 sits on
	1	Linear Slide #3	parallel to Linear slide #1, helps guide motion of linear slide #1 and maintain height of linear slide #2
	1	Linear Slide #2	perpendicular to #1 and #2, parallel to drawer slide, main control of motion along the threads, motor #2 controls this, actually two slides joined together
	1	Motor #2	drives the motion along the threads, sits on linear slide #1
	1	Small Gear (Gear #2)	attached to motor #2
	1	Drawer Slide	parallel to Linear slide #2, helps guide motion
	1	Large Gear (Gear#3)	attached to long square rod which contains the threaded pieces, links with gear #1 to control thread to thread motion
	2	Angle Gusset	located on either end of linear slide #1, helps it sit within the hole, structural component of the threaded rod system, see picture here: http://www.vexrobotics.com/products/accessories/structure/angle-gusset.html
	2	Delrin	black plasic pieces, three holes, attached to angle gussets, allow long square

		Bearing	rod to turn, picture here: http://www.vexrobotics.com/products/accessories/motion/276-1209.html
	1	Long Square Rod	long black metal rod with square cross section, threaded plastic pieces are put on here to create the threaded rod
	12	Threaded Rod Pieces	cylindrical pieces that are put into the long square rod to make the threaded rod
	1	Plastic Spacer	Threaded onto long square rod with threaded rod pieces to make the threaded rod the right length
	1	Gear Bracket	C shaped think piece which fits over linear slider #1 and holds gear #4 in place
	1	Medium-Small Gear (gear #4)	Sits against threaded rod, held in place with gear bracket,
	2	Lock bar	small black bearing attached to the gear bracket, short square rod goes through here and is prevented from turning
	2	Motor Bracket	metal curved pieces used to mount the two motors
	2	Silicone Strip	used as a cushion between the motors and motor brackets
	1	Motor #2 Platform	clear polycarbonate rectangle which is mounted on linear slide #1, motor #2 sits on here
	1	Connector Bracket	small thin rectangle of metal used to join the two halves of linear slide #2
	1	Aluminum Spacer #1	aluminum bar used to join linear slides #1 and #3
	1	Aluminum Spacer #2	aluminum bar used to join linear slide #2 and drawer slide
	1	Pipette	pyrex pipette used to hold tubing upright
	1	Aluminum Square	holds pipette, attaches to linear slide #2
	1	Pipette Heat Shrink	around pipette, keeps pipette and proper level
	8	Gear Track Pieces	attached to linear slide #2, connected to gear #2
Attachment	17	Track Screws	used to attach gear track pieces to linear slide #2: #6-32
	10	Standard Socket Cap Screws	long, large cylindrical heads, used to attach linear slider #1 as well as the motor brackets and motor #2 platform. #8-32
	23	Button Head Screws	shorter, rounder/flatter heads, used to attach spacer #1 and various other smaller things. #8-32
	19	Nuts	almost always #8-32, used to attached screws and also for spacing with gear #4
	5	Large Washer	used for spacing in threaded rod and gear #4
	4	Small Washer	used for spacing with gear #3

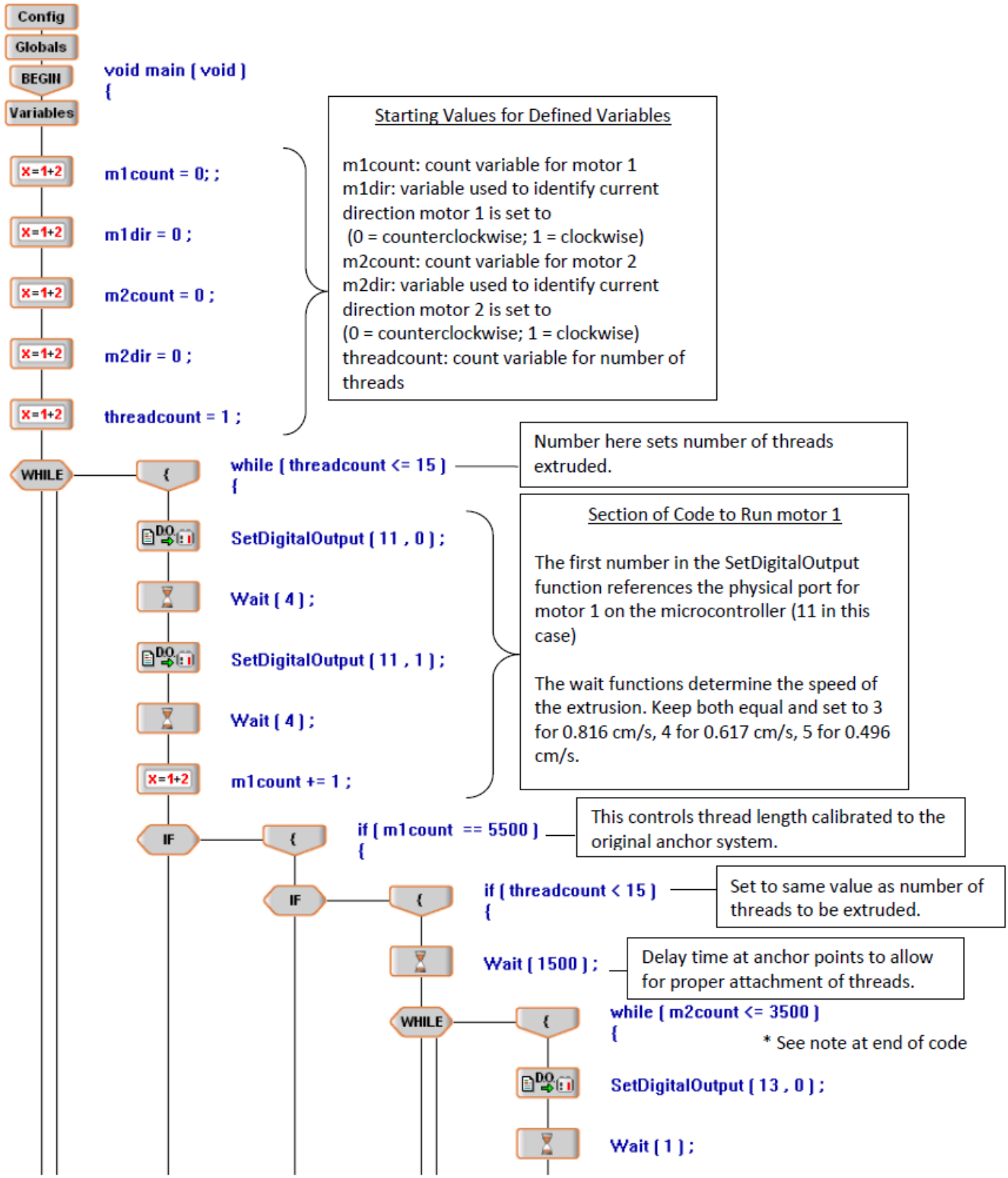
Other	1	Immersion Heater	self-explanatory
	1	Microcontroller	self-explanatory
	1	Battery	self-explanatory
	1	Syringe Pump	self-explanatory
	1	Extrusion Tubing	thin tubing used for extruding the collagn
	1	Collagen Syringe	syringe filled with collagen

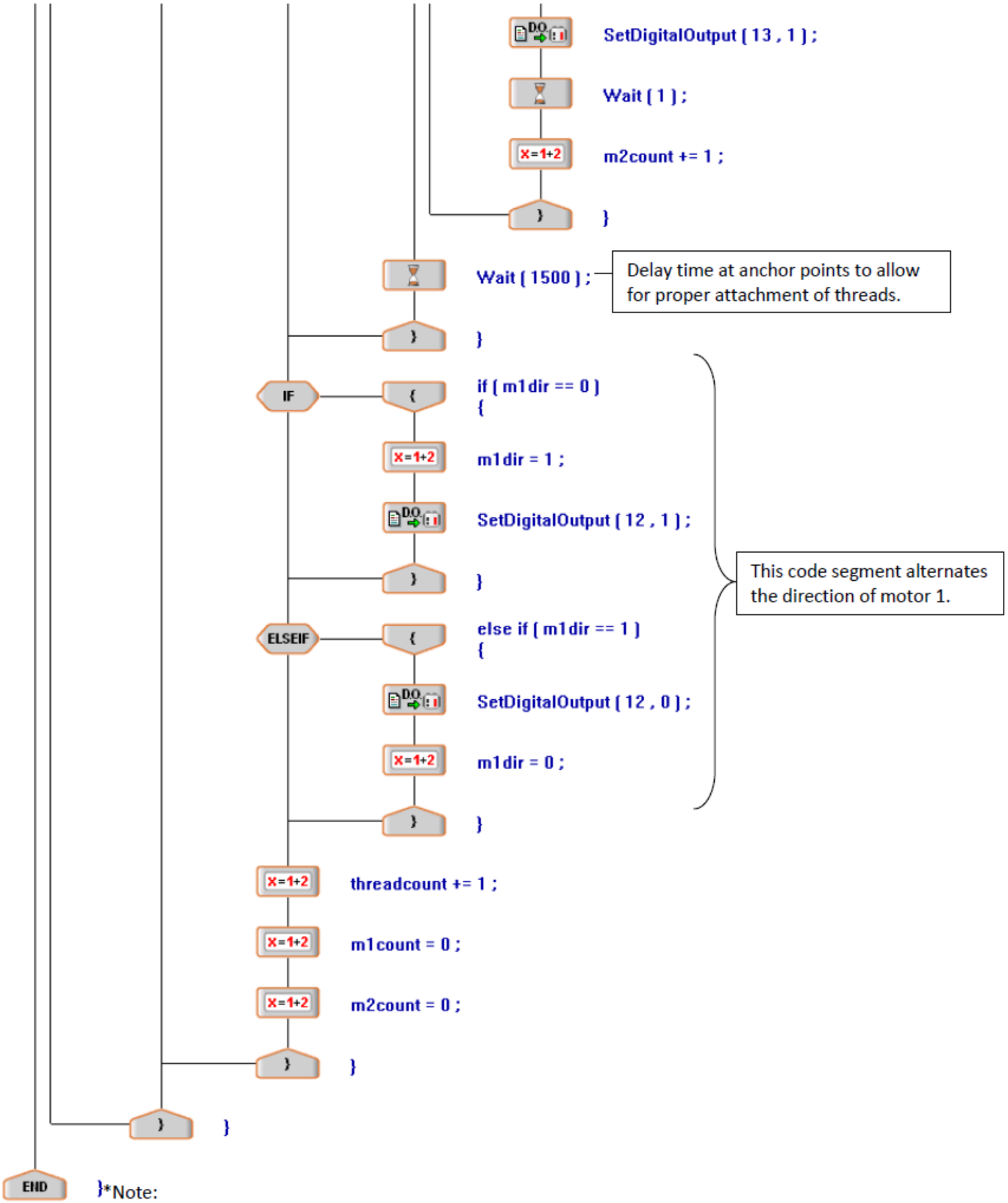
Appendix G: Budget List

Category	Material	Cost ($)
Already Spent	Polycarbonate and Glue	$135.31
	Screws	$28.03
	Rods	$11.96
	Plastic Tub	$2.00
	Valves/Tubing/Screws/Rubber/Braces	$45.00
	Drawer Slide	$12.49
Need to Buy		
	Vex Microcontroller	$149.99
	Motors (x2)	$30.00
	Drivers (x2)	$30.00
	Battery and Charger	$40.00
	4 Linear Sliders	$40.00
	Gear Bracket (x2)	$10.00
	Programming Hardware Kit	$50.00
	Hopper Bottles	$0.00
	Total Budget	$624.00
	Spent	$584.78
	Money Left	$39.22

Appendix H: Operating Instructions

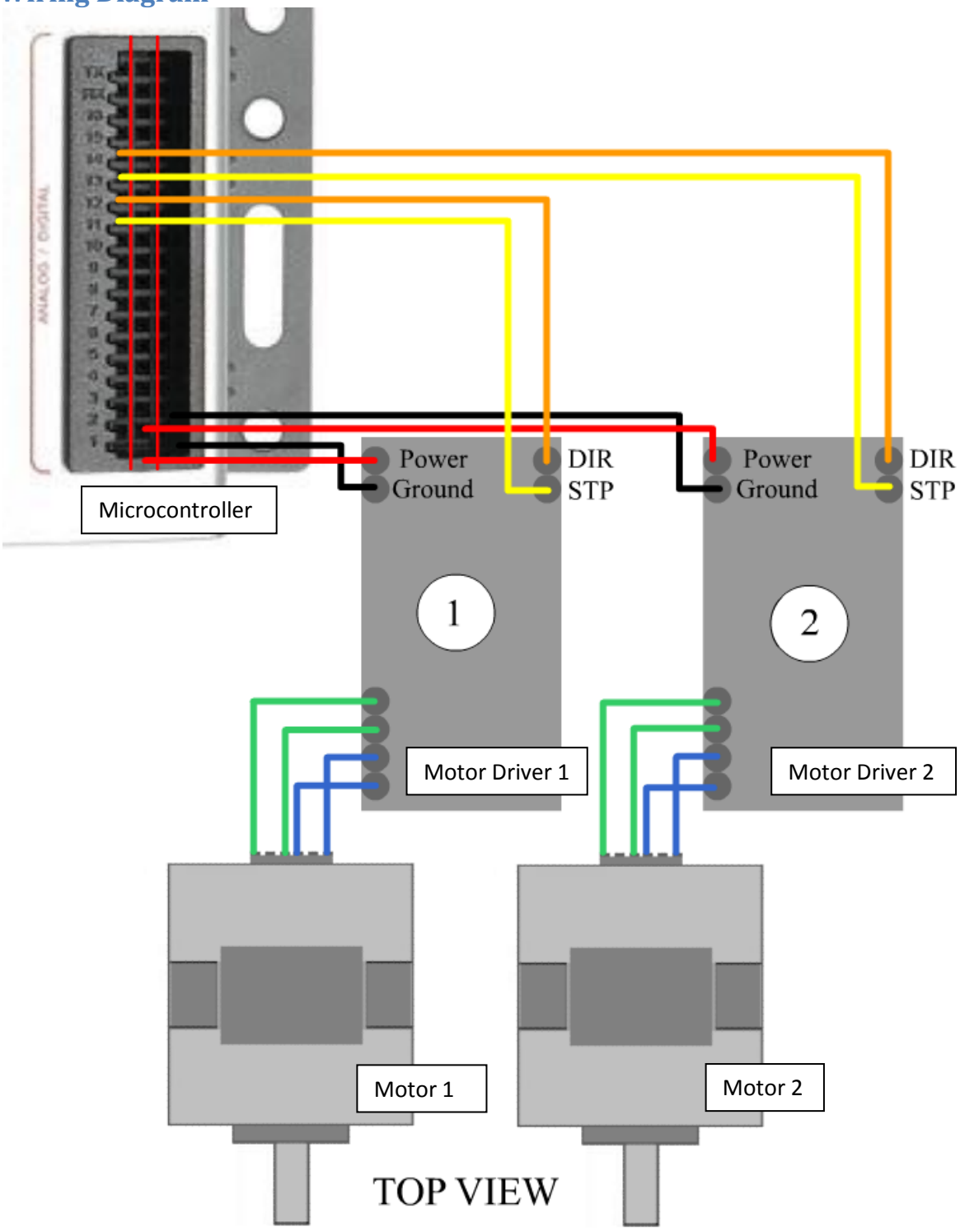
Reprogramming





}*Note:
The code segment "while (m2count <= 3500)" controls the distance between threads. 3500 approximates to 1cm distance between threads; a value of 1750 would yield half centimeter spacing.

Wiring Diagram



Before Use

Before operating this microthread extrusion device the following must be prepared:

- 10 mg/mL type I collagen
- Fiber Formation Buffer
- Fiber Incubation Buffer

Preparing Type I Collagen

Type I collagen can be obtained from rat rails. For full instructions on preparing 10 mg/mL type I collagen, see previous methods outlined by Kevin Cornwell, Ph.D. [Cornwell, 2007c].

Preparing Buffer Solutions

Necessary components:

- 135mM Sodium chloride (NaCl)
- 30mM Tris HCl
- 30mM Tris Base
- 5mM Sodium phosphate ($NaPO_4$) dibasic

Table 81: Amounts of Components for Buffer PreperationTable 81 below shows the mass of each component that is necessary to make 1X and 10X solutions of each buffer. Each batch of collagen microthreads requires 1 L of each buffer solution. If making only a single batch of microthreads, 1 L of 1X solution is necessary. If making multiple batches, 4 L of 1X solution can be prepared and stored in the device's two 4 L carboys, or 1 L of 10X solution can be prepared and stored in the 4 L carboys after dilution.

Table 81: Amounts of Components for Buffer Preperation

Component	Mass / Liter (g/L)			
	Fiber Formation Buffer		Fiber Incubation Buffer	
	1X solution	10X solution	1X solution	10X solution
135mM Sodium chloride (NaCl)	7.89	78.90	7.89	78.90
30mM Tris HCl	3.43	34.30	1.14	11.40
30mM Tris Base	1.00	10.00	0.33	3.30
5mM Sodium phosphate ($NaPO_4$) dibasic	0.71	7.10	4.26	42.6

Procedure:

Note: If 1X buffer solutions are already prepared, skip all steps
If 10X buffer solutions are already prepared, skip to step 10

1. Fill a graduated cylinder with an amount of de-ionized water that is slightly less than the final volume of the buffer solution
    a. For example, to make 1 L of final buffer solution, fill the graduated cylinder initially with only 800 mL of de-ionized water.
2. Place a magnetic stirrer bar inside the graduated cylinder
3. Place the cylinder on a magnetic stirrer plate running at medium speed
4. Measure out appropriate amounts of each of the components based on Table 81 above
5. Add each component to the graduated cylinder of de-ionized water SLOWLY IN SMALL INCREMENTS
    a. **This is extremely important, especially when making 10X solution, to ensure that your components will dissolve properly**
6. Allow the solution to stir until all components have completely dissolved. This could take several minutes.
7. Once all components have fully dissolved, pH the solution to 7.4

8. Add de-ionized water to bring the solution to the correct final volume
9. If 1X solution was prepared, buffers are now ready for use
10. If 10X solution was prepared, buffers must be diluted before use
    a. Determine the final volume of buffer necessary (example: 1 L)
    b. Pour 1/10th of this final volumes worth of 10X buffer solution into a graduated cylinder (example: 100 mL)
    c. Add de-ionized water to the graduated cylinder until the desired final volume is obtained (example: 900 mL)

Device Operation

A. Bath and Anchor System Set-Up

Necessary Components:

- Outer bath
- Inner bath
- Wedge pins
- Anchor block
- Anchor frame
- Immersion heater
- Approximately 12 L of tap water
- Outer bath lid
- Inner bath lid

Procedure:

Note: If the outer bath system is already in place and heated, skip steps 1 and 4-10
If the inner bath is already in place, skip step 2
If anchor system is already in place, skip step 3

1. Align outer bath so floater wedge marked with “A” is on the right
2. Align and secure inner bath
    a. Rotate inner bath so anchor post marked with “A” is in upper right hand corner
    b. Align the inner bath so it is between the two outer bath floater wedges
    c. Gently slide the inner bath between the two floater wedges until flush with bottom of outer bath
    d. Insert wedge pins into holes at the top of floater wedges
3. Align and secure anchor system
    a. Align and secure anchor platform
        i. Rotate black anchor platform so “A” edge is facing away and holes are facing down
        ii. Slide anchor platform into two short anchor posts in middle of inner bath wedge until it is flush with top of inner wedge
    b. Align and secure anchor frame
        i. Rotate anchor frame so “A” is in the upper right hand corner and roughened anchor points are facing up
        ii. Slide anchor frame on four tall anchor posts around the edge of the inner bath wedge until it lies flush with top of inner wedge
4. Mount immersion heater
    a. Align immersion heater so it faces to the left

    b. Slide heater onto the right hand side of outer bath until pins on the back of the immersion heater sit flush with the top of the side
    c. Check to make sure the immersion heater is slightly raised above the bottom of the bath
5. Fill outer bath so the water level is approximately 1.5 inches below the top of the inner bath (approximately 12 Liters)
6. Plug in immersion heater
7. Turn on immersion heater
8. Cover inner and outer baths
9. Allow immersion heater to run for at least 20 minutes
10. Bath and anchor system are now ready for use

B. Collagen and Extrusion Pump Set-Up

Necessary Components:

- 5mL of 10mg/ml type I collagen (from rat tails)
- 5CC plastic syringe
- Extrusion tubing
    - approximately 3 ft. of 0.0338 in (0.86 mm) diameter polyethylene tubing
    - 21 gauge syringe needle
- KD Scientific extrusion pump
- Sharp wire cutters

Procedure:

Note: If extrusion tubing is already assembled, skip step 1
If extrusion pump is already programmed, skip step 10

1. Assemble extrusion tubing
    a. Using very sharp wire cutters, carefully remove the sharp tip from the syringe needle
    b. Ensure the needle is still open at the end
        i. If the needle has been closed off, gently pinch the end with pliers in the opposite direction that was used for cutting until the needle is opened at the end
    c. Gently side the needle into one end of the extrusion tubing until it is fully inserted
2. Check collagen to ensure there are no bubbles
    a. If bubbles are present, centrifuge container for approximately 5 minutes
3. Slowly fill syringe with 5 mL of collagen
4. Check syringe to ensure there are no bubbles
    a. If bubbles are present, empty syringe and repeat step 2a
5. Attach the needle-end of the extrusion tubing the collagen-filled syringe
6. Pull up on the small knob located on the right-most black block (stationary block) on top of the syringe pump
7. Turn knob 90° so it rests on the raised triangular portion of the stationary block
8. Turn the knob located at the top of the middle black block (driver block) clockwise until the drive block is released and slides back and forth easily
    a. Do not force the block, if it does not move try turning the knob further first
9. Slide the driver block until it is relatively close to the stationary block
10. Place the collagen-filled syringe into one of the wedges in the stationary block so the extrusion tubing is pointing outwards (to the right) and the plunger lines up with the driver block
    a. Ensure that the wings of the syringe cylinder rest within the small slit on the left-hand side of the block

i. If not, loosen the two knobs on the front and back of the stationary block to expand the slit slightly

b. Ensure the plunger of the syringe rests within the small slit on the right-hand side of the driver block
   i. If not, loosen the knob on the right-hand side of the driver block to expand the slit slightly or move the drive block closer to the stationary block

11. Rotate the top knob back 90° so it now rests on top of the collagen-filled syringe and holds it firmly in place
12. When the collagen-filled syringe is fully in place and secured, rotate the knob on top of the driver block counterclockwise until it no longer slides freely
13. Plug in and turn on extrusion pump (black switch at the back)
14. Program extrusion pump
    a. Press the "Select" button
    b. Use the arrow buttons to scroll to the 'Table" option and press the "Select" button
    c. Use the arrow buttons to scroll and choose the type of syringe being used (most likely a "Bec.Dic. plastic") and press the "Select" button
    d. Use the arrow buttons to scroll and choose the size of the syring (most likely "5cc 11.9mm") and press the "Select" button
    e. Use the numbered keypad to enter the volume of collagen being extruded (most likely 5.0 mL) and press the "Enter" button
    f. Use the numbered keypad to enter the extrusion rate (most likely 0.225)
    g. Use the arrow buttons to scroll and choose the units for the extrusion rate (most likely mL/min) and press the "Enter" button
15. The collagen is now ready for extrusion

C. Extrusion

Necessary Components:

- Prepared bath and anchor system
- Prepared collagen and extrusion pump
- Extrusion system
- Carboy with fiber formation buffer and associated tubing

Procedure

1. Align extrusion system with the bath system so the extrusion head runs parallel to the inner bath in both directions
2. Place syringe pump containing collagen-filled syringe behind the bath system
   a. Ensure that the extrusion tubing can freely move along the entire length and width of the inner bath
   b. If not, adjust accordingly
3. Remove the lids from the inner and outer bath
4. Fill the inner bath with 1 L of fiber formation buffer
   a. Attach carboy tubing to the inner bath
   b. Open the valve on the carboy spigot and allow solution to flow into the inner bath until it reaches the fill line on the front of the bath
   c. Close the valve
   d. Remove the carboy tubing from the inner bath
5. Insert free end of the extrusion tubing into the Pyrex® pipette of the extrusion head
6. Continue to push the tubing into the pipette until the tape marker reaches the top of the heat shrink

    a. This ensures the tubing sits the correct height above the inner bath
    b. Do not force the tubing further into the pipette
7. Press “Run” button located on the syringe pump
8. Wait approximately 2.5-3 minutes until the collagen solution reaches the far end of the extrusion tubing, approximately 0.6 mL of collagen will have been extruded
9. Turn on the extrusion system by flipping the grey switch located on the front of the microcontroller underneath the extrusion platform
10. Allow the system to run until the program ends (approximately 15 minutes)
    a. Watch the extrusion during the first few threads to ensure that initial adherence occurs correctly
    b. If threads do not adhere and become tangled, gently move them out of the way of the extrusion head with a pair of forceps to prevent them from affecting the formation of further threads
    c. If threads become extremely tangled, it may be necessary to remove them from the bath entirely
    d. If moving threads, handle them as little as possible
11. After the program finishes, turn off the microcontroller
12. Gently lift the pipette so it is clear of the inner bath solution
13. Gently push linear slide 2 back to its starting position
14. Turn the crank on gear 3 clockwise until linear slide 2 is back to its starting position (aligned with the black mark)
15. Turn the crank on gear 3 counterclockwise one turn so the system moves freely
16. Remove extrusion tubing from the extrusion head
17. Place the lids back on the inner and outer bath
18. Allow threads to sit for 24 hours

D. Extrusion Clean-Up

Necessary Components:

- Extrusion tubing
- 10 CC plastic syringe
- Syringe pump
- Microcontroller battery and charger

Procedure

1. Remove syringe from the syringe pump by lifting up the knob in the stationary block
2. Detach extrusion tubing from the syringe and dispose of the syringe in the proper sharps container
3. Fill 10 mL plastic syringe with water
4. Attach extrusion tubing to 10 mL syringe
5. Push water through the extrusion tubing
6. Repeat steps 3-5 at least 3 times
7. Fill 10 mL plastic syringe with air
8. Attach extrusion tubing to 10 mL syringe
9. Push air through the extrusion tubing
10. Repeat steps 7-9 at least 3 times
11. Store extrusion tubing in a Petri dish with the 10 CC syringe for future use
12. Turn off the syringe pump
13. Every 10 batches, unplug the microcontroller battery and allow it to charge overnight

E. Buffer Changes

Necessary components

- Carboy with fiber incubation buffer and associated tubing
- Carboy with de-ionized water and associated tubing
- Aspirator system
    - 1000 mL aspirator flask with associated tubing
    - Vacuum pump

Procedure

1. Remove lids from outer and inner bath
2. Attach aspirator system to the inner bath
    a. Ensure that tubing reaches all the way to the bottom of the bath in the lower right-hand corner
3. Aspirate out fiber formation buffer
4. Fill the inner bath with 1 L of fiber incubation buffer
    a. Attach carboy tubing to the inner bath
    b. Open the valve on the carboy spigot and allow solution to flow into the inner bath until it reaches the fill line on the front of the bath
    c. Close the valve
    d. Remove the carboy tubing from the inner bath
5. Replace lids on inner and outer bath
6. Allow threads to sit for 24 hours
7. Repeat steps 1-6 to replace the fiber incubation buffer with de-ionized water
8. Allow threads to sit for 24 hours

F. Drying and Storage

Necessary components

- Bath system
- Anchor frame
- Cardboard box
- Forceps

Procedure

1. Grasp the anchor system firmly by the handles
2. Lift up in a vertical, gentle, fluid motion until all threads have cleared the de-ionized water in the inner bath
3. Place the anchor frame on the cardboard box so none of the threads touch the cardboard
4. Using forceps, gently separate any threads which have stuck together
5. Allow threads to dry in this position overnight
6. Once dry, remove from the anchor system by gently pulling the threads with forceps close to the anchor points
7. Wrap threads in tinfoil, label, and store in a desiccator until use

Mechanical testing

Sample Preparation

Necessary components

- Velum paper
- Scotch tape
- Medical grade silicone glue

- Hole punch
- Scissors
- 1 CC plastic syringe
- Forceps
- Paper cutter
- Ruler

Procedure

1. Create velum paper frames
    a. Cut velum paper into strips 2 cm x 6 cm
    b. Create two holes in the center of each strip spaced 2 cm apart (from outer edge to outer edge)
    c. Cut away the velum paper between these two holes leaving a 2 cm. x 6 cm. strip of velum paper with a 2 cm. long cutout in the center (Figure 106).

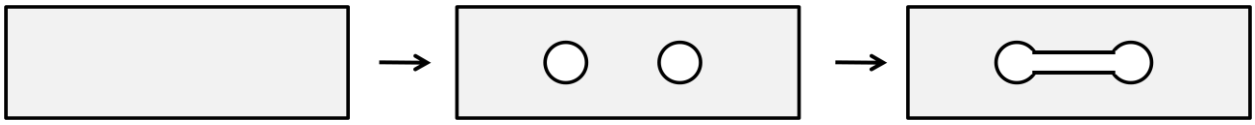


Figure 106: Diagram of Tensile Test Sample Setup

2. Cut thread into a piece approximately 5 cm long
3. Lay a thread sample on top of the opening in the velum paper strips
4. Tape down the ends of the thread
    a. Leave space between the tape and the opening in the center
    b. Ensure thread is taught and straight
5. Fill 1 CC syringe with silicone glue
6. Deposit a small amount of silicone glue onto the two ends of the thread between the opening and the tape
7. Repeat steps 1-6 for each test sample
    a. At least 12-15 samples should be prepared for each experimental group
    b. Number each sample
8. Allow glue to cure for 24 hours

Testing Procotol

Necessary components

- 12-15 prepared samples for each experimental group
- 1X phosphate buffered saline (PBS) (pH 7.4)
- Small, shallow square dishes (such as a rectangular well plate)
- Forceps
- Tensile testing machine (Instrol ElectroPuls E1000) with 1 N load cell
- Microscope (Nikon Eclipse E600) with video camera and associate software (RT Color SPOT Diagnostics and SPOT Advanced) or calibrated eyepiece

Procedure

1. Lay samples in the shallow dish
    a. They can overlap slightly, the collagen will not stick to itself
2. Pour enough PBS into the dish to fully cover all the samples
3. Allow the thread samples to rehydrate in PBS for at least 45 minutes
4. Using the microscope and associated equipment, measure the diameter of each sample

a. Take at least three random diameter measurements along the length of the thread
b. Average this random sampling

5. Load sample into the tensile testing machine (Figure 107)
    a. Lay each end of the velum strip on opposite metal plates
        i. Be very careful with the side attached to the load cell
    b. Secure them down with plastic clamps
        i. On the side with the load cell, do not heavily tighten
    c. Cut out the remaining portions of the middle section of velum paper so only the thread sample remains between the two ends

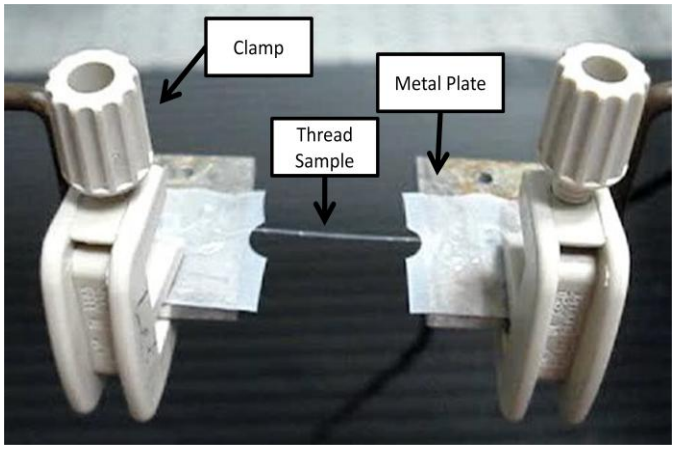


Figure 107: Tesnsile Test Example

6. Run test according to the necessary protocol
7. After breakage, remove sample remnants and repeat steps 5-6 for all samples

Appendix I: Statistical Data

For all analyses a one way ANOVA was run.

A Brown-Forsythe test was conducted to equality of variances among the four groups (not shown). In each case, the test failed to reject the null hypothesis of equal variances at the 0.05 significance level.

Normality was assessed using normal quantile plots of residuals and the Shapiro-Wilk test (results not shown). For UTS, the test rejected the assumption of normality
($p<0.0001$). this appears due to two outliers. For SD it did not ($p=0.9228$). Nevertheless, we report the results for UTS, as we verified these using a permutation test.

For each case in which the ANOVA F test indicated a significant ($p<0.05$) difference in group means, a Tukey honest significant difference post-hoc test was conducted at an overall 0.05 significance level for pairwise differences of group means.

**********STRAIN**********

The GLM Procedure

Dependent Variable: Strain Strain to Failure (mm/mm)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.29277576	0.09759192	1.82	0.1507
Error	73	3.91014978	0.05356370		
Corrected Total	76	4.20292554			

**********UTS**********

Significant F test indicates differences in means of the four types.

Dependent Variable: UTS Ultimate Tensile Strength (MPa)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	8.92209191	2.97403064	7.93	0.0001
Error	71	26.63220737	0.37510151		
Corrected Total	74	35.55429928			

R-Square	Coeff Var	Root MSE	UTS Mean
0.250943	42.96847	0.612455	1.425360

The GLM Procedure

Brown and Forsythe's Test for Homogeneity of UTS Variance
ANOVA of Absolute Deviations from Group Medians

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Type	3	1.6749	0.5583	2.55	0.0624
Error	71	15.5357	0.2188		

The GLM Procedure

Tukey's Studentized Range (HSD) Test for UTS

NOTE: This test controls the Type I experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	71
Error Mean Square	0.375102
Critical Value of Studentized Range	3.72071

Comparisons significant at the 0.05 level are indicated by ***.

Type Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
Machine 0.816 cm/s - Machine 0.617 cm/s	0.4950	-0.0397	1.0297	
Machine 0.816 cm/s - Machine 0.496 cm/s	0.7813	0.2346	1.3280	***
Machine 0.816 cm/s - Hand-drawn	0.9512	0.4045	1.4980	***
Machine 0.617 cm/s - Machine 0.496 cm/s	0.2863	-0.2239	0.7965	
Machine 0.617 cm/s - Hand-drawn	0.4562	-0.0540	0.9664	
Machine 0.496 cm/s - Hand-drawn	0.1699	-0.3528	0.6927	

The GLM Procedure

Tukey's Studentized Range (HSD) Test for UTS

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	71
Error Mean Square	0.375102
Critical Value of Studentized Range	3.72071
Minimum Significant Difference	0.5288
Harmonic Mean of Cell Sizes	18.57164

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Type
A	2.0029	16	Machine 0.816 cm/s
A			
B A	1.5079	21	Machine 0.617 cm/s
B			
B	1.2216	19	Machine 0.496 cm/s
B			
B	1.0516	19	Hand-drawn

**********SD**********

The GLM Procedure

Dependent Variable: SD Standard Deviation

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	145.6116605	48.5372202	7.71	0.0013
Error	20	125.9009218	6.2950461		
Corrected Total	23	271.5125823			

R-Square	Coeff Var	Root MSE	SD Mean
0.536298	23.35464	2.508993	10.74302

The GLM Procedure

Brown and Forsythe's Test for Homogeneity of SD Variance
ANOVA of Absolute Deviations from Group Medians

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Type	3	5.0765	1.6922	0.91	0.4536
Error	20	37.1701	1.8585		

The GLM Procedure

Tukey's Studentized Range (HSD) Test for SD

NOTE: This test controls the Type I experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	6.295046

Critical Value of Studentized Range	3.95829
Minimum Significant Difference	4.0545

Comparisons significant at the 0.05 level are indicated by ***.

Type Comparison		Difference Between Means	Simultaneous 95% Confidence Limits		
Hand-drawn	- Machine 0.496 cm/s	3.223	-0.832	7.277	
Hand-drawn	- Machine 0.617 cm/s	5.293	1.239	9.348	***
Hand-drawn	- Machine 0.816 cm/s	6.497	2.443	10.552	***
Machine 0.496 cm/s	- Machine 0.617 cm/s	2.070	-1.984	6.125	
Machine 0.496 cm/s	- Machine 0.816 cm/s	3.274	-0.780	7.329	
Machine 0.617 cm/s	- Machine 0.816 cm/s	1.204	-2.851	5.258	

The GLM Procedure

Tukey's Studentized Range (HSD) Test for SD

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	6.295046
Critical Value of Studentized Range	3.95829
Minimum Significant Difference	4.0545

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Type
A	14.496	6	Hand-drawn
A			
B A	11.273	6	Machine 0.496 cm/s
B			
B	9.203	6	Machine 0.617 cm/s
B			
B	7.999	6	Machine 0.816 cm/s

ANOVA test for difference in diameter of hand-drawn amd 3 machine speeds:

	Hand-Drawn	Machine 0.496 cm/s	Machine 0.617 cm/s	Machine 0.816 cm/s
Diameter (µm)	83.7	68.6	69.6	59.1
	85.4	72.1	80.8	65
	78.3	75.5	79.9	61
	77.2	72.9	87.1	71.2
	75.1	80.6	79.2	52.2
	76.5	82	73.5	53

Anova: Single Factor

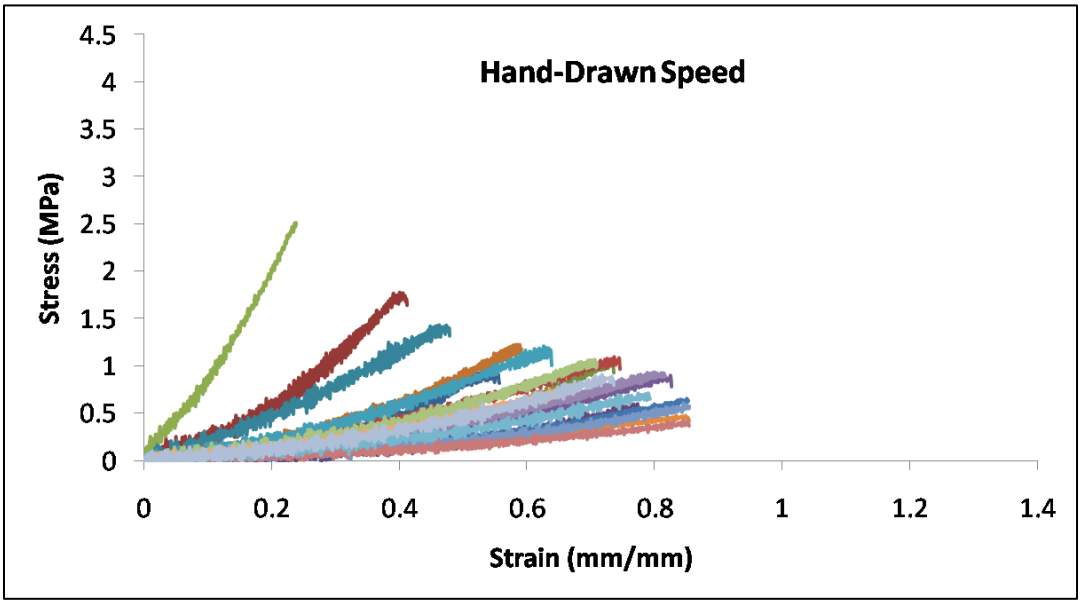
SUMMARY

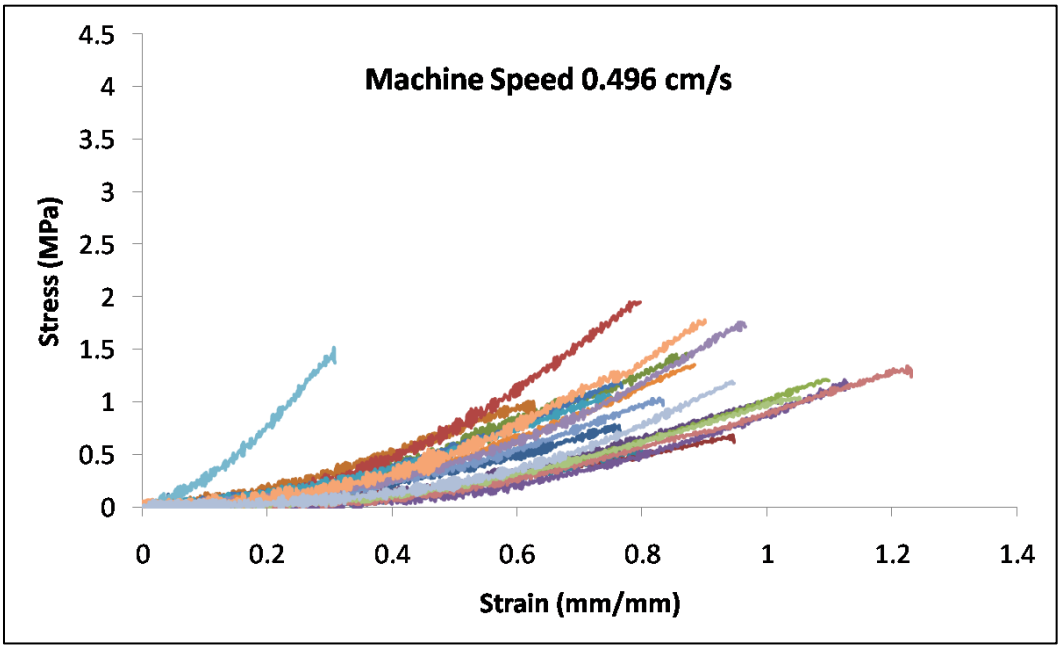
Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	6	476.2	79.36666667	17.48666667
Column 2	6	451.7	75.28333333	26.78166667
Column 3	6	470.1	78.35	37.155
Column 4	6	361.5	60.25	52.343

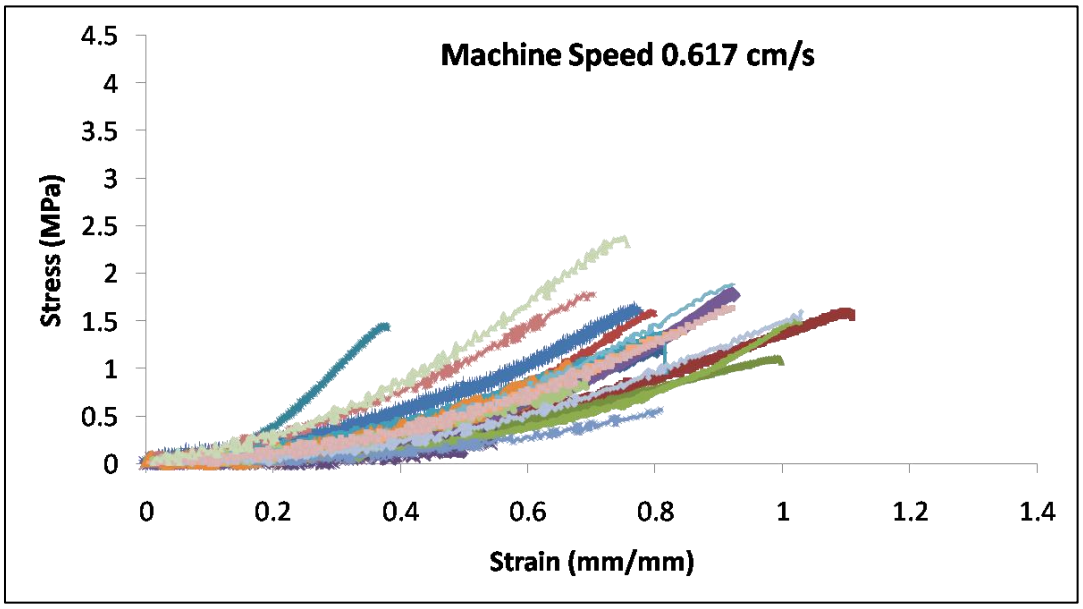
ANOVA

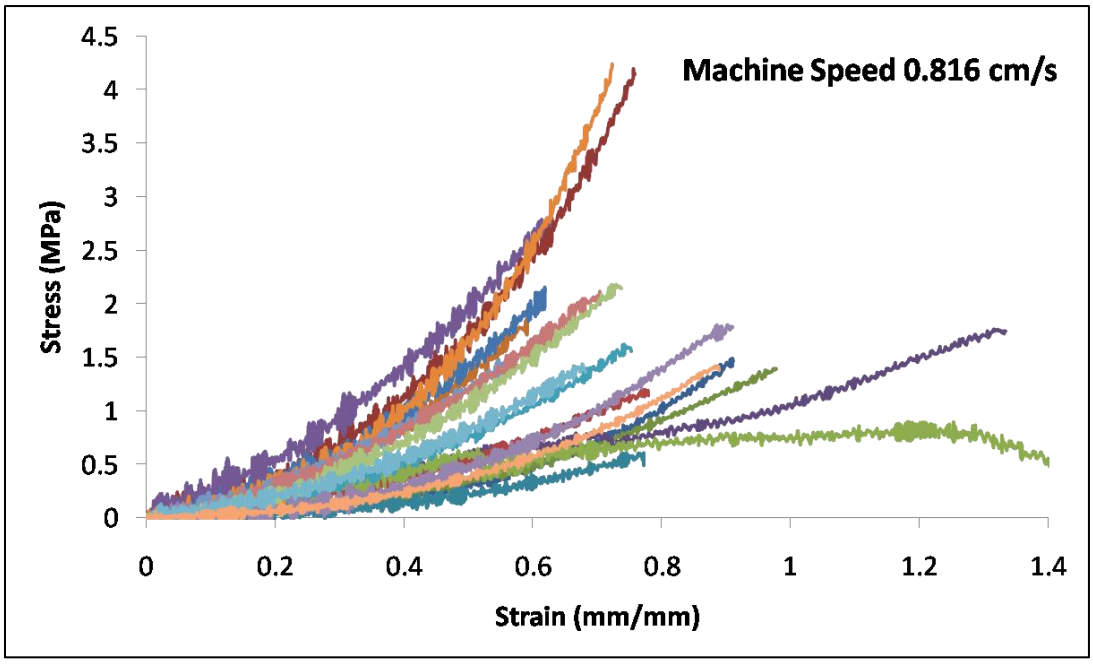
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1419.2545 8	3	473.084861 1	14.1466047 3	3.54E-05	3.09839122 4
Within Groups	668.83166 7	20	33.4415833 3			
Total	2088.0862 5	23				

Appendix J: Mechanical Testing Graphs






Machine Speed 0.617 cm/s
Stress (MPa)
Strain (mm/mm)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0
0.2
0.4
0.6
0.8
1
1.2
1.4


Machine Speed 0.816 cm/s
Stress (MPa)
Strain (mm/mm)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0
0.2
0.4
0.6
0.8
1
1.2
1.4