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Fixation Device to Measure the Contractile Force of Skeletal Muscle in Live Animals

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FIXATION DEVICE TO MEASURE THE CONTRACTILE FORCE OF SKELETAL MUSCLE IN LIVE ANIMALS

A Major Qualifying Project submitted to the faculty of Worcester Polytechnic Institute in partial fulfillment of the requirements for the Degree of Bachelor of Science

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April 26th, 2012

Abstract

The goal of this project was to design a relatively inexpensive, minimally invasive fixation device for the hind limb of a mouse that uses topical electrical stimulation of skeletal muscle to accurately and repeatedly quantify the force generated by muscle contractions. The device also had to be compatible with surgical procedures visualized using a stereomicroscope. Testing on anesthetized animals was performed and repeatable force measurements were acquired following multiple series of electrical stimulation by placement of bipolar electrodes on the tibialis anterior muscle surface. There was no visible evidence of tissue damage at either the knee anchor point or at the point of attachment of the ligature to the foot, which was connected to the force transducer. Further, there was no visible damage to the muscle tissue due to electrode placement.

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Authorship Page

Section	Author	Editor
1. Introduction	Jirom	Dylan
2. Literature Review	All	Gregory/Dylan
3. The Design Process	All	Jirom
4. Conceptual Designs	All	Bryan
5. Design Verification	Bryan	Jirom
6. Final Design and Validation	Bryan	Dylan
7. Discussion	Dylan	Gregory
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1. Introduction

Skeletal muscles are often called voluntary muscles because the contractions are consciously controlled. These muscles are usually attached to both ends of the bone by means of tough connective tissues called, tendons. When a muscle contracts, it forces the tendons to undergo tension which allow the skeleton to move. However when skeletal muscles are damaged and recovery is sought, several muscle regeneration processes are available. Mechanical conditioning for example involves weeks of repetitive stretching to improve muscle fiber and orientation, on the injured limb.

In order to obtain several observations of the contractile force on a live test subject, a non-invasive device is needed. Currently techniques for assessment of skeletal muscle repair are limited to histological examinations of injured tissue on contractile force measurements of dissected muscle fibers. Histological examinations lack direct accurate quantitative data to assess the degree of neither the injury, originally inflicted nor the recovered.

Accurate techniques for measuring the contractile force of the muscle in lab rats have been developed, but are inconvenient for this specific project. Repeated measurements are not permitted due to the invasiveness of the method. The procedure requires the muscle and nerve to be isolated and attached to a force transducer by a ligature.

The muscle this project will be focusing on is the tibialis anterior. The tibialis anterior is the muscle that is most near to the tibia (shinbone), and most responsible for dorsiflexion and inverting the foot. This muscle plays an essential role in activities such as running and balancing. Comparisons of force measured in injured muscles to contralateral limbs do not offer the desired accurate reading of contractile force due to a putative therapy gap for muscle wound repair.

Preliminary observations suggest up to a 30% difference in contractile force between the uninjured (control) contralateral tibialis anterior muscles in the mouse.

The goal of this project was to develop a device and methodology that enables comparative functional measurements for the muscle at defined time points. The device measures the force generated by the tibialis anterior prior to the injury, then the reduction in force due to injury, and then the recovery of force due to treatment at specified time points of the regeneration process. The device involves minimal invasive electrical stimulation at defined locations on the muscle. The instrument uses bipolar electrodes and non-invasive fixation attachment points to measure the force generated by the tibialis anterior muscle. The anchor points that secure the limb only cause minimal tissue damage or edema during the procedure. The most significant injury the mouse should undergo for the sake of the procedure is the removal of skin and intervening fascia. This is done to properly expose the muscle to permit the electrodes to stimulate the muscle. The device must be designed to be built on a platform suitable for stereomicroscopic examination of the surgical procedure. Also in order to ensure the subject is kept still during the procedure general anesthesia is applied.

The upcoming sections involve background information regarding general skeletal muscle anatomy, current methods for measuring presented *in-vivo* muscle function, and modern muscle regeneration treatments. These sections are discussed to provide context to further the understanding of the results and effectiveness of the device.

2. Literature Review

In order to gain a substantial knowledge of the composition and function of skeletal muscle, as well as similar devices already made, an extensive literature review was conducted at the start of this project.

2.1 Structure of Skeletal Muscle

Skeletal muscles are composed of several different layers. The outermost layer is the fascia. Directly under the fascia is as sheath of irregularly arranged tendons called the epimysium. Tissue from the epimysium extends deeper into the muscle and divides into fascicles. These fascicles are then surrounded by another connective tissue, the perimysium. (Fox, 2009).
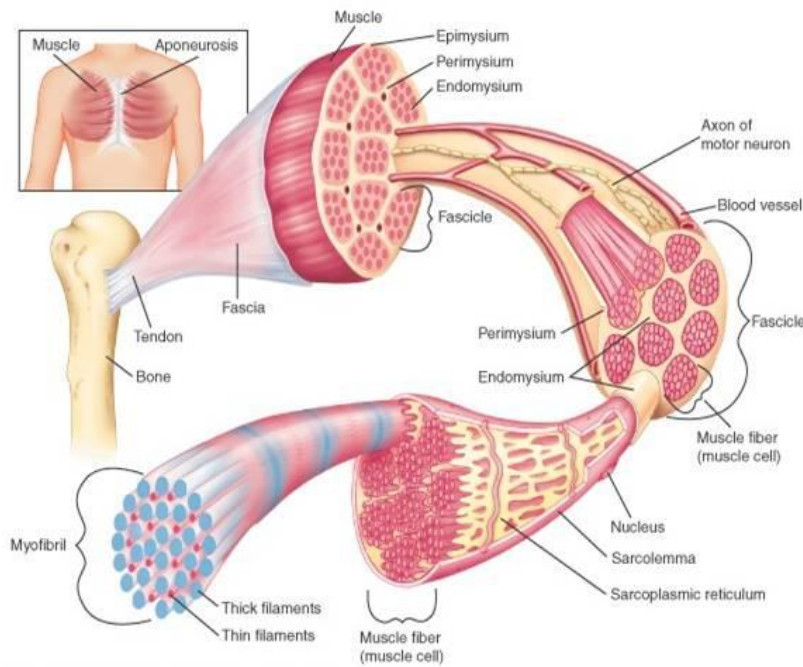
Figure 1: Muscular Hierarchy

Fascicles are made up of small muscle fibers, or myofibers. Myofibers are made of long protein molecules called myofilaments, and classified as thick or thin. The myofibers are surrounded by a plasma membrane called the sarcolemma, which in turn is covered in a layer of tissue known as the endomysium. The sarcolemma is the membrane of a muscle cell, and performs several important functions. It maintains a membrane potential that allows impulses to travel. The muscle cell impulses cause contraction.

2.1.1 Characteristics of Muscles

Muscles have four main characteristics. They are excitable, which allows it to respond to electrical stimuli; contractility, which gives muscle the ability to shorten; extensibility, which allows the muscles to stretch; and elasticity, which lets it return to its original shape. Skeletal muscle attaches to the bone and allows for movement of the skeleton (Fox, 2009).

2.1.2 Sarcolemma and Sarcomeres

A major part of the sarcolemma is the sarcoplasmic reticulum (SR). The SR contains calcium pumps, and stores Ca^{+2}. In a relaxed muscle, there is a high concentration of calcium in the sarcoplasmic reticulum and a low concentration in the sarcoplasm (Fox, 2009). If an electrical signal is sent through the sarcolemma to the sarcoplasmic reticulum, the calcium will diffuse out of the reticulum and into the sarcoplasm. These signals travel along transverse tubules, which are holes in the sarcolemma. The tubules weave around the myofibrils and release on the other side (Fox, 2009).

Each muscle cell is made of multiple subunits known as myofibrils. Each myofibril is approximately 1 micrometer in diameter, and they extend in parallel rows from one end of the muscle cell to the other. Myofibrils are composed of thick and thin myofilaments. Thick myofilaments are made mostly of myosin and are approximately 110 angstroms thick, thin myofilaments are made mostly of actin and are approximately 50 angstroms thick (Fox, 2009).

The overlapping of myofilaments gives the muscle a striated appearance that can be viewed by light microscopy. These striations appear as alternating light I-bands and dark A-bands. I-bands are comprised mostly of thin filaments and A-bands are comprised mostly of thick filaments (Fox, 2009). The thin filaments in the I-band extend partially into the A-band, where they overlap and form a central region known as an H-band. In the center of each I-band is the Z-line. The Z-line defines the boundary between the two bands. The subunits in between two

Z-bands are called sarcomeres. Protein filaments at the center of the thick filaments produce M-lines. M-lines anchor thick filaments and keep them together during muscle contraction. The sarcomere also contains titin, an elastic protein that runs through thick filaments from M-lines to Z-line.
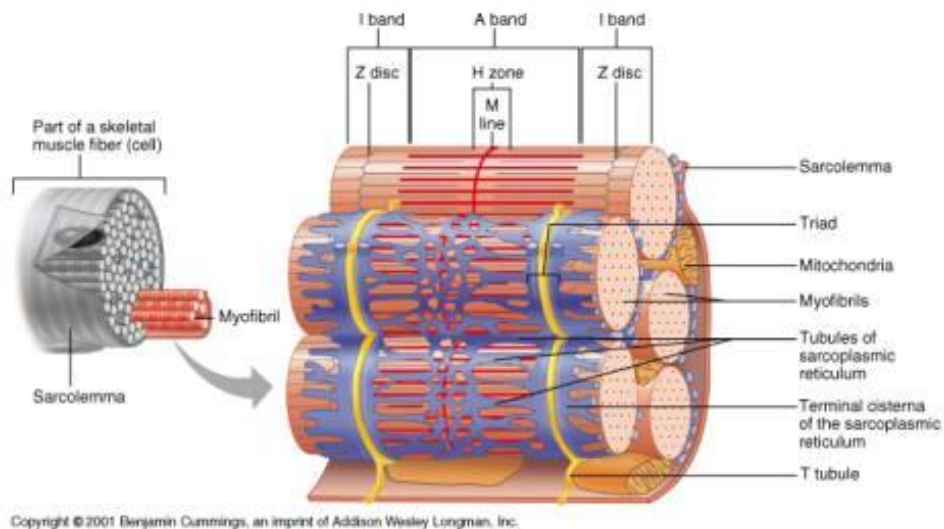
Figure 2: Muscle Striations

2.1.3 Muscle Contraction

When a muscle contracts, the individual fibers decrease in length, caused by the shortening of the smaller myofibrils. During contraction, the Z-lines in the sarcolemma move to overlap each other. Although the myofibrils decrease in length during contraction, the thick and thin filaments do not. When sarcomeres shorten, the thick and thin filaments slide and overlap each other (Fox, 2009).

Myosin is the protein that makes up thick myofilaments. It has a tail that forms the body and a head that extends from the body towards the actin. The myosin heads, cross bridges, are orientated so that they can pull the actin in the opposite direction to cause contraction. When muscles are at rest, the myosin heads are not connected to the actin. This allows muscles to stretch easily. Each myosin head has an ATP-binding site and an actin-binding site. These sites

allow the head to function as a myosin ATPase enzyme, which splits incoming ATP into P and ADP (Fox, 2009). When ATP is hydrolyzed by the enzyme, the myosin head shifts its configuration, which gives it the energy that it needs to allow contraction. This shift binds the myosin head to the actin and releases one of the phosphates from the hydrolyzed ATP. This produces power stroke that causes the thin filaments to slide. After the power stroke, the ADP is released, and the myosin head detaches from the actin. Another ATP molecule then binds to the ATPase enzyme and the cycle repeats. A single power stroke pulls the actin filaments about 6 nanometers, and the combination of all the power strokes at once shortens the muscle by about 1%. Muscles can shorten by about 60%, so the entire power stroke cycle must be repeated multiple times (Fox, 2009).

2.1.4 Tropomyosin and Ca²+ in Muscle Contraction

In order to cease muscle contraction, the attachment of cross bridges between actin and myosin must be stopped and blocked. The actin filament F-actin is a polymer composed of multiple G-actin subunits that are arranged in a helical formation. The protein tropomyosin is situated between the grooves of the G-actin. Attached to the tropomyosin is the protein troponin. Together, troponin and tropomyosin regulate the binding of cross bridges (Fox, 2009).

When the Ca^2+ concentration rises in the sarcoplasm, some of it attaches to troponin. This attachment causes the troponin complex and tropomyosin to move out of the way so that the cross bridges can begin to attach.
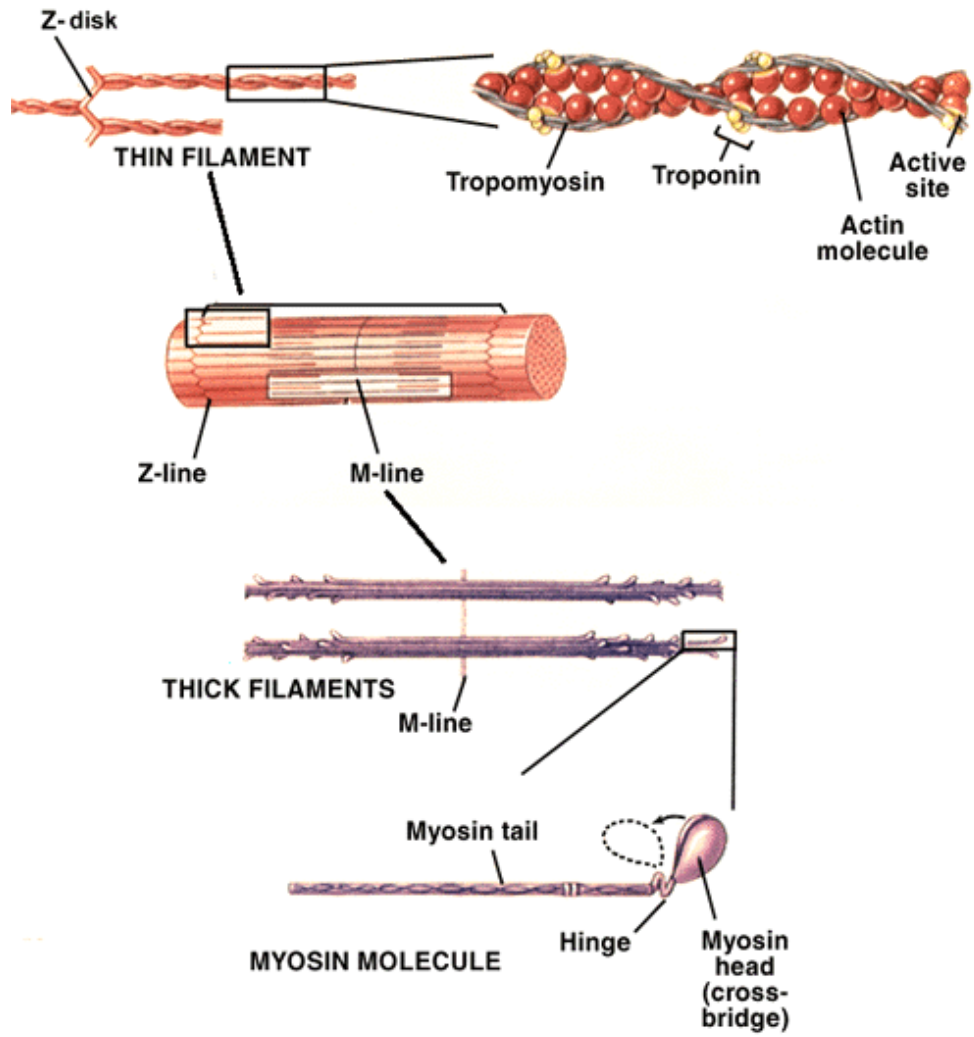
Figure 3: Myosin Molecule

2.1.5 Muscle Structure of a Rodent Leg
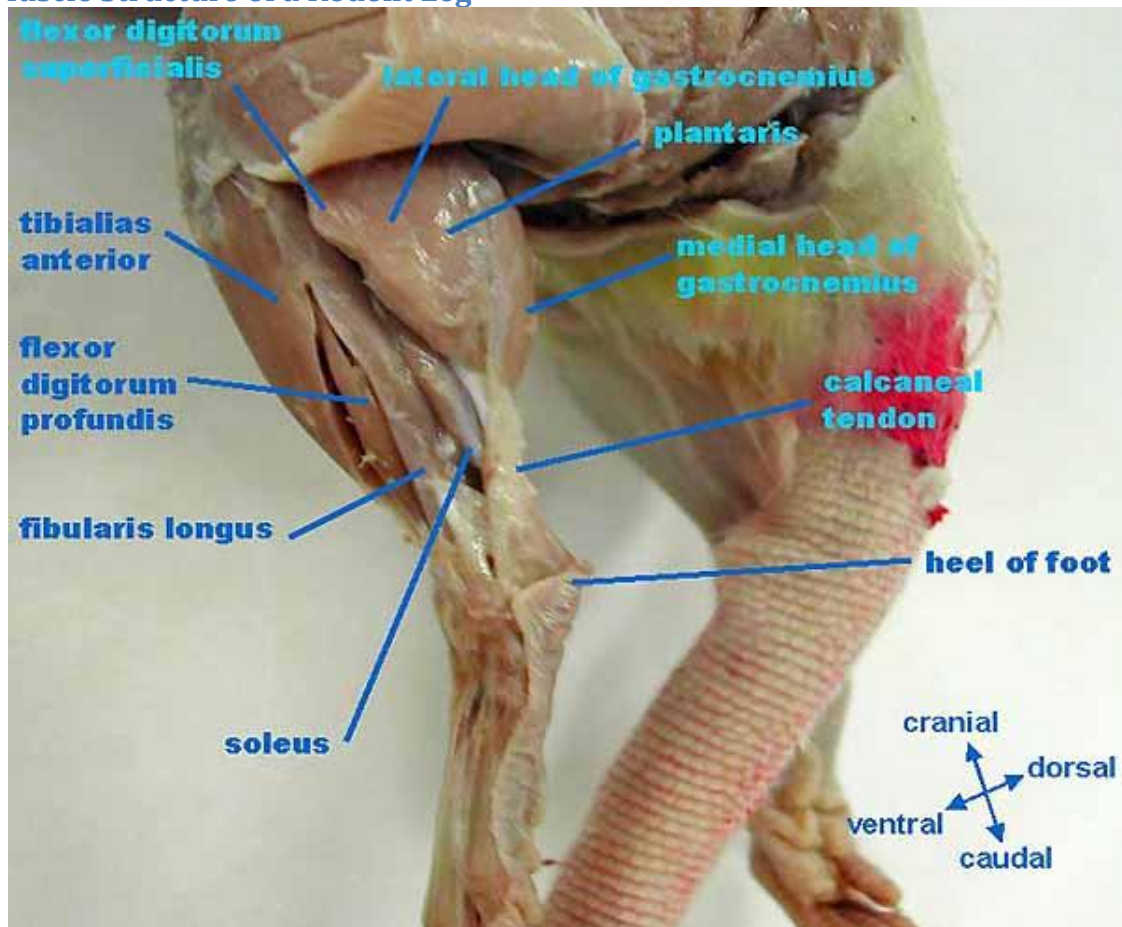
Figure 4: Rat Muscles of the Left Leg, Lateral View

In figure 4, a dissected view of the left leg of a rat is presented. In the design of a fixture to measure contractile force, the tibialias anterior will be stimulated and force will be measured.

2.2 Skeletal Muscle Injury

The repair of skeletal muscle is an essential function of the body. To understand how skeletal muscle is repaired it is important to understand how an injury is incurred. There are

several features that characterize injury, they include: loss of muscle function, altered morphology noticed with or without a microscope, altered intracellular protein levels and localization, and the loss of intracellular muscle proteins (Tiidus, P.M. 2003). Tiidus defines muscle injury as "the loss of muscle function caused by the physical disruption of muscle structures involved in producing or transmitting force". In high-force eccentric contractions several changes occur; these include the disruption of sarcomeres, disruption of cytoskeletal elements responsible for force transmission, damage to the muscle cell membrane, impaired excitation-contraction coupling and a loss of overall force production. With this type of muscle damage, the muscle can be repaired back to the original state where evidence of the injury is undetectable. With contraction-induced injury, the muscle has been conditioned to previous contractions and therefore can recover more rapidly after its initial injury. It has been shown that muscle tension and muscle length are important factors when determining the injury induced by contraction.

2.2.1 Natural Skeletal Muscle Repair

Muscle may become damaged due to mechanical trauma, or exposure to toxins or infections. It is essential for an organism's survival to quickly repair the damaged tissue. The disturbance of muscle regeneration may lead to more decline of muscle tissue, inflammation, or fibrosis. After an injury is incurred in the skeletal muscle tissue, cytokines and growth factors are released from the injured blood vessels and the inflammatory cells. This causes an increase in inflammatory cells at the site of injury and control cell survival and proliferation. At this point, phagocytosis will occur if there is any cell debris in the site of injury. New muscle fibers are formed by the proliferation and differentiation of satellite cells. Until injury occurs, satellite cells are quiescent beneath the basil lamina and reside immediately outside the sarcolemma. Once an injury is incurred the satellite cells activate and begin to proliferate to replete lost myonuclei and

eventually fuse with damaged myotubes. The satellite cells will differentiate and mature to form new myotubes (Parker, et al., 2003). The transcriptional factors PAX3 and PAX7 are known to be satellite cell markers, however the dynamics of activation from quiescence to induction remains unknown. Some research suggests that the protein myostatin negatively regulates satellite cells (McCroskery, et al., 2003).

Another key component to muscle repair is fibroblasts. The fibroblasts form the extracellular matrix (ECM), which serves as a scaffold to help stabilize new muscle fibers as they form. Basement membranes as well as the temporary ECM are essential in forming new neuromuscular junctions. The ECM will then be degraded once its task is complete; proteases and specific inhibitors control this process. The degradation of the ECM contributes important protein fragments that are essential in facilitating normal tissue repair. Lastly, while new muscle fibers grow and mature, the vascular network is developed.

2.2.2 Fibrosis

Fibrosis is muscular scar formation, which can occur after skeletal muscle tissue injury. Fibrosis is the result of an excessive accumulation of ECM components, usually collagen. The scar formation can be detrimental to the muscle by impairing tissue function and possibly causing disease in many vital organs and tissues. Fibrosis can occur in many different muscle types but there are several common factors that can occur, such as: cell and tissue degradation, leukocyte inflammation, chronic inflammation of the tissue, and excess build-up of collagen tissue. Because of this, the microenvironment of the tissue is disturbed and connective tissue will constantly remodel, destroy, and replace the normal tissue (Mann, et al., 2011).

2.2.3 Dystrophy

Muscle fibrosis is most often associated with muscular dystrophy. Muscular dystrophy is a group of diseases characterized by skeletal-muscle inflammation and skeletal-muscle wasting.

In many cases the disease is caused by a mutation that affects the protein links between the cytoskeleton and the basal lamina. The sarcolemma then becomes very fragile, especially during intense contractions. This causes damage to the fibers due to an increased entry of calcium ions (Mann, et al., 2011). Myosatellite cells are known to contribute to regenerated muscle cells. However, in muscular dystrophy the satellite cell population is diminished over time, or the cells can lose their capabilities to repair tissue. The result of this is a buildup of adipose and fibrotic tissue. Currently, the only effective treatment of severe dystrophy is the injection of corticosteroids. However this leads to unwanted side-effects, such as: irritability, weight gain, and cushingoid symptoms, which is a hormonal disease. As a result there is not an effective treatment for fibrosis associated with muscular dystrophy (Angelini, 2007).

2.2.4 Aging

Sarcopenia is muscle tissue loss, natural fibrosis, and ECM deposition due to aging. The cause of sarcopenia can be changes in hormone status, inflammation, and changes in caloric and protein intake. The effects of sarcopenia are continued atrophy of muscle tissue and loss of individual muscle fibers. The decrease in muscle mass allows for infiltration of adipose tissue and collagen into muscle tissues (Mann, et al., 2011).

2.2.5 Assisted Skeletal Muscle Repair

Finding ways to assist skeletal muscle in repairing itself is the subject of much research effort. The following section will describe a few of the techniques that are currently being studied. The skeletal muscle was given a severe injury by both myotoxin-mediated direct damage and regional ischemia. The scaffold contained VEGF and IGF-1 and was able to deliver these factors locally. The scaffold was able to transplant and disperse the cultured myogenic cells, enhance their engraftment, limit fibrosis, and ultimately accelerate muscle regeneration. The VEGF/IGF-1 drastically increased the extent of muscle regeneration, due to the formation of new

blood vessels, and the return to normal tissue perfusion levels. Afterwards the muscle increased in mass and showed improved contractile function (Borselli, C. 2011).

2.2.6 Stem Cells and Skeletal Muscle Regeneration

The muscle stem cell (MuSC) represents another cell type that contributes to muscle regeneration, along with satellite cells. It is believed that cells from the circulation and vasculature give rise to MuSCs, which have the ability to differentiate into skeletal muscle fibers. Human synovial stem cells are a type of stem cell that is responsible for regeneration of muscle fibers and reconstituting the satellite cell pool. These stem cells have been shown have a small effect on the regeneration of skeletal muscle after being injected into cryodamaged muscles in mice. They could play a role in treating muscular dystrophies and defects in extracellular matrix proteins (Meng, J. 2010).

2.3 Current Methods for Stimulating and Measuring Skeletal Muscle *In-Vivo*

Traditionally to stimulate the skeletal muscle tissue of a rodent the process would call for a very invasive procedure which would include the total dissection of the muscle tissue from the bone to isolate the peroneal or tibial nerve for stimulation. Often the rodents that were being used for such experiments would either already be dead or so much damage would be dealt to the native tissue that a second procedure would be impractical. Variations of these experiments would also include the complete removal of the skeletal tissue from the animal so that it may be harnessed at both ends in order for stimulation and contractile strength measurements to be made. It is clear from the brief descriptions of these procedures that they are quite invasive and may not be particularly accurate.

Scientists now are interested in discovering the full contractile force of skeletal muscle while it is still attached to a living organism. A non-invasive *in-vivo* procedure would be far more accurate as to ascertaining the total amount of contractile force the muscle is capable of

generating. This method would also supply the opportunity to do repeat testing on an individual animal so that multiple measurements can be taken. This proves especially valuable if therapeutic applications are to be tested on damaged skeletal muscle tissue. As of now there is no standard means to non-invasively stimulate and measure skeletal muscle force *in-vivo*. However, researchers have begun to create their own devices in order to accomplish this task.

Current methods include using an isometric torque sensor to measure in situ contractions of plantar or dorsal flexors of intact mouse hindlimb via measuring muscle torque (Gorselink et al., 1999). The second method involves an apparatus that quantifies the biomechanical behavior of the dorsi- and plantarflexor muscles of the ankle, by measuring movement of the ankle during isometric, isovelocity shortening, or isovelocity lengthening contractions after stimulation (Ashton-Miller et al., 1992). The third and last of the current methods involves the use of a dynamometer to measure the force output of the plantar flexor muscles during stimulation (Cutlip et al., 1997; Willems and Stauber, 1999). As this section progresses a more detailed account of each of these methods will be given so that a general understanding of what we hope to accomplish with our device.

2.3.1 Isometric Torque Sensor

As mentioned above, this method involves the development of an isometric torque sensor that measures the *in situ* contractions of the plantar or dorsal flexors of intact mouse hindlimb to measure the muscle torque during stimulation. Hindlimb fixation was key in this model due to the fact that it allowed for the gathering of accurate measurements during stimulation. Mice were fixated to a thermostatic measurement platform via a hip and foot fixation system. A schematic of the device that used by Gorselink et al. is shown.
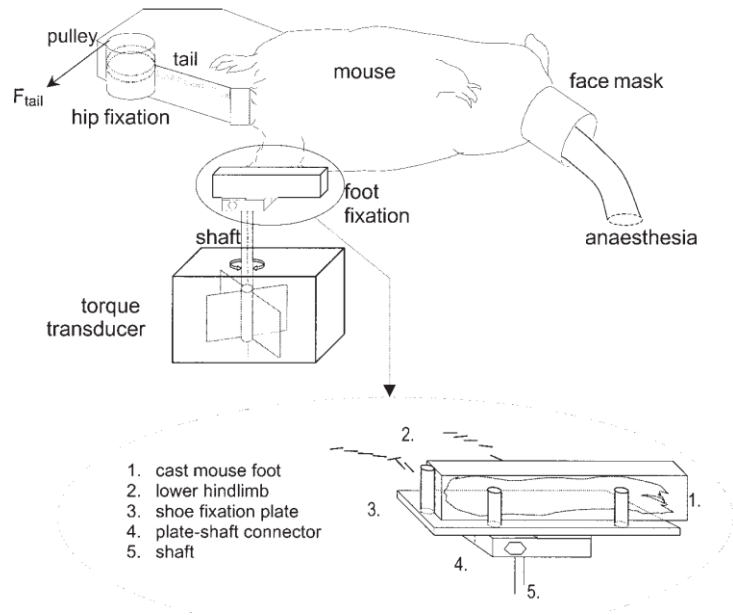
Figure 5: Schematic for Measuring Torque of Dorsal and Plantar Muscle

Once the mouse had been secured properly in the apparatus, the device would measure the knee and ankle displacements during a contraction. In order to stimulate a contraction a piece of skin was removed to allow a small incision in the hollow or the lateral part of the knee to make access to the tibial or peroneal nerve. Once the nerve was exposed a bipolar platinum hook electrode was attached to it allowing a pulse generator to stimulate the muscle complex. After data was gathered a mathematical muscle model was used to calculate the *in situ* measurements of isometric contractions of intact dorsal and plantar muscle complexes and it was found that these measurements are reliable assessments of the contraction parameters set forth by the ankle flexors of mice (Groselink et al., 1999).

2.3.2 Measurement of Biomechanical Contractile Force

The second method described is considered to be one of the initial developments in the field of measuring skeletal muscle *in-vivo*. In this method an apparatus was designed and developed to quantify the biomechanical behavior of the dorsal and plantar flexor muscles of the ankle of a mouse and compare those findings to that of invasive or in situ findings. The

anesthetized rat was placed on either its right or left side to test its right or left ankle respectively. The femoral condyle was secured to a platform using screw clamps making sure not to compromise the musculature of the leg. Once the knee was fixated, the foot was placed into the shoe plate for alignment. With the leg fixated, needle electrodes were inserted through the skin and placed on either side of the peroneal or tibial nerve to stimulate the dorsiflexor skeletal muscle complex. An outline of the device is shown.
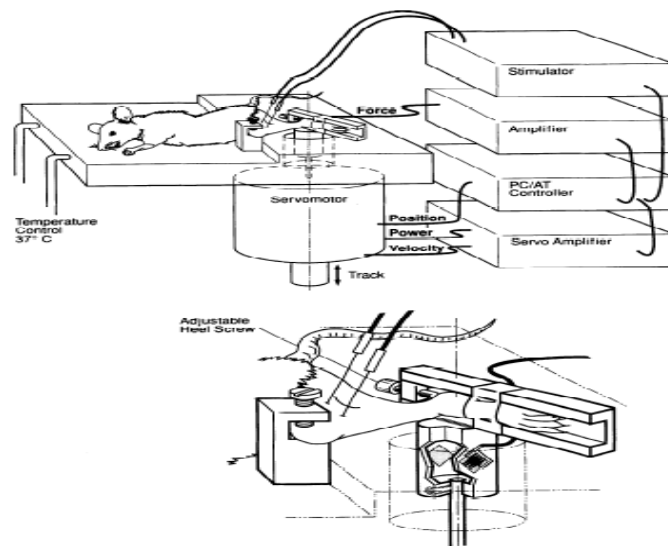
Figure 6: (Top) Layout of Entire Device, (bottom) close-up view of the knee and ankle fixation

With the mouse fixated and undergoing stimulation the device would then enable measurement of the moment development about the ankle joint during isometric, isovelocity shortening, or isovelocity lengthening contractions of the muscle. By measuring the isometric tetanic (maximum) force, power output, and power absorption it was determined that the data corresponded to data from in situ procedures and therefore it was concluded that the device was a valid way to measure the force and power of the dorsal and plantar flexor muscle complexes *in-vivo* (Ashton-miller et al., 1992).

2.3.3 Dynamometer Testing

The third method for stimulating and measuring the contractile force of skeletal muscle *in-vivo* is by using a device called a dynamometer. Several researchers including (Cutlip et al., 1997) and (Willems and Stauber, 1999) have used dynamometers to conduct their testing. A dynamometer is designed to measure the force output during static and dynamic actions of the plantar flexor muscles. The system in Cutlip et al., is ran by a computer controlled DC servomotor that adjusts the range of motion, angular velocity, and electrical stimulation of the plantar muscle complex, all the while keeping track of the force output at the plantar surface of the foot. An animal positioning platform was fabricated to hold both the piezo electric load cell which measured the force output of the stimulated muscle as well as fixate the foot of the rat while providing holding clamps to fixate the knee. A diagram of the device with a mouse foot inserted can be seen below.
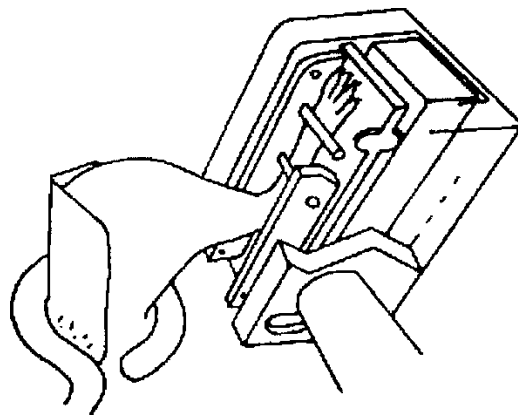
Figure 7: Position of Mouse Foot in Load Cell Fixture with Knee Fixation

Electrical stimulation was achieved by the placement of platinum needle electrodes or implanted nerve-cuff electrodes. Willems and Stauber specifically used bipolar cuff electrodes which require a mid-line incision in the posterior aspect of the hindlimb to allow blunt dissection until the tibial nerve was exposed. The connective tissue and adipose tissue surrounding the

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nerve was removed and the common peroneal/sural nerves were cut to allow for the bipolar cuff electrode to be placed around the tibial nerve.

Once the electrodes were placed, the stimulation was controlled by a computer program and could be turned on and off as a function of either time or position of the load cell. The force output was measured by a piezo-electric load cell while angular velocity and position were measured by a DC tachometer and potentiometer, respectively. These instruments allowed for an accurate and reliable system that was able to measure static and dynamic forces *in-vivo* of a rodent plantar flexor muscle complex.

2.4 Clinical Motivation

Skeletal muscle controls voluntary movement, protects internal organs, and is the most abundant muscle in the body. While it can sometimes regenerate under certain conditions it does not. Physical injury can be too traumatic for full healing (Stern-Straeter, 2007), when the muscle is too damaged to repair itself. Congenital defects can also cause a lack of muscle growth (Stern-Straeter, 2007). Compartment syndrome is a condition in which the pressure from swelling or bleeding within the muscle cuts off blood supply to the muscle resulting in nerve and muscle tissue death. Rhabdomyosarcoma, cancer of the muscle, can be surgically removed, but the procedure is invasive enough to cause tissue damage which cannot be healed naturally. About 350 cases of Rhabdomyosarcoma occur annually in the United States.

Recently new techniques have been discovered which show promise to regenerate muscle tissue. These include cell transplantation and tissue engineered skeletal muscle constructs. These therapies can theoretically do numerous things, including regenerate lost skeletal muscle tissue. While tissue regeneration can have great benefits for humans with skeletal muscle damage, animals trials are performed to test these technologies on living systems first. Because mice are inexpensive, small, and share similar physiological and cell biological properties with humans

they have become a widely used in *in-vivo* testing (Willis-Owen, 2006), including the testing of

skeletal muscle regeneration.

Non-invasive detailed testing of skeletal muscle healing progress over time can be

difficult. Skeletal muscle regeneration in often tested via the force the muscle is able to produce.

As the muscle must be electrically stimulated and the joint isolated from movement from the rest

of the body, even in the least invasive methods the mouse subject is to surgery requiring

additional tissue damage to permit repeat testing. This is problematic when tracking the

progression of muscle regeneration as a function of time, as variation in force between different

mice or different limbs of mice can be difficult to control for. Creating a methodology and

apparatus to test the contractile skeletal muscle force in mice can improve testing of tissue

regeneration treatments in mice, and can expedite to the translation of regenerative therapies for

human skeletal muscle.

2.5 Project Goals and Strategy

The goal of this project is to design a fixture that will allow for the non-invasive

measurement of the contractile force in the tibialias anterior of a mouse through topical electrical

stimulation without requiring the muscle to be detached from the bone. To realize this design a

base fixture must be created, as well as a means to stimulate the skeletal muscle, immobilize the

necessary parts of the mouse, and obtain accurate data for the contractile forces measured. This

chapter was written to explain design process and how the team utilized it to create objectives,

functions, means, and constraints in order to make the final product design.

3. The Design Process

One of the most integral aspects of engineering is the design process. The goal of any

design process is to create a finalized product in a way that is safe, cost effective, useful, and

satisfactory to the client. Without any kind of process, the creation of any kind of complex product would be impossible. By utilizing a series of design tools, such as pairwise comparison charts, Gantt charts, lists of objectives, means, functions, and constraints, a team can create a well thought out, well documented plan of action to create any product. The following section describes in detail the process of selecting and pruning objectives, functions, means, and constraints, revising the client statement, and then weighting each objective against the others using a series of sub objectives and pairwise comparison charts.

In order for a design process to be effective, a team must recognize who the client is and learn exactly what they want and expect. In any design, there are stakeholders. In this particular case, the stakeholders are the team designing the project, the final users of the product, and the client who expressed a need for the product. The client for this particular design process is Professor Raymond Page. At the beginning of the project, Professor Page provided an initial client statement to lay out a general foundation of what the product must be able to accomplish. In this statement, current strategies for assessment of skeletal muscle repair and the pros and cons of each method were discussed. Most methods for assessing skeletal muscle repair are very invasive, so one of the main goals of the final design is to be non-invasive and repeatable on the same muscle. The potential users of the final product would be students and faculty of WPI doing research on skeletal muscle regeneration. The design team, made up of Bryan Choate, Gregory Gonzalez, Dylan Pinnette, , and Jirom Yibrah, plan to understand the desires of the client and create a final product that meets all objectives and satisfies the client statement.

3.2 Initial Client Statement

After receiving the initial client statement, steps must be taken to fully understand exactly what the client wants. This was achieved by both asking questions of the client, Professor Page,

and by using pairwise comparison charts and pruned objectives to decide exactly what the

product should do. The initial client statement as follows:

"Current strategies for assessment of skeletal muscle repair or regeneration rely on histological examination of injured/treated tissue and/or contractile force measurements of dissected muscle fibers or whole muscle placed in organ culture systems. These techniques do not enable the direct quantitative assessment of the degree of injury originally inflicted nor the recovery due to intervention therapies such as cell transplantation or tissue engineered cell/tissue constructs. Methods have been developed to measure the contractile force exerted by partially dissected muscle where the distal portion is removed from the tendon and bone and attached to a force transducer by ligature. While this method can very accurately determine the force of contraction due to electrical stimulation of the innervating nerve, and with the circulation intact if performed under anesthesia, repeated measurements on the same animal are not permitted due to the invasiveness of the procedure required to isolate the muscle and nerve. While contralateral muscle comparisons might offer a solution to the problem of obtaining contemporary comparative force measurements, for example at the time of animal sacrifice, our preliminary observations suggest that as much as a 30% difference in contractile force can be measured between uninjured (control) contralateral tibialis anterior muscles in the mouse. Therefore, the goal of this project is to develop a device and methodology to acquire comparative functional measurements that can be used to quantify the initial (uninjured) force generated by muscle contractions, the reduction in force due to injury, and the recovery of force due to treatment at selected time-points during the recovery/regeneration process. This method involves topical electrical stimulation of the muscle at defined locations using customized bipolar electrodes and non-invasive fixation of attachments points to measure force generated by the muscle. Therefore, the material of construction and anchor points for the limb must cause minimal tissue damage or edema during the procedure. This procedure must be applicable under general anesthesia and requires only exposure of the muscle surface by removing a skin flap and intervening fascia which can be replaced surgically yielding complete recovery. Furthermore, the device must be built onto a platform suitable for stereomicroscopic examination of the surgical procedure."

The initial client statement was rather long and non-specific, which makes it much more

difficult to design a product around. Therefore, in order to fully understand what specific

requirements the product must meet, the client statement must be condensed into a much simpler,

more concise form. This was achieved through asking the client questions during meetings, as

well as discussion among the team about which functions and objectives were the most

important.

3.3 Objectives, constraints, and functions

Once design goals are established, objectives and constraints are listed along with a list of possible functions. These functions must be within the parameters of the design objectives and constraints. Simply put functions are actions that a successful design must perform. Constraints are strict limits that a design must meet for it to be acceptable. Finally objectives are desired attributes and behaviors of a design.

Objectives:
- Create inexpensive device.
- Establish total noninvasive fixation of mouse limb.
- Noninvasively stimulate skeletal muscle complex of mouse limb electrically.
- Measure and accurately quantify strength of muscle contractions.
- User friendly
- Cleanable and Reversible
- Safe

Constraints:
- Must be built to bench top for use with stereomicroscope
- Not damage knee
- Must be non-invasive or minimally invasive not resulting in damage of muscle /tendon
- Applicable under general anesthesia
- Limited budget
- Completed in 25 weeks (Ideally)

Functions:
- Fixate knee of mouse
- Stimulate skeletal muscle
- Measure force generated by muscle contractions
  - Pre Injury, post injury, at selected time points during recover/regeneration process

3.4 Pruned Objectives

After creating a comprehensive list of objectives, constraints, and functions, it became clear to the design team that this list must be reorganized and more concise. With the help of the client, the design team was able to prune the objectives list into six main objectives with sub categories for each. The six main objectives were:

1. Cost

2. Fixation

3. Electrical Stimulation

4. Accurately Quantify Strength of Isometric Muscle Contractions

5. User Friendly

6. Cleanable and Reversible

Cost

- Relatively inexpensive

Possibly the easiest objective to decide on was the cost being relatively inexpensive. Each group member is budgeted $156 that will be reimbursed by the WPI Biomedical Engineering Department, there are four group members so the maximum cost was set to be less than $624. It is important to note that $100 automatically goes towards lab supplies, so the cost of materials other than lab supplies has to be less than $524.

Fixation

- Adjustable to fit different sized mice limbs
- Consistent positioning of attachment site for force transducer
- Allow for electrical stimulation without tissue damage
- Minimize invasiveness (no damage to ligaments or muscles)
- Allow for multiple testing [Repeatability]

Knee joint fixation is the central concept of this project; the design team generated several sub objectives for the fixation component. The knee fixation must be flexible and adjustable so that mice of different size can fit onto it, but rigid enough not to deflect contractile force. It must also have consistent positioning so that data from different tests may be compared with each other. The device must be reusable or have replaceable components. Possibly the most important sub objective for fixation was that the device must be minimally invasive so that the

muscle or surrounding tissue is not permanently damaged, and must allow for electrical

stimulation.

Electrical stimulation

- Consistent electrical output
- Consistent electrode placement
- Maximize muscle stimulation

The design team determined that the electrical stimulation must be consistent in the

output and placement. These two sub objectives are very important if the results of multiple tests

are to be compared. The stimulation must also maximize the muscle contractions, by doing so

different tests can be examined with the knowledge that the muscle was fully stimulated in all

tests.

Accurately quantify muscle strength

- Pre damage
- Post damage
- During recovery at defined time points

If multiple tests are to be analyzed the device must permit accurate and reproducible

quantification of muscle force. The muscle force data must be able to be collected pre damage,

post damage, and at selected time points during recovery.

User friendly

- Ease of use
- Safety
    - Streamline design
- Cleaning
- Minimal procedure time

Making the device user friendly was an important objective to the design team so that

users would not require excessive training or experience to handle the device. Keeping the user

and animal safe was critical to the design team, if the animal were to be injured it would defeat

the purpose of the device, if the user were to be hurt then the procedure would be halted. To ensure safety to the user the device must have a streamline design without sharp corners that could injure the user. The streamline design must allow for easy cleaning so that the device can be used repetitively without risk of infection to the animal and user. Making the procedure time minimal was an important as well, since the animal is sedated the procedure time must not exceed the time that the animal is unconscious.

3.5 Qualitative Analysis of Objectives

Pairwise comparison charts allow for the comparison of the importance of different design objectives. When designing a device, alternative designs can have different advantages, with whichever can best fulfill different goals of the project. By ranking objectives, alternative designs can be weighed by how much they seem to meet the different objectives of the project. Pairwise comparison charts are a simple way to rank all the objectives and sub-objectives against each other, to find their relative importance to one another.

For the main objectives, and each grouping of sub objectives, every objective was compared against every other objective. If the objective was deemed of greater importance to the objective it was compared against, it was given a score of 1, while if the objective was deemed of lesser importance, it was given a value of 0. A score of scores of .5 was given when an objective was compared with another of equal importance. The scores of each objective were totaled, and from those scores the objectives were ranked, with a ranking of 1 indicating the objective was of the greatest importance among the group.

Main Objectives	1	2	3	4	5	Total	Ranking
1. Cost	■	0	0	0	0	0	5
2. Ideal Fixation	1	■	0.5	1	1	3.5	1
3. Ideal Electrical Stimulation	1	0.5	■	1	1	3.5	1

						Total	Ranking
4. Accurately quantify muscle contractions	1	0	0	■	1	2	2
5. User Friendly	1	0	0	0	■	1	4

As the pairwise comparison chart above shows, some objectives were deemed more important than others. Ideal fixation and ideal electrical stimulation were the objectives deemed most important and held the greatest exigency for innovation. Accurate quantification of the muscle contractions was ranked equally with them because of how importance it was to the verification of the design. Of the other objectives, a user-friendly design ranked next, followed by minimal procedure time, and minimal cost. The objective of a streamlined design ranked last.

2. Ideal Fixation	A	B	C	D	E	Total	Ranking
A. Adjustable	■	0	0	0	0	0	5
B. Consistent Positioning	1	■	0.5	0.5	0	2	3
C. Allows for Electrical Stimulation	1	0.5	■	1	1	3.5	1
D. Minimum Invasiveness	1	0.5	0	■	1	2.5	2
E. Allows for Multiple Tests	1	0	0	1	■	2	3

For the sub-objectives of ideal fixation, having a fixation device which allows for proper electrical stimulation was deemed most important, followed by minimizes the invasiveness of the procedure. Allowance for multiple tests was ranked equally with consistent positioning, while the adjustability of the device ranked last.

3. Electrical Stimulation Sub Objective PCC	A	B	C	D	E	Total	Ranking
A. Consistent Electrode Placement	1	■	0	0.5	0.5	2	4
B. Maximum Muscle Stimulation	1	1	■	1	1	4	1
C. Flexible Electrode	1	0.5	1	■	0	2.5	2
D. Electrode doesn't puncture tissue	1	0.5	0	1	■	2.5	2

For the sub objectives of Ideal electrical stimulation, allowance for the maximum amount of muscle stimulation was ranked first. Having an electrode which is flexible and doesn't

puncture the tissue ranked next with equal weight. The least important sub objective was

consistent electrode placement.

4. Accurate Quantification of Muscle Contractions	A	B	C	Total	Ranking
A. Pre Damage Quantification		0	0	0	2
B. Post Damage Quantification	1		0.5	1.5	1
C. Quantification During Recovery	1	0.5		1.5	1

For the sub objectives of quantification of muscle contractions, quantification during

recovery and post damage were given equal weight as the most important, followed by

quantification before damage.

5. User Friendly	A.	B.	C.	E.	Total	Ranking
A. Ease of Use		0	0.5	0	0.5	2
B. Safety	1		1	0	2	1
C. Cleaning	0.5	0		0	0.5	2
E. Minimal Procedure Time	0	0	0		0	3

For the sub objectives of user friendliness, the safety of the device ranked first, following

by the ease of using the device. The minimal procedure time and ease of cleaning the device

were determined to be the least important sub-objectives of equal importance.
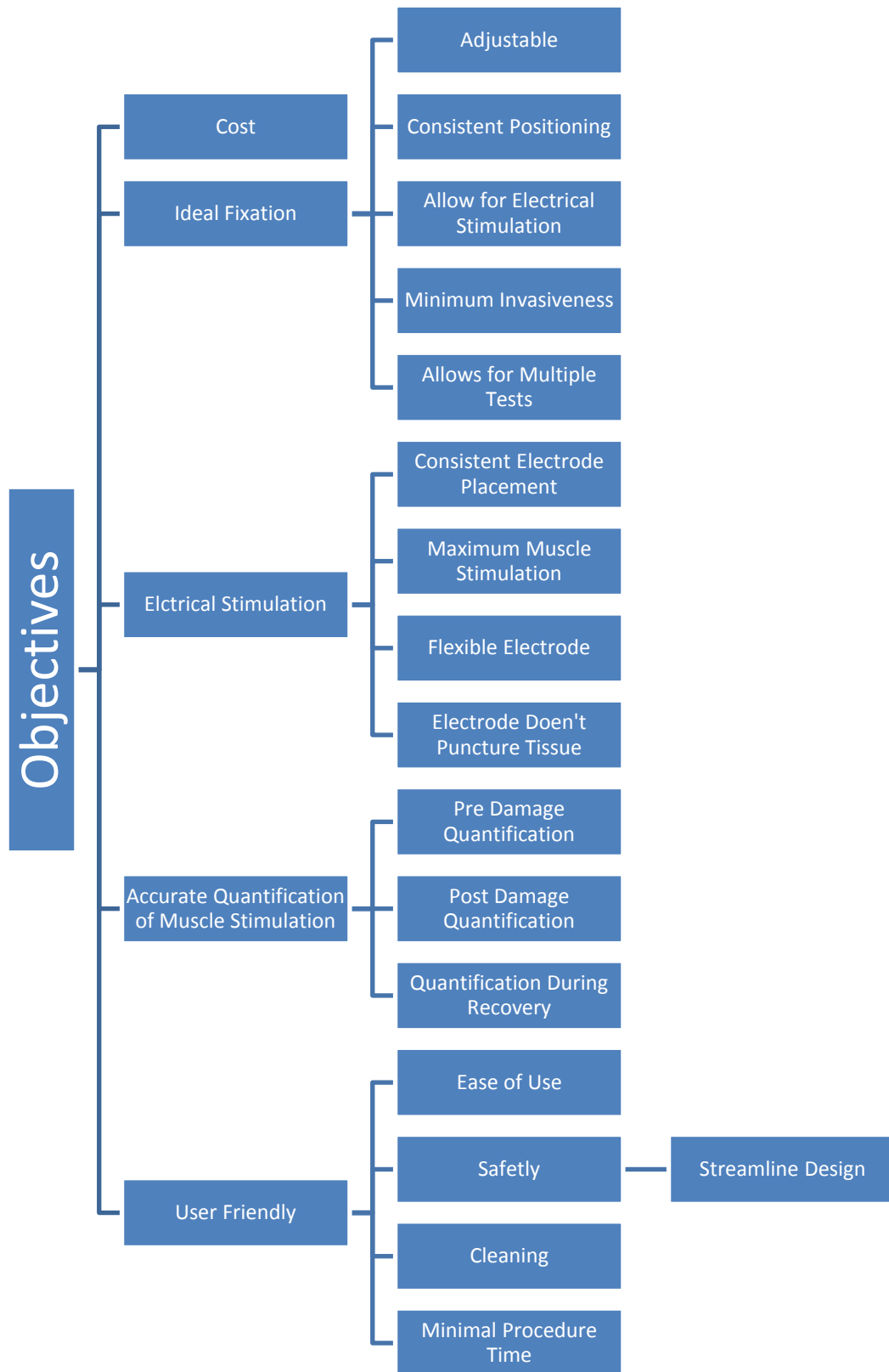
Figure 8: Objectives Tree

3.6 Revised Client Statement

After meeting with the client and user to determine which particular aspects should be incorporated into the device, along with the analysis of the objectives using pairwise comparison chart, the original client statement was revised into a more clear and concise statement which is:

Design a relatively inexpensive, minimally invasive fixation device for the hind limb of a mouse that uses topical electrical stimulation of skeletal muscle to accurately and repeatedly quantify the force generated by muscle contractions.

3.7 Project Approach

The group developed a three step process that would fixate the knee joint of the mouse effectively with minimal tissue damage, cause contractions of skeletal muscle tissue via the placement of topical electrodes onto the muscle surface of the mouse, and lastly a transducer that feeds the force output into a computer program that accurately quantifies and analyzes the force generated by the contraction. A representation of the system is shown below.
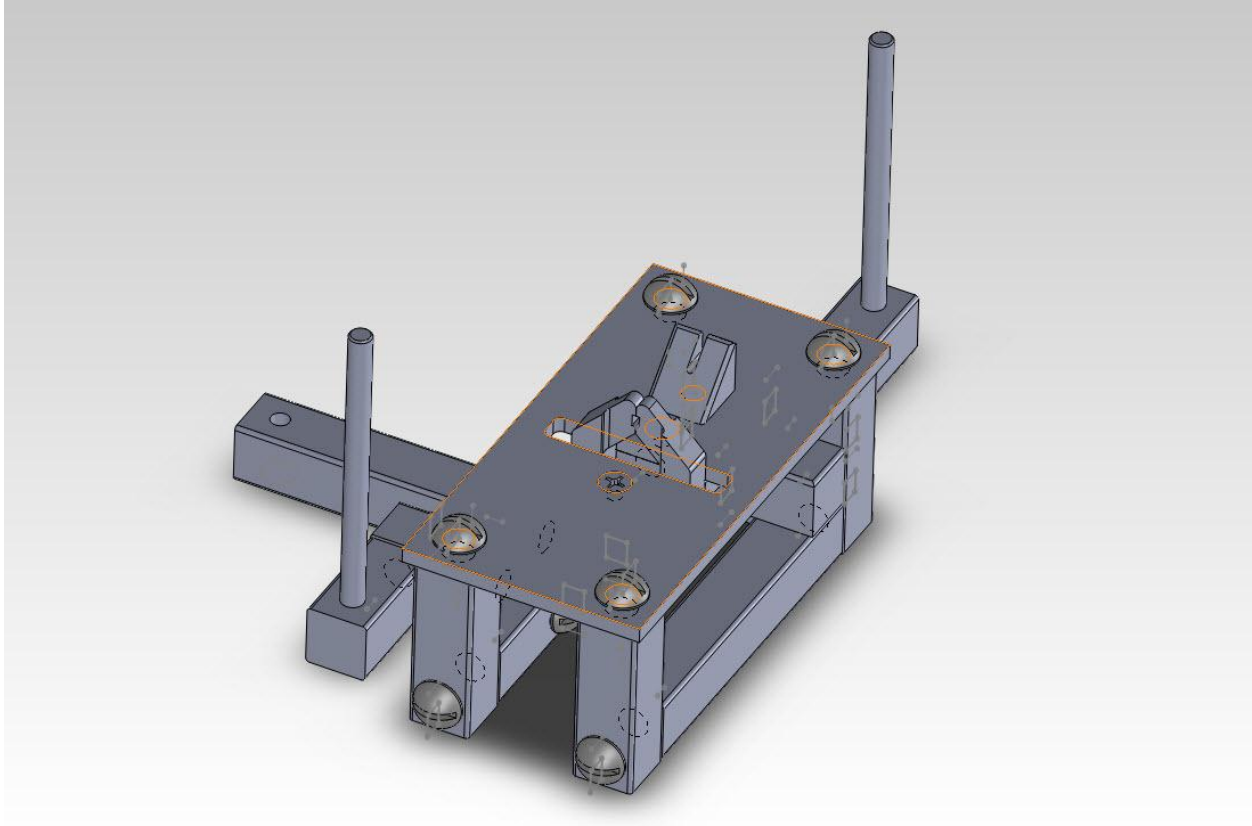
Figure 9: CAD Representation of Design

The main aims that must be achieved for this approach to be effective are:

1. **Total fixation of hind limb of mouse**

   To properly and accurately measure the force generated by the muscle contraction adequate fixation must be achieved. The knee joint of the hind limb was fixated with an adjustable clamp system that can be used to fit any size mouse. The next phase of total fixation was to create a rest platform to allow the same initial starting point for the ankle joint for every test. The third part was a plate to sit directly atop of the ankle joint so that the angle of the foot and leg are consistently the same for every test.

2. **Topical electrical stimulation**

Once the hind limb of the mouse has been completely fixated specially designed topical electrodes will be placed at pre-designated location atop of the tibialis anterior muscle to cause stimulation. The electrodes will be designed with a blunt edge to make sure no penetration of the muscle tissue occurs during tests. These electrodes will be connected to wires that have been placed in a stiff yet flexible wire shielding that allows for adjustability. There will also be a spring-loaded system incorporated into the placement of the electrodes so that they may stay in contact with the muscle even during the strongest contractions.

3. **Force output measurement**

With the muscle stimulated, the force generated will feed into a force transducer which will transmit the readings into a specially designed computer program using MATLAB (Mathworks®, Inc.). AqcKnowledge™ another program will take those readings to interpret and quantify the force generated via the muscle contractions. Measurements will be taken pre-injury, post-injury, and during the recovery/regenerative process to form a comparative analysis of how the strength of skeletal muscle in affected by trauma.

4. Conceptual Designs

After a brainstorming session was held, several alternative designs were conceptualized for aspects fixation component of the device. The following conceptual designs were considered for the final design but ultimately were not sufficient for the final design.

4.1.1 Spring-loaded Fixation System

In the first design much emphasis was placed on the total fixation of the mouse hind limb. The system utilizes several spring-loaded clamps to hold the leg in place during stimulation. The first spring-loaded clamp will fixate the knee joint of the mouse and will be positioned on the base design. The second clamp will be positioned about half way down where

the leg would lay to ensure total fixation. Ideally even during the strongest contractions the spring action in both clamps would be strong enough to keep the leg firmly fixed. Also, guides will be incorporated into the base design for the integration of a stereomicroscope for better observations during usage.

In other areas of the design, there is to be an electrode guide plate that will be placed directly through the leg clamp. Once in position the guide plate will allow for accurate placement and fixation of electrodes when contacting the tibialis anterior muscle of the mouse. The electrodes will be designed and manufactured to have a blunt tip to ensure that no penetration into muscle tissue occurs. Preferably the wires connecting the electrodes to the stimulator will be encased in a stiff yet flexible shielding to allow for the retention of position and shape. Lastly a footrest will be built to a predetermined angle to allow for the resting foot to begin in the same position for each subsequent test.
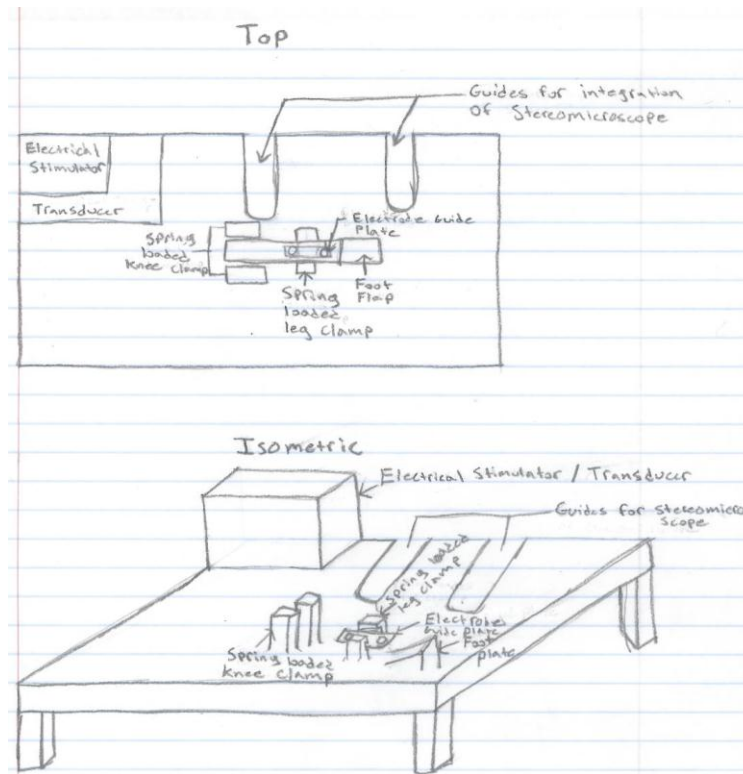
Figure 10: Early concept of the spring-loaded fixation system

Table 1: Table of pros and cons from the early spring-loaded fixation system.

Pros:	Cons:
Total fixation of hind limb	Depending on spring, clamps may cause damage to tissue
Guide and fixation of electrodes	Electrode guide plate may interfere with leg clamp
Guides for attachment of stereomicroscope	
Footrest for consistent initial angle of foot/limb	
Adjustable fixation clamps	
Total fixation of hind limb	

4.1.2 Simple Clamp Fixation system:

In order to properly and accurately measure the force generated by the muscle contraction, adequate fixation must be achieved. The device in figure 11A is meant to lock the knee joint via adjustable clamp. The one-dimensional clamping surface area would be greater

than that of the tibia and femur. The device has ridges at the base of the bar, which indicates that the width may be adjusted depending on the size of the test subject. The ankle joint is fastened to a foot petal, so that the angle of the foot and leg are consistently the same for every test.

Flexible topical electrodes will be placed at pre-designated location atop of the skeletal muscle complex to stimulate the tibialis anterior muscle. The electrodes in figure 11C are flexible to prevent any and all tissue damage. Stainless steel electrodes are considered ideal because of its bendability, flexibility, and electro conductive properties. With the muscle stimulated, the force generated will be transferred into a voltage into the force transducer. The force transducer will be attached to the twine on the foot pedal. Whatever contractions the computer reads will come from the slight movements of the foot pedal, refer to figure 11B.
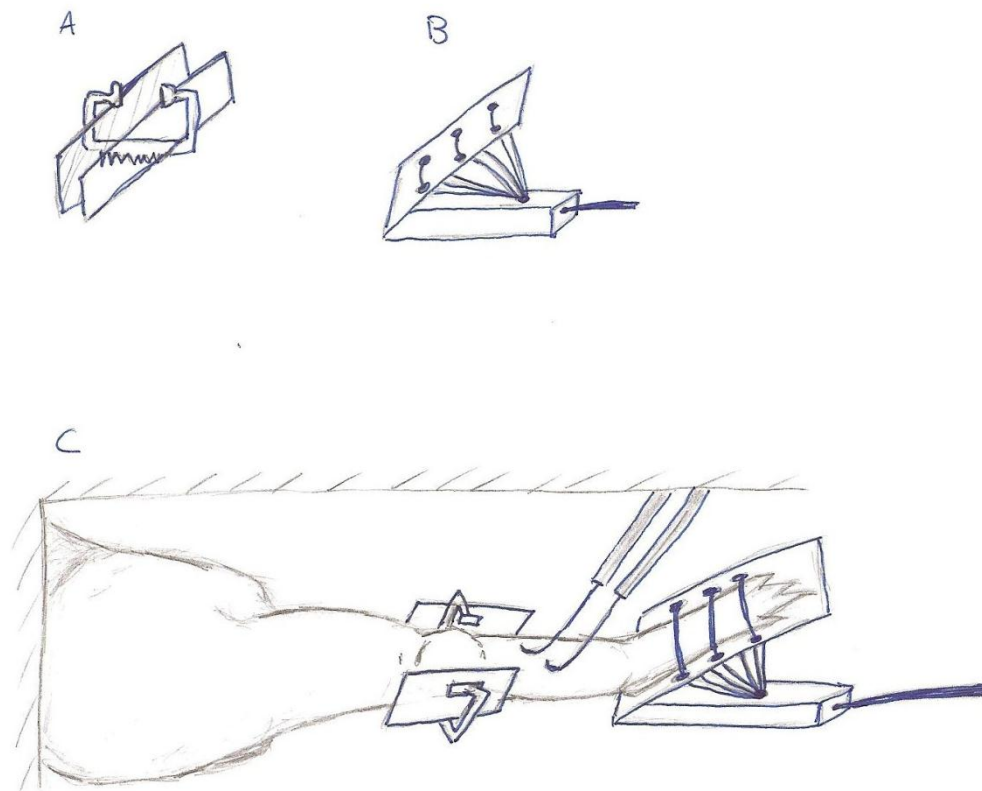

Figure 11: Illustration of (A) knee clamp, (B) foot pedal, (C) the knee clamp and foot pedal fixating a mouse leg with electrodes.

4.2.1 Smooth Clamp Design

The smooth clamp design has a flat surface that grips both ends of the test subject's knee. At the base of this design is a spring base system that keeps the clamp closed. The spring-loaded clamps hold the leg in place during stimulation. An advantage of this concept is its simplistic structure and easy machinability. Ideally the clamps should be able to keep the knee in place under the most forceful contractions. This clamp should fixate the knee, however may be improved upon. A drawback to the parallel plates design is the surface of the clamp; because the surfaces are flat it may cause tissue damage and affect the performance of the test subject.

4.2.2 The Countersink Track Design:

The countersink-track design consists of a altering the knee clamp so that a countersink and track align with the murine knee and the track aligns with the thigh and lower leg of the mouse countersink hole with a track to hold the mouse knee and limb respectively. This holds the knee in place while the track aligns the limbs. The countersink would be at a 45% angle with a centimeter diameter. The track would be a centimeter in length. A spring clamp will provide the force within the clamp, as in the flat design.
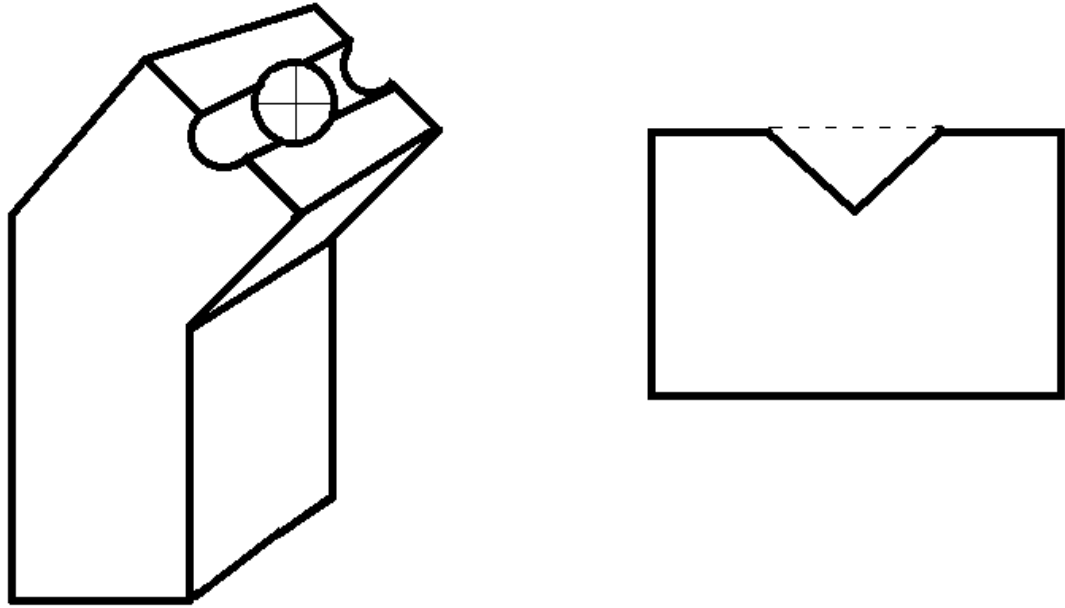
Figure 12: Design of countersink-track

Table 2: Table of pros and cons of the initial design.

Pros:	Cons:
Holds knee and legs it place with more control than the parallel plate method.	Countersink and track may not fit all mice limbs well, and the mouse knee is not shaped like the countersink is shaped.
More general fit allows for irregular limbs to have good fit.	Edges may increase damage to tissue.
	Differing length of limb size problematic with immobile footrest alternative.

4.2.3 Plaster fixation

In a study done by Drost, et al. in 2003, they were able to fixate anaesthetized mice on

their side by creating a plaster shoe cast. The cast was made of SES creative molding powder,

and a mold of shrink tubing (dimensions 4x4x20 mm). The shoe was then glued to a fixation plate, which connected to their torque transducer.

After researching the possibility of using a plaster fixation approach to fixate the mice legs the design team concluded it would not be an efficient or an effective method. Plaster fixation would require a different mold for each mouse specimen. A generic mold would not suffice because the knee must be fixated securely. One of the original goals of the project was to have a minimal procedure time and by including a plaster mold it would add to the preparatory time. Also if a mold were to be made for each mouse, they would have to be anaesthetized first to create the mold and again to conduct the experiment. We ruled out the possibility of creating different size molds for different size mouse knees because we want a very secure fixation about the knee.

4.3 Electrode Holder

The electrode holder components will be machined to model those available from companies such as Narishige (Tokyo, Japan). The electrode guide plate will be placed directly through the leg clamp. The guide will allow for accurate placement and fixation of electrodes when contacting the skeletal muscle complex of the mouse. The electrodes will be designed to have a blunt flexible tip to ensure that no penetration of the muscle tissue is possible. Preferably the wires connecting the electrodes to the transducer/stimulator will be incased in a stiff yet flexible shielding. These specifications would allow for optimum retention of position and shape. Finally the foot pedal will be built at a predetermined angle to allow for the resting foot to begin in the same position for each proceeding test. This pedal will not interfere with the force transducer's readings because there will be a hole for the suture to poke through and connect with the other components in this project. These components include the force transducer and the tension knob which will be used to modify the tension of the suture.

4.4 Methodology

The surgery procedure used was used before in Professor Page's mouse testing (Page, et al., 2011). The surgery was accomplished by anchoring the knee joint using the device created by the design team. A silk ligature was attached to the cleft between digits 1 and 2 that was anchored to a force transducer (Harvard Apparatus) at the other end. The exposed TA muscle was stimulated using 2 custom needle electrodes placed at the proximal muscle surface. Electrical stimulation was applied at 5 volts, 4 ms pulse duration, at 500 ms intervals and the resultant tetanic force (g) recorded was recorded at 200 points/s using a BioPac MP-100 (Harvard Apparatus) and accompanying software (AqKnowledge™). The muscle was kept hydrated during the procedure using sterile saline. Maximum tetanic force was measured by reducing the stimulation interval to 20 ms, thus generating continuous stimulation simulating the tetanus condition. Four measurements per animal were made and values obtained, both before and after the muscle was dissected away from the bone. Data are reported as mean normalized force [+ or -] standard deviation, and can be found in Section 7, Discussion. The mouse was then sacrificed by cervical dislocation.

5. Design Verification

In order to verify our design, the functionality of each individual component and the whole device itself was tested and weighed. At this point if there were changes to be made the individual components would either be redesigned completely or modified wherever possible. The following section described the fabrication and verification process of the original design and revised design before a finalized product was constructed.

5.1 Fabrication of device

With our preliminary design set it was determined to begin the manufacturing and fabrication process. Before machining took place choosing the right material was key to the construct of our device. Judging from industry standards in the medical device industry there were two clear choices: 316L Stainless Steel or 6061 Multi-Purpose Aluminum. Each of the materials was evaluated on four specific criteria: cost, machinability, sterilizability, and mechanical properties. In terms of cost, stainless steel is significantly more expensive than the aluminum and with a limited budget it was more economically sound to go with the aluminum. In terms of machinability, the aluminum bested the stainless steel due to its softer texture and ease of fabrication. When it came to sterilizability both materials would be able to be sterilized however this aspect was a secondary concern during the decision process when choosing the material. The final category was to consider the mechanical properties of each material. For our given application either of the materials had significantly stronger mechanical properties than we needed, so either material would have worked perfectly fine. After evaluating both of these materials it was determined that the 6061 aluminum would provide the team with a cost effective, easily machinable material with sufficient mechanical properties to construct the device.

5.2.1 Spring-loaded Clamps

The focal point and arguably the most important part of the device are the clamps, which will be fixating the knee of the mouse during stimulation. A spring mechanism was chosen to control the clamps. The system distributes the clamping force generated by the spring evenly across the knee to create a uniform fixation. This system allows for the interchangeability of varying springs depending on strength to choose a spring that would adequately fixate the knee

during stimulation but not create too much pressure where tissue damage could occur. Figure 14 shows an image of the spring-loaded clamps.
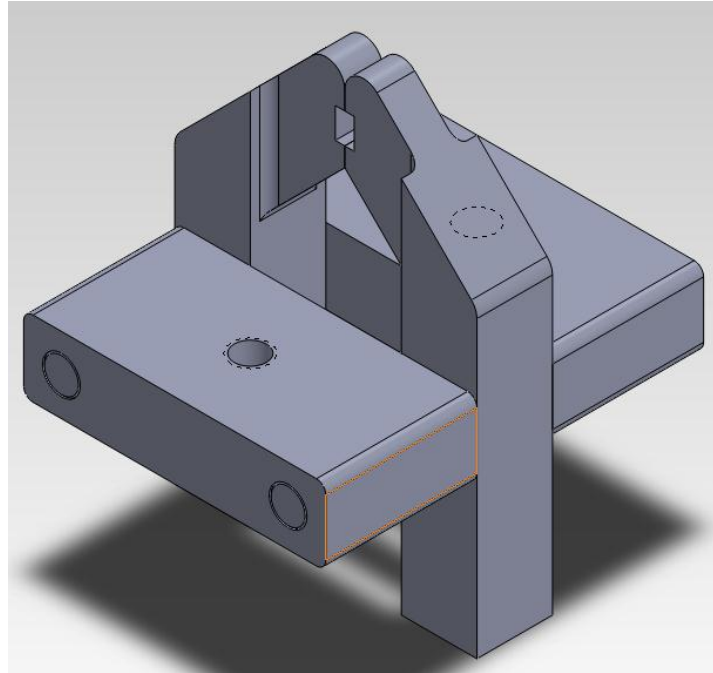
Figure 14: CAD model of the initial spring-loaded clamps.

5.2.2 Microscope Compatibility

One of the key features to include on this device was to allow for the attachment of a stereomicroscope. The magnification that the stereomicroscope would provide allows the user to have a larger viewing area allowing for a more intuitive procedure and overall test of the contractile force generated in the skeletal muscle complex when stimulated. In order to make the device compatible with stereomicroscopy, a support arm with a thru hole was attached to the base of the device allowing for the microscope shaft and head to attach to it. Figure 15 depicts this portion of the device.
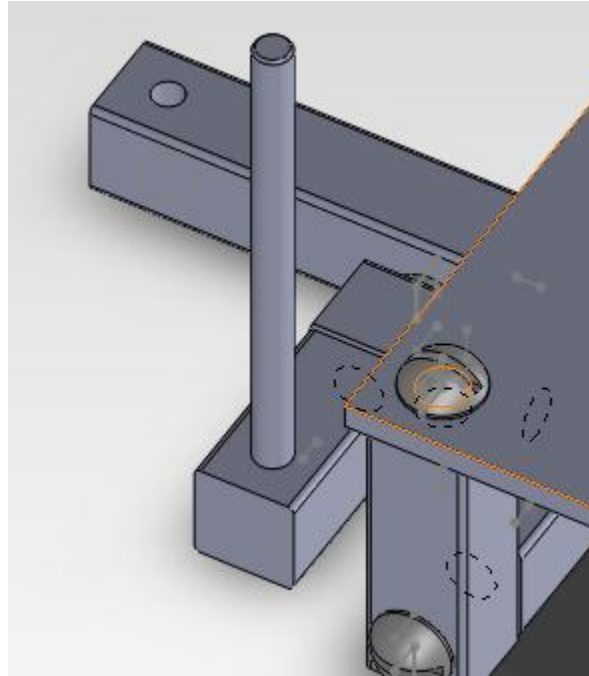
Figure 15: CAD model of the micromanipulator arm support.

5.2.3 Force Transducer Compatibility

The purpose of this project was to measure the contractile force of the skeletal muscle complex during stimulation. In order to accomplish this, a force transducer must be incorporated into the device to measure those readings. The back portion of the device is where the force transducer is housed which is represented by the long rectangular block and shaft connected to the back of the device. Figure 16 displays this interface for which the force transducer will be attached to.
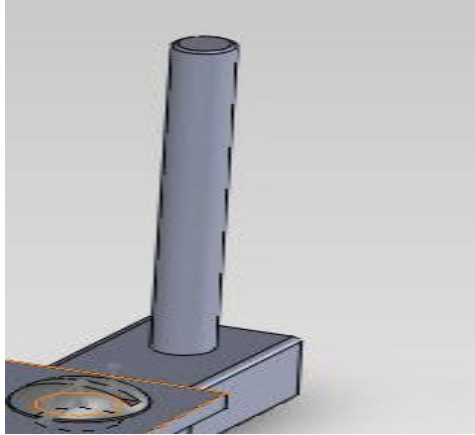
Figure 16: CAD model of force transducer support.

5.2.4 Footrest

During stimulation there was a need to allow for the standardization of the initial angle of the foot of the animal. The standardization of this angle would ensure that each animal's foot was starting at the same position to allow for more consistent testing when conducting several experiments. Figure 17 shows the footrest relative to the base plate and spring-loaded clamps.
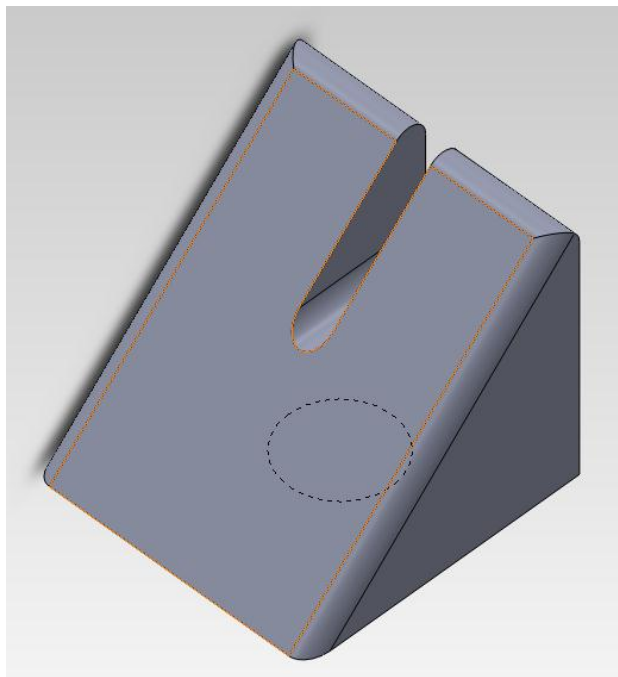
Figure 17: CAD model of initial footrest

5.2.5 Micromanipulator Arm

The micromanipulator arm would be the instrument that would allow for the fixation of the electrodes during stimulation as well as allowing the movement of the electrodes in any direction. The micromanipulator arm itself was a spare component that Professor Page allowed us to use. However, the support that fixated the arm was created by connecting two rectangular blocks and a precision ground shaft to the base of the device. Figure 18 presents a representation of what this component looks like.
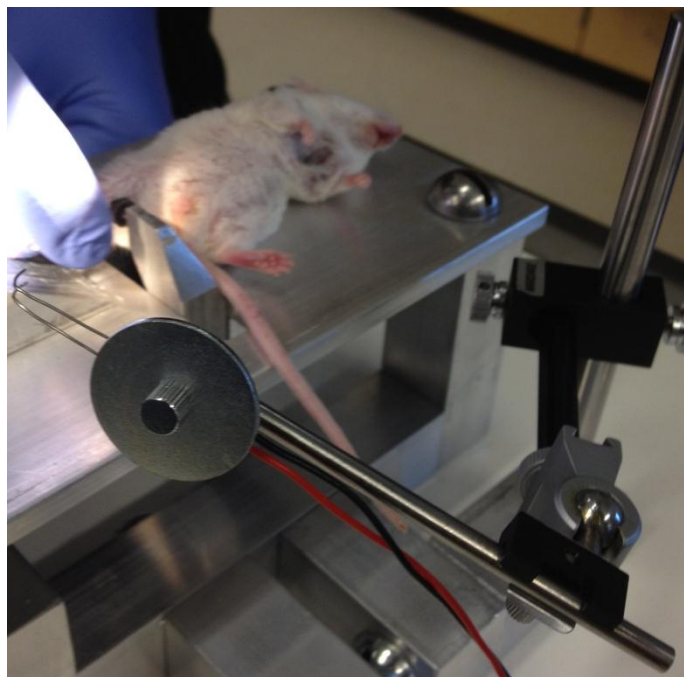
Figure 18: Photo of micromanipulator arm with electrodes attached

5.2.6 Preliminary Pencil Test

Once the initial design was completed the team decided to do a crude but effective test to gauge the strength and effectiveness of the spring-loaded clamps which would be the focal point

of the device. The clamps ideally would only cover a small portion of the leg as to leave a

significant area to apply the electrodes during stimulation and more importantly minimize the

amount of movement of the leg during stimulation. A pencil was determined to be roughly the

same size of a mouse limb and was used to demonstrate a proof of concept that the clamps would

limit the covering of surface area and securely hold the pencil in place. Figure 19 below displays

this experiment as the pencil was held in place by the clamps. As a result of this experiment, it

was clear that the force generated by the spring-loaded clamps would firmly fixate the knee,

however the clamps were simply too large and would overtake too much of the skeletal muscle

complex not allowing for enough surface area to stimulate the muscle.

Figure 19: Photograph of initial knee fixation design during preliminary pencil test

5.2.7 Functional Revisions to Address

As a result of assessing each individual component a list of functional revisions to the design were generated. These changes would be implemented in the revised design and included: a total redesign of the spring-loaded clamps, an adjustable footrest with a smaller angle, the offset of the force transducer attachment, the ability to operate the clamps with one hand, and the overall fixation of many connecting parts. The reasoning behind these revisions was motivated by a few concerns.

For instance, a total redesign of the spring-loaded clamps was needed. The redesign would incorporate a modular system which would streamline the clamping mechanism itself and allow for much smaller fixation points. This decrease in size permitted a much more desirable clamp overall and granted more muscle surface area to work with during stimulation. Varying lengths of mice hind limbs and an angle closer to a neutral position of the mouse foot were the inspiration to incorporate an adjustable feature and decrease the initial angle of the footrest. It appeared as though the force transducer was not in direct line with the foot which could possibly throw the measurements askew, that it why to address that problem the force transducer attachment would be offset at a predetermined dimension. The operation of the clamps was not particularly very convenient to operate with one hand. As a result, it was determined that a handle would be attached to allow for the operation of one hand by the user accessible while the users other hand can place the knee of the mouse in the correct position.

5.3 Revised Design

Generating a list of functional concerns of the initial design allowed for the team to focus on those key aspects for the revised design. Once those concerns were addressed, a revised version of the device was established to go through further testing. The testing also allowed the

team to validate the functionality of the newly designed components to ensure the device was performing adequately.

5.3.1 Modular Spring-loaded Clamps

The major concern that was addressed for the revised design was the spring-loaded fixation clamps. Rather than a singular component that would incorporate a spring-loaded mechanism and fixate the knee, it was decided to move to a modular system. This modular system allowed for a streamlined clamping mechanism, which gave the users the flexibility to swap inserts in and out depending on how well they fixated the knee of the mouse. The inserts were made out of Delrin® which is a versatile engineering polymer. Delrin® was chosen for its high mechanical strength and rigidity, while still a soft material that would not cause any tissue damage during fixation. A handle was also incorporated into the clamps to which allowed for a more intuitive interface when operating the clamps. It was foreseen that the user would operate the clamps with one hand while the other positions the knee properly. Figure 20 demonstrates the modular clamps with interchangeable inserts.
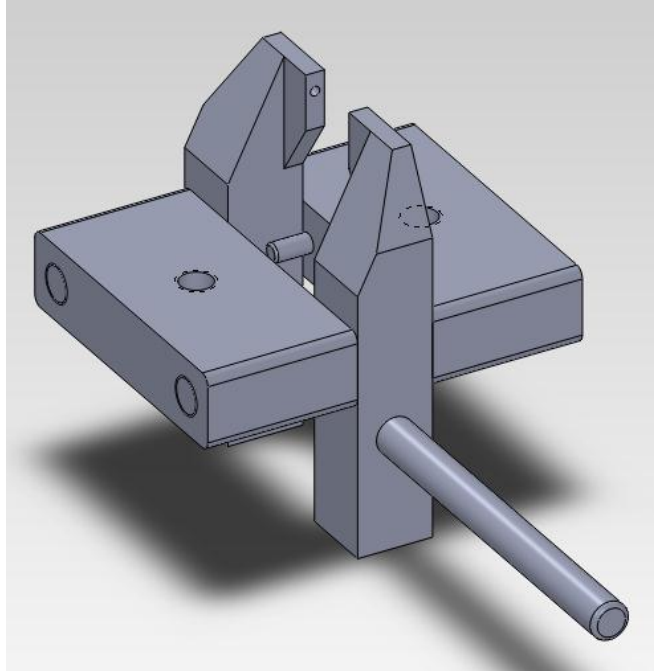
Figure 20: CAD model of revised modular spring-loaded clamps with handle

5.3.2 Force Transducer Compatibility

For the revised design the force transducer support was offset the width of the transducer to align the force transducer with the foot of the mouse during fixation. The way the support was positioned before would cause inaccurate readings due to the misalignment. Figure 21 shows an updated representation of the force transducer support.
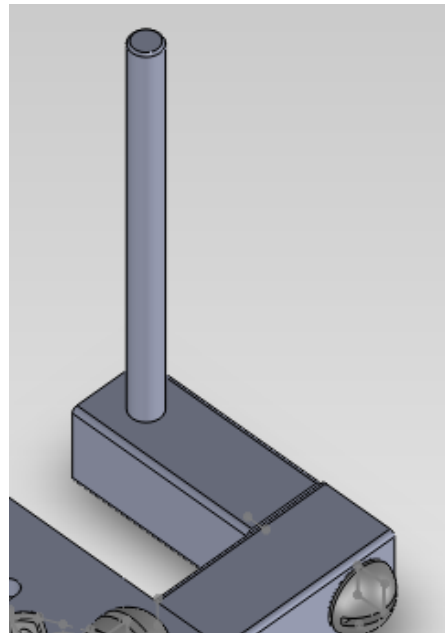
Figure 21: CAD model of force transducer support

5.3.3 Adjustable Footrest

The footrest was also a component that went through a substantial redesign. It was determined that the initial footrest which was constructed with an angle of 45° was too much and could cause inaccurate readings during stimulation. To address this, a new footrest was designed to be constructed with a 30° platform as well as the ability to adjust the footrest in the positive or negative X-direction depending on the length of the animal. A slot was also milled out of the middle of the platform to allow for the suture to run freely from the foot of the mouse to the force transducer. Figure 22 shows an image of the updated footrest.
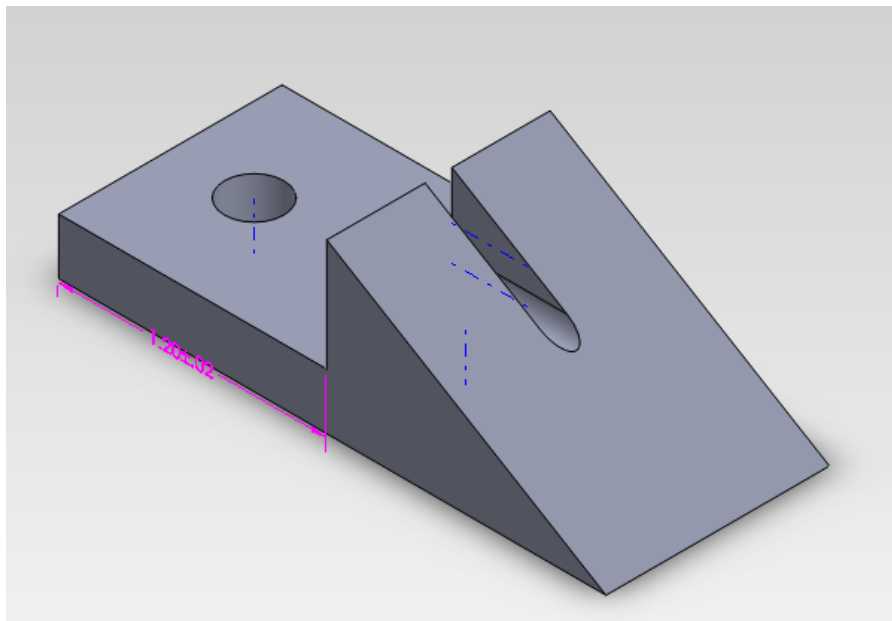
Figure 22: CAD model of revised footrest

5.3.4 Deceased Mouse Preliminary Test

In order to determine the overall effectiveness of the revised device a deceased mouse test was planned and conducted accordingly. Following the methodology that was presented in previous studies conducted by Professor Raymond Page, the mouse was anesthetized using a combination of ketamine and xylazine according to the animal's body weight. Once anesthetized, the hair and fascia layer was removed from the leg to allow for a more accurate contact surface. The knee of the mouse was then fixated in between the clamps while a suture was attached to its foot and anchored around the force transducer. Once the test had begun, it appeared that the clamps were fixating the knee securely. The contractions were viewable under the microscope which established the device's compatibility with microscopy. The test was successful and a proof of concept was achieved. Figure 23 below displays the knee of the mouse properly fixated during this preliminary test.

Figure 23: Photo of deceased mouse knee fixated between modular spring-loaded clamps

5.3.5 Functional Revisions to Address

Although the revised device was a success there were still a few minor issues that had to be addressed. The first and most notably was the size of the Delrin® knee anchor inserts. At the time of the first preliminary test, the diameters of the inserts were significantly larger than ideal. The oversized inserts had the potential to clamp a lot of fascia tissue rather than simply the knee itself. This aspect of the inserts also covered up significant amounts of the skeletal muscle complex making it more difficult to contact and stimulate the muscle. A secondary issue of the device was that the footrest itself was an obstruction for the user so it was removed from the device. Another issue with the device was maneuverability of the micromanipulator arm, the support post which the arm was attached to was considerably loose. It was determined to fixate the support to make sure there was no movement of the electrodes during stimulation. The final aspect was focused on the incorporation of a tension knob that would allow for adjustability of the tension of the suture to streamline future testing.

6. Final Design and Validation

After verifying the individual components and overall effectiveness of the original and revised design it was time to tackle any remaining functional short comings. Modifications and new components were incorporated to solidify the team's final design which would be validated through a series of tests. The tests conducted would include the standard experiment of fixating the knee and stimulating the skeletal muscle complex. By stimulating the muscle tissue a force output was generated which was compared to several trails to ensure the device was accurate and consistent in its measurements.

6.1 Delrin® Inserts

The incorporation of the Delrin® inserts was a great addition to the spring-loaded clamps. They offered a mechanically stable yet soft material that would not cause any damage to

the tissue during fixation. However, although the material was satisfactory, the overall diameter

of the insert was too large. For the final design, the Delrin® insert's diameters were decreased

about half to create a more localized fixation point around the knee, rather than clamping

extraneous fascia tissue. Figure 24 displays the final Delrin® inserts after the diameter was

decreased.
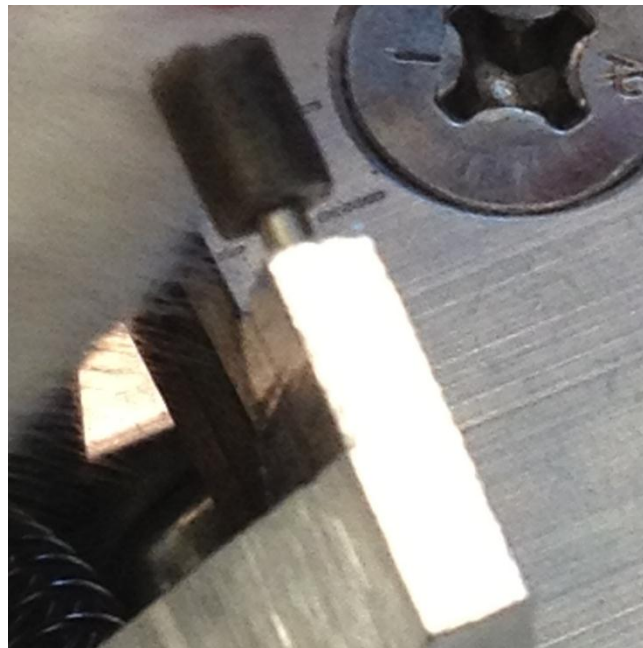
Figure 24: Decreased diameter Delrin® inserts

6.1.1 Suture Wheel

A suture wheel was also added for the final design of the device. This aspect of the

device would allow for the suture, which was connected to the foot of the fixated leg to be

stationed at a downward angle to ensure that the suture stayed around the foot. The suture would

then run through the wheel, providing a frictionless surface to direct the suture towards the force

transducer. The component overall allowed for a more secure connection between the suture and

the foot of the animal, thereby aiding in the streamlining of testing.

6.1.2 Tension Knob

The last component of the final design to be integrated was the introduction of a tension knob. The ability to set a standard tear load on the suture and foot was a must to standardize testing. A wing nut system attached to a threaded rod was fastened to the surface plate of the device, which can be seen in Figure 25. Ideally once the suture was secured to the force transducer it would tie to the wing nut and depending on the amount of wing nut rotations the tension of the suture could be adjusted accurately.
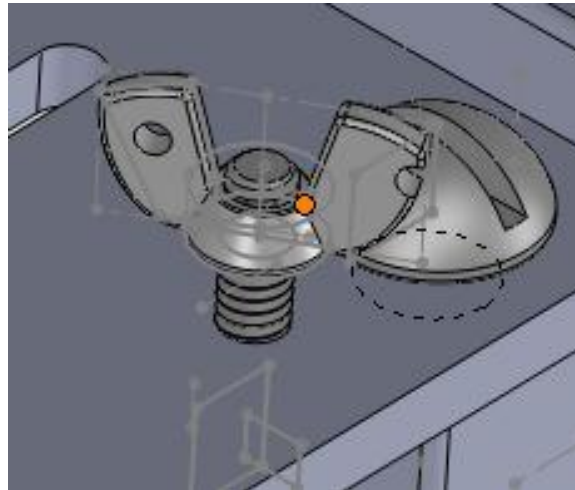
Figure 25: CAD model of tension knob

6.2 Validation

In order to validate the final design of the device, it was used for its intended purpose: fixating the knee of a hind limb of a mouse, using topical electrical stimulation of the skeletal muscle complex to induce a contractile force, and accurately measuring that contractile force generated in the muscle tissue. Additionally, an invasive procedure was also done where the muscle was dissected and connected directly to the force transducer. This would allow for a comparison to be made between the minimally invasive procedure and the invasive procedure. The results from the minimally invasive test should be able to accurate predict the standard invasive approach and therefore validate the device completely.

6.2.1 Live Mouse Test

Like in the previous mouse test, this test was to determine the overall effectiveness of the device. The same procedure as before would be followed which included anesthetizing the mouse using a combination of ketamine and xylazine according to the animal's body weight. Once anesthetized, the hair and fascia layer was removed from the leg of the animal to allow for a more accurate contact surface. The knee of the mouse was then fixated in between the clamps while a suture was attached to its foot at the cleft between the first and second digits, directed around the suture wheel, and anchored around the force transducer. The mouse complex was stimulated which began the recording of data through the AqcKnowledge™ software. Figure 26 below displays the knee of the mouse properly fixated during this final test.

Figure 26: Knee fixation during live mouse test

6.2.2 MATLAB Script

Once the data was collected from AqKnowledge™ the design team chose to use MATLAB (Mathworks®, Inc.) as its primary program for data analysis. MATLAB is accessible on most of the WPI computers and available for download through the WPI network. It is a powerful analysis program that can be easy to use depending on the operator's skill level. The first portion of the script simply imports columns and labels them as voltage and force. Time was not being recorded for the second set of our data collection, so an array of equal data points was created instead. The second portion of the script uses the "findpeaks" command to determine the value and location of the peaks in the force data. The peaks must have a minimum height of 8 and a minimum distance between peaks of 50 data points. These variables can be easily modified so that the script can be used for different data files. The values of the peaks are then created in a column with the file name "MaxF". The next command displays the number of peaks found so that the user can confirm that the data is accurate. Lastly, the script creates a figure of the force versus time data, with peaks labeled with a red triangle, and the voltage versus time. The complete code can be found in Appendix C: MATLAB code.

6.2.3   Biomechanical Analysis

A biomechanical analysis was conducted after the final surgery was completed. The free body diagram and equations seen in figure 27 were given to the team by Professor Page to assist in the analysis. F_A=the actual force of the muscle, F_M=the measured force when the suture was attached to the foot, d_A=the distance from where the tendon innervates the muscle, to the base of the heel, and d_M=the distance from where the suture was attached around the digits to the base of the heel. In theory, by using this equation the user could predict the full contractile force of the muscle without performing the invasive dissection procedure. The two distances d_A and d_M were found to be 5.5mm and 12mm, respectively. The following equation solves for F_A:

$$F_A = \frac{(F_M)(d_M)}{d_A} = \frac{(18.0g)(12mm)}{5.5mm} = \boldsymbol{39.3g}$$

Equation 1: Mathematical representation of the calculated actual force.

F_A should be equal to the force found after the muscle had been dissected and attached directly to

the force transducer.

$F_A\, d_A = F_M\, d_M$

F_A = actual muscle force (dissected)
F_M = measured muscle force (intact)
d_A = distance from joint of tendon attachment
d_M = distance from joint of measurement
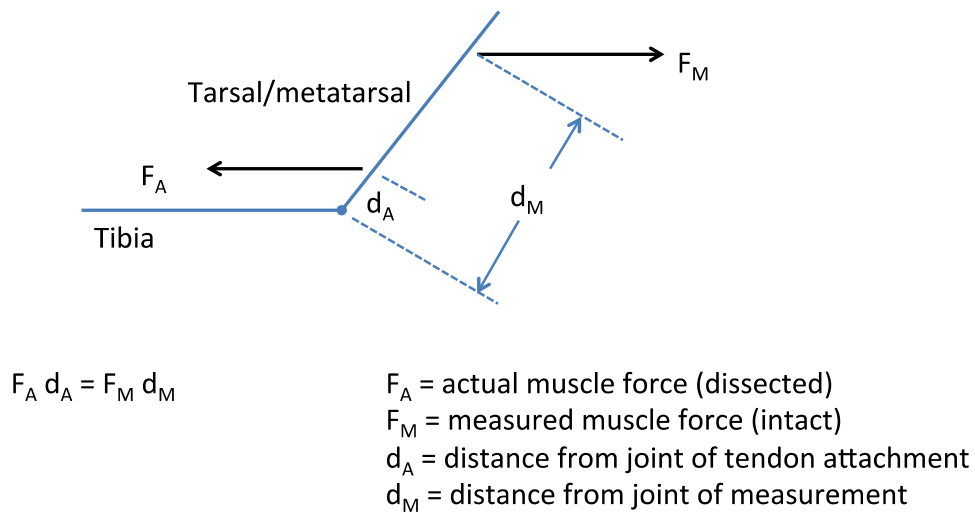
Figure 27: Free body diagram of the mouse tiba and foot.

6.3 Results

The final test proved the concept of the design and completely validated the device. The

data was inputted through the MATLAB script effectively, thereby producing an array of peak

forces generated by the skeletal muscle at every contraction during the test. The mean peak

contractile forces, and standard deviations, from the individual tests using the non-invasive

55

methodology can be found below in table 3. The mean peak contractile forces, and standard deviations, for the invasive test can be found below in table 4. The overall average of the non-invasive procedure was 18g, while the overall average for the invasive procedure was 22.3g. The live animal test was considered a success. The device was able to minimally invasively fixate the knee of a mouse to accurately and repeatedly quantify the contractile force generated in the skeletal muscle complex during stimulation.

Table 3: Table of the mean force and standard deviations for the regular testing, when the suture was attached to the foot.

Stimulation #	1	2	3	4
Mean Force (g):	17.7	18.7	17.8	17.7
Standard Deviation:	0.673	0.546	0.786	0.673

Table 4: Table of the mean force and standard deviations for the isolated muscle testing, when the suture was attached directly to the muscle.

Stimulation #	1	2	3	4
Mean Force (g):	23.4	22.5	22.0	21.5
Standard Deviation:	0.702	0.572	0.818	0.582

Chapter 7: Discussion

The following chapter will discuss various portions of the project as well as many of the influences and implications the project may have in the future. A general project discussion serves as a summary to what the device was able to accomplish. An impact analysis was conducted to analyze the influence of the project on seven different criteria.

7.1 Project Discussion

The goal of this project was to design, produce and verify a methodology and apparatus for minimally invasive measurement of contractile force in murine tibialis anterior skeletal muscle. After the apparatus was machined it was tested on laboratory mouse subjects. The contractile tests were performed in 4 sets of 10 contractions. The averages for the sets were 17.7, 18.7, 17.8 and 17.7g. Overall, the tests showed a range of 16.0 to 19.6g of peak contractile force produced, with an overall average force of 18.0 gs of force. Table 3 depicts these data as well as the standard deviations. This is within the magnitude of the visual results from Cutlip et al, which used a dynometer and produced 17-18 gs in force from the dorsal flexor contractions. This magnitude is similar to the results found by Page et al, which used the same force transducer as this project. The device measures dorsal flexion to the same magnitude as literature.

After completing the initial tests using the non-invasive methodology, an invasive procedure to isolate the muscle was performed. The tibialis anterior muscle was isolated and the force was measured directly. The forces produced in these tests ranged from 20.6g to 24.5g with an average of 22.3g. The 24.2% increase in force is accounted for by the indirect method of attachment.

Proper fixation of the knee was a top ranked design objective. While no quantitative tests were performed for the fixation of the knee, video recording of the experiment showed no change in knee position for the duration of electrical stimulation. The clamp design fulfills all sub-objectives as well. It allows enough room for proper stimulation of the muscle without clamping an excessive amount of muscle tissue. It is easily adjustable via the handle bar, and is

not strong enough to cause muscle damage to the clamped tissue. Thus the "ideal fixation" objective was met.

Ideal electrical stimulation was the second top ranked objective. The electrodes used were able to properly stimulate the muscle to comparable forces found in literature. They do not puncture the muscle tissue whilst doing so due to blunted tips, fulfilling the sub-objectives of ideal muscle stimulation and minimal invasiveness. The consistent electrode placement sub objective was not met, but it was ranked last.

Accurate quantification of muscle was the third ranked objective. While the muscle force measured is only an indirect measure of the muscle, testing showed consistent results and results consistent with the data from other plantar flexion experiments.

User friendliness was the fourth ranked objective. The design was to be as easy to use as possible while retaining functionality. A handle was added to the knee fixation clamp to ease in the setting of the knee in the clamp. The electrodes can be secured into place so that the user does not need to hold them while applying electrical stimulations. During the testing only Professor Page conducted the surgeries and operated the device. This proves that the device could was user friendly and could be operated by one user.

Minimal cost was the last ranked objective. The total budget of the project was $398.65, leaving $125.35 remaining. The full budget can be found in Appendix A. Over the course of the project, cheaper materials such as aluminum were chosen to minimize the cost of the project. Also, since the device underwent several different designs, some of the materials were rendered useless. If the device were to be manufactured from scratch again, the final design model could be used and some money would be saved on material that was not necessary for final fabrication.

Limitations of the device include an indirect measurement of muscle force readings. Because the measurement of force generated is not direct it is not directly comparable to other literature, which used more invasive procedures. However, the device can effectively be used to compare measurement from the same device at different time points, which was an objective. Thus the accuracy was sufficiently met, as the actual force can be calculated from the force measured. A detailed description of limitations and recommendations can be found in Chapter 8.

7.2 Impact Analysis

An Impact analysis was conducted which analyzed the relationship of the project to seven spheres of concern on a global scale: economic, environmental, societal, political, ethical, health and safety issues, manufacturability, and sustainability.

7.2.1 Economic

The economic impact of the project depends on the need for the product. This includes both the size of consumer base and the amount of money they are willing to pay to purchase it. Since the device proved to be easy to use by one user, researchers concerned with the tibialis anterior muscle in mice may be interested in purchasing the device. Since the device proved to be relatively inexpensive there may be some serious interest by researchers in purchasing the device.

7.2.2 Environmental Impact

The device does not have a significant direct environmental impact. The amount of materials used was relatively minimal. Only 3 mice were sacrificed in the testing of the device. A potential indirect impact is allowing for better skeletal muscle regeneration therapies to be produced, which may allow humans to live longer, more able lives. By leading longer, more able

lives, humans may consume more natural resources. However, in the context of this project for right now, the environmental impact is very low.

7.2.3 Societal Influence

The device may have an influence in the scientific community, both in saving time in testing skeletal muscle therapy, and in improving practices in skeletal muscle regeneration therapy.

7.2.4 Political Implications

The device has no direct political impacts. Improved skeletal muscle regeneration therapies supported by the apparatus could cause political implications in the realm of military medicine or stem cell usage.

7.2.5 Ethical Concerns

The device is associated with two major ethical issues: animal testing and stem cells. The device is intended for the use of animal testing.

7.2.5.1 Animal Testing

Many activist groups openly protest the use of animal testing, based on the violation of animal rights and perceived cruelty in the experiments. The methodology used includes an anesthetic dosage to ensure the mouse feels no pain for the duration of the experiment. The WPI Institutional Animal Care and Use Committee (IACUC) approved the methodology. Furthermore the device, in comparison to alternatives, allows the test mice to live longer lives by letting them stay viable test subject by the end of a single contractile force test.

7.2.5.2 Stem Cell Use

The device is meant for the testing of generic skeletal muscle regeneration therapies. One major method of producing skeletal muscle regeneration is to use stem cells. Certain groups denounce the usage of certain types of stem cells, but because the device is not specifically for stem cells, it is shielded from such controversies.

7.2.6 Health and Safety Issues

The device's intention is to provide a way for skeletal muscle regeneration therapies to be tested. Any successful regeneration therapies which move into the general market have the potential to allow for the healing of patients with skeletal muscle damage. The device has minimal sharp corners and edges to inflict damage on the user. The electrodes have blunted edges so that they could not cause damage to the mouse muscle. The user must always be careful when handling, anesthetizing, and dissecting the skin of the mouse.

7.2.7 Manufacturability

The manufacturing process used to machine the device consisted of about 48 hours of machining the initial device and numerous more hours during revisionary machining. The device should take 40 hours to machine with the use of a mechanist. Using mass production methods would save time, if the customer base were wide enough to make the option viable.

7.2.8 Sustainability

Sustainability is the use of materials and processes, which do not deplete the earth's resources of natural cycles. The main material used in the device is aluminum, with a small amount of stainless steel and the polymer Delrin®. Aluminum is regarded as a very sustainable metal because of the ease and frequency at which it is recycled. Stainless steel is made from over 60% recycled material. Delrin® (Polyoxymethylene) however, is chemically manufactured via acid catalysis with formaldehyde, which is produced from methanol. Methanol is usually

produced from methane, a non-renewable natural gas, but can also be produced from renewable biomass gasification. The usage of Delrin® in the device is minor, so the sustainability of the device is not impacted strongly.

Chapter 8: Conclusion and Recommendations

The goal of this project was to create a relatively inexpensive, minimally invasive fixation device for the hind limb of a mouse. The device had to be able to accurately and repeatedly quantify the force generated by muscle contractions by topical electrical stimulation of the muscle. The device also had to be compatible with a stereomicroscope. The surgery platform that the team created features a knee clamp system that causes little to no damage to the knee and surrounding tissue. Electrodes were fashioned that were able to topically simulate the targeted muscle without damaging the muscle. Electrode holders were established so that the user could fixate the electrodes into position. A pulley was also added to the underside of the device for the suture to be connected to the force transducer. Repeated tests confirmed that the results were accurate and repeatable. Overall, the team created an effective, minimally invasive way to quantify the force generated by muscle contractions *in-vivo*. Despite the device accomplishing the goals set forth, there were several recommendations that must be taken into account for future studies.

During the design stages of the project it was assumed that the same microscope would be used for every test. It was not until the design verification stage that the team learned that different microscopes, of varying size, were to be used. Because of this, the different microscope heads may not always align to the desired position on the surgery platform. A recommendation to solve this dilemma in future studies would be to incorporate Y-axis movement on the microscope stand. This would enable the microscope head to move towards and away the

surgery platform so that the user could find an optimal position. Due to time constraints and a limited budget the team was not able to incorporate this modification.

Another big portion of the design stage was focused on creating and implementing a footrest to keep the angle of the foot constant throughout multiple tests. Two different footrests were created, but both had flaws that rendered them useless during testing. The first footrest had too large of an angle and was fixed at one position. The second footrest had a smaller angle and could move in a slot to accommodate mice of different sizes. When a preliminary test was conducted using this footrest it was found to be too tall and impeded the suture. The footrest was then removed from the final design. The pulley was then added to the underside of the device so that the suture could pass through the device and to the force transducer. To remedy this problem there were two strategies that may be considered. The first solution is to redesign the footrest to a smaller height with a larger channel for the footrest. The force transducer could be kept level with the foot in this case. Alternatively it would be possible to place the force transducer directly underneath the foot below the base of the device. This would eliminate the need for the footrest and pulley all together. The downside to this strategy is that the foot would not be kept at a constant angle; instead it would be hanging above the force transducer. As long as the basal tension in the suture is maintained throughout tests the results should be accurate and consistent. To deal with this issue, a small ankle support post could be incorporated to keep the foot in the same position for each text.

A tuning knob was also created that features a wing nut with a hole drilled in it for the suture to be tied to, and a slot for the suture to be wound in. As the wing nut is turned the suture would stay in the slot and the basal tension could be modified, at the discretion of the user. When this component was tested in the design verification stage it was found that two slots would be

necessary for it to work as desired. It was also found that the screw the wing nut was attached to had the potential to impede the user during parts of the surgery. The team recommends a complete redesign of the tuning knob, including the location so that it would not obstruct surgeries. A wing nut is not recommended; instead the component should be modeled after a guitar-tuning knob. This can be achieved by drilling a hole into a thumbscrew for the suture to be secured in. Due to time constraints, the tuning knob was not redesigned; instead surgical tape was used to hold basal tension.

Another component of the device that underwent several modifications was the clamp system. A handle was added to assist in clamping but the handle only opens the proximal side of the clamp. The distal side remains static unless moved by the operator. This made it difficult to align both parts of the clamp while holding the mouse leg in the right position. The team recommends another handle for the distal component of the clamp that can be opened simultaneously with the proximal component.

The electrode holders were designed to allow for movement in all directions. While we accomplished this goal there is no way to slowly and carefully insert the electrode tips onto the muscle tissue. Because of this it may be possible to cause unwanted and unnecessary damage to the tissue. A solution to this would be to have a micromanipulator on the holder that can gently insert the electrodes into contact with the muscle tissue. This would require new holders or a component that is capable of moving the electrodes in such a way. Another recommendation to improve the electrode holders is to reduce the size of the washer. The washer is used to secure the electrodes in the holder. Currently, the washer is excessively large and a smaller one would facilitate the surgeries. Due to time constraints and a limited budget the team used the electrodes that were available and made sure not to cause excessive damage to the muscle.

The biomechanical analysis in section 6.2.3 yielded a force of 39.3g; however, the experimental results gave an average force of 22.3g. The experimental and calculated results had a difference of 17.0 g. One possible reason for this discrepancy could be due to the accuracy of measuring the two distances. A centimeter ruler was used instead of a caliper so the precision of the measurements was not favorable. It is recommended to use a caliper in future measurements to get more accurate distances. Since this analysis was only conducted on one mouse, the accuracy of this prediction is difficult to quantify. It is recommended to make the same biomechanical analysis in further tests to establish a correlation between the two forces, and validate the equation used.

The end goal of this project was to create a device to fixate and stimulate the hind limb of a living mouse using topical stimulations in a non-invasive fashion, and work in conjunction with a stereomicroscope. The results had to be accurate and repeatable readings of the force generated by electrical stimulations. As seen in the results section, the team was able to generate several accurate readings from multiple mice. The accuracy can be confirmed when analyzing the results of a regular test and the test of a separated muscle. The resulting force increased when the muscle was separated as expected.

References:

Angelini C, (200). The role of corticosteroids in muscular dystrophy: a critical appraisal. *Muscle Nerve*, 36, 424-435

Ashton-Miller, J. A., He, Y., Kadhiresan, V., McCubbrey, D., & Faulkner, J. (1992). An apparatus to measure in vivo biomechanical behavior of dorsi-and plantarflexors of mouse ankle. *Journal of Applied Physiology, 72*(3), 1205.

Borselli, C., Cezar, C. A., Shvartsman, D., Vandenburgh, H. H., & Mooney, D. J. (2011). The role of multifunctional delivery scaffold in the ability of cultured myoblasts to promote muscle regeneration. *Biomaterials, 32*(34), 8905-8914. doi:10.1016/j.biomaterials.2011.08.019

Cutlip, R., Stauber, W., Willison, R., McIntosh, T., & Means, K. (1997). Dynamometer for rat plantar flexor musclesin vivo. *Medical and Biological Engineering and Computing, 35*(5), 540-543.

Fox, S. I. (2009). Skeletal muscles. In C. Wheatley (Ed.), *Human physiology* (11th ed., pp. 355-355-375). New York, New York: McgGraw-Hill.

Gorselink, M., Drost, M., De Louw, J., Willems, P., Rosielle, N., Janssen, J., & Van der Vusse, G. (2000). Accurate assessment of in situ isometric contractile properties of hindlimb plantar and dorsal flexor muscle complex of intact mice. *Pflügers Archiv European Journal of Physiology, 439*(5), 665-670.

Mann, C. J., Perdiguero, E., Kharraz, Y., Aguilar, S., Pessina, P., Serrano, A. L., & Muñoz-Cánoves, P. (2011). Aberrant repair and fibrosis development in skeletal muscle. *Skeletal Muscle, 1*, 21.

McCroskery, S., Thomas, M., Maxwell, L., Sharma, M., Kambadur, R. (2003). Myostatin negatively regulates satellite cell activation and self-renewal. *The Journal of cell biology*, 162 (6), p. 1135-1147.

Meng, J., Adkin, C. F., Arechavala-Gomeza, V., Boldrin, L., Muntoni, F., & Morgan, J. E. (2010). The contribution of human synovial stem cells to skeletal muscle regeneration. *Neuromuscular Disorders, 20*(1), 6-15.

Page, R, et al. (2011). "Restoration of Skeletal Muscle Defects with Adult Human Cells Delivered on Fibrin Microthreads". *Tissue engineering. Part A (1937-3341)*, p. 2629-2640.

Parker, Maura H. (2003). Looking back to the embryo: defining transcriptional networks in adult myogenesis. *Nature reviews. Genetics*, 4 (7), p.497-508.

Stern-Straeter, J., Riedel, F., Bran, G., Hormann, K., & Goessler, U. R. (2007). Advances in skeletal muscle tissue engineering. *In Vivo, 21*(3), 435.

Tiidus, P. M. (2003). Influence of estrogen on skeletal muscle damage, inflammation, and repair. *Exercise and Sport Sciences Reviews, 31*(1), 40.

Willems, M., & Stauber, W. T. (1999). Isometric and concentric performance of electrically stimulated ankle plantar flexor muscles in intact rat. *Experimental Physiology, 84*(2), 379.

Willis-Owen, S. A. G., & Flint, J. (2006). The genetic basis of emotional behaviour in mice.

European Journal of Human Genetics, 14(6), 721-728.

Appendix A: Budget

Date	Cost	Subtotal	S&H	Total	Part #	Description
11/7/11	$ 54.49				9008K26	6 feet of 1in x 1in stock Aluminun
	$ 11.94				6023K191	4in of 1in x 0.5in stock of Aluminum
	$ 11.77				8975K425	¼ x 4in x 12in plate of Aluminum
	$ 7.46				6061K11	2 Hardened Shafts 0.25in x 6in
	$ 7.71				90145A551	2 ¼ x 2.5in Dowel Pin for pack of 5
	$ 9.21				9657K231	2in Compression Spring
	$ 5.28				90276A720	7 ½-13 2in Machined Screws for pack of 10
	$ 11.69				90276A716	4 ½-13 1.5in Machined Screws for pack of 25
	$ 9.94				91783A714	4 ½-13 1.25in Machined Screws for pack of 5,
	$ 7.03				90276A619	1 3/8-16 0.5in Machined Screws for pack of 25
	$ 7.73				93085A558	2 ¼-28 0.625in Machined Screws for pack of 10,
		$ 143.59	$ 18.89	$ 162.48		
12/7/12	$ 5.16				9657K171	2.5in Compression Spring
	$ 8.18				90276A712	2 Hardened Shafts 0.375in x 6in
	$ 5.31				6061K12	½-13 x 1in Machined Screws = $5.16 for pack of 1(
		$ 18.65	$ 4.90	$ 23.55		
1/24/12	$ 5.30				50345K76	High-Performance Blend Rubber/Plastic Tubing Va
	$ 1.37				5239K25	Extreme-Temp Tubing w/ PTFE & Teflon® PTFE 3/3
	$ 1.24				51845K54	High-Purity White Silicone Rubber Tubing Nonrein
		$ 7.91	$ 4.78	$ 12.69		
1/27/12	$ 14.80				89215J486	½" x ¾" x 1' stock of aluminum
		$ 14.80	$ 5.11	$ 19.91		
2/2/12	$ 1.56				8579k12	PTFE-Filled Delrin ® Acetal Resin Rod 1/4" Diamete
		$ 1.56	$ 4.78	$ 6.34		
2/3/12	$ 5.25				95150A190	18-8 Stainless Steel Round Knurled Thumb Nut 3/8
		$ 5.25	$ 4.78	$ 10.03		
2/10/12	$ 2.69				92815A115	Black-Oxide Steel Round Knurled Thumb Nut 1/4"-
		$ 2.69	$ 4.76	$ 7.45		
2/17/12	$ 5.27				97395A414	1/16in x 3/4in Dowel Pins
	$ 7.50				93180A220	¼-20 x 1in Carriage Bolt
		$ 12.77	$ 4.76	$ 17.53		
	$ 123.67	$ 123.67	$ 15.00	$ 138.67		12 Mice
		$ 330.89		$ 398.65		Grand Total
				$ 125.35		Left Over

Appendix B: Surgical Procedure

Preoperative procedures include anesthetizing the mouse, visual assessment of the level of anesthesia, securing the knee to the operating platform, removing hair from around the surgery region, cleaning the surface with 70% isopropyl alcohol. All surgical instruments (microdissection forceps and microdissection scissors) are cleaned with Zephirin, rinsed with water and 70% isopropyl alcohol and air dried in a sterile field. Bleeding is controlled by applying direct pressure using a sterile cotton swab. Mice are monitored for bleeding and signs of distress.

Non-survival surgery – tibialis anterior partial resection. Adult mice (CD-1) will be anesthetized (Ketamine 50mg/kg/Xylazine 5mg/kg). Animals will be placed in the prone position on the aforementioned customized operating platform enabling the fixation of the knee joint and visualization under a stereomicroscope. The tibialis muscle is exposed by cutting a ¾ circle skin flap leaving vasculature intact and maintaining flap hydration with sterile saline. The foot is anchored at the cleft between digits 1 and 2, through a pulley located underneath the platform,f and to a force transducer using silk ligature. A custom bipolar electrode made from sterile 30 gauge needles is placed in contact with the exposed muscle and contraction stimulated using 10 pulses at 5 volts, 4 ms duration at 1 sec intervals. The force of each contraction is measured and recorded (200 points/sec). Maximum tetanic force was measured by reducing the stimulation interval to 20 ms, thus generating continuous stimulation simulating the tetanus condition. As an additional control, the tetanic force will be measured on the contralateral leg using the same procedure. Following data collection the animals will be euthanized by cervical dislocation.

Appendix C: MATLAB code

```matlab
%%Data Analysis

clear
clc
close all
%% Import Data
fileName='Mouse 04.csv';
%fileName='Mouse iso 04.csv';

data=dlmread(fileName,',',1,0);
Voltage=data(:,1);
Force=data(:,2);
T=(1:1999)/200;
%If time data is present in data file, the above line is
unnecessary,
%import data column as T instead.

[MaxF, locs] = findpeaks(Force, 'minpeakheight',8 ,
'minpeakdistance', 50);
%Value and location of peaks with a minimum height of 8 and a
distance
%between peaks of 50.

%MaxF= MaxF';

noPeaks=length(MaxF)
%number of peaks

%% Plot experimental results
figure(1)
subplot(2,1,1)
plot(T,Force);
xlabel('Time(s)'); ylabel('Force(g)');
title(fileName)
%title('Force v Time')

hold on
plot(T(locs),MaxF,'k^','markerfacecolor',[1 0 0]);

subplot(2,1,2)
plot(T, Voltage)
%title('Voltage v Time')
xlabel('Time(s)'); ylabel('Voltage(mV)');
```

Appendix D: CAD Drawings
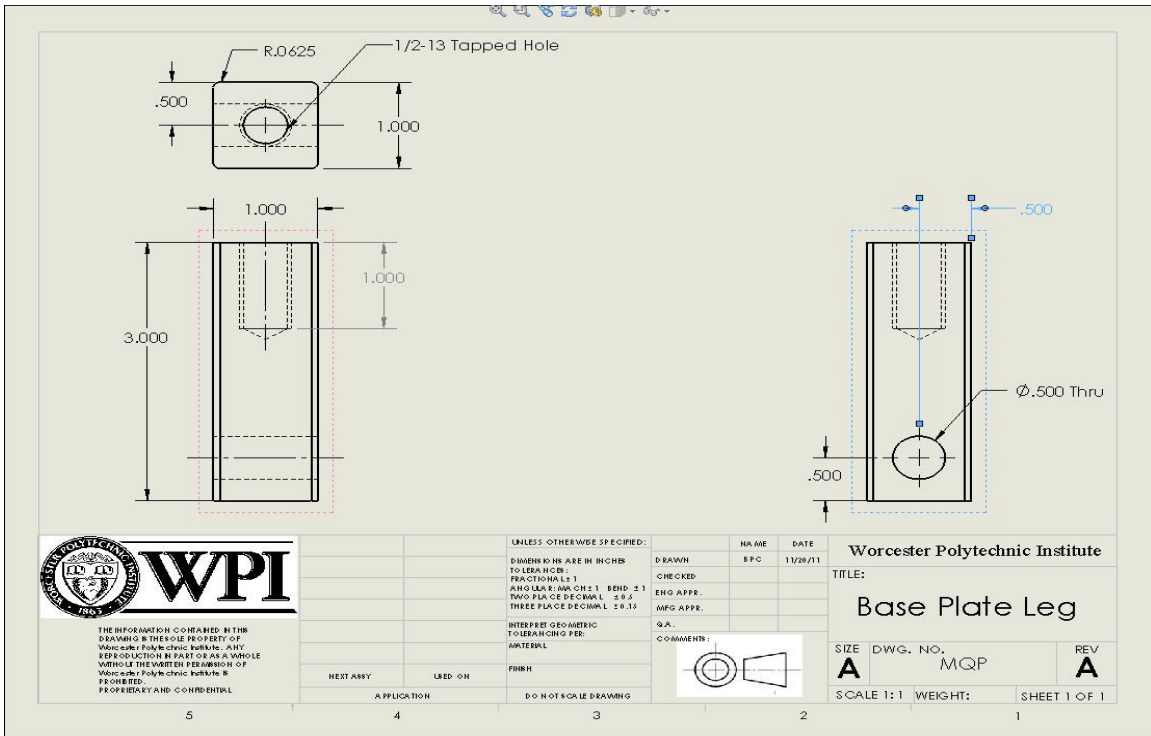
Figure 28: Final drawing of base plate leg design
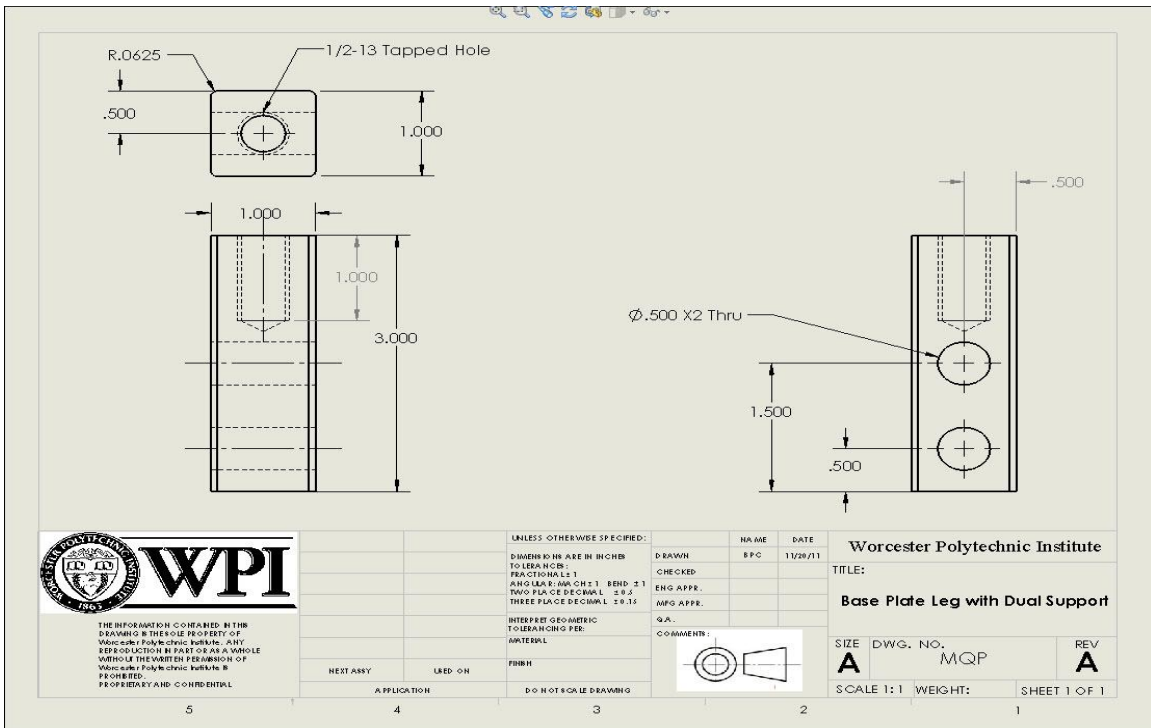
Figure 29: Final drawing of base plate leg with dual support
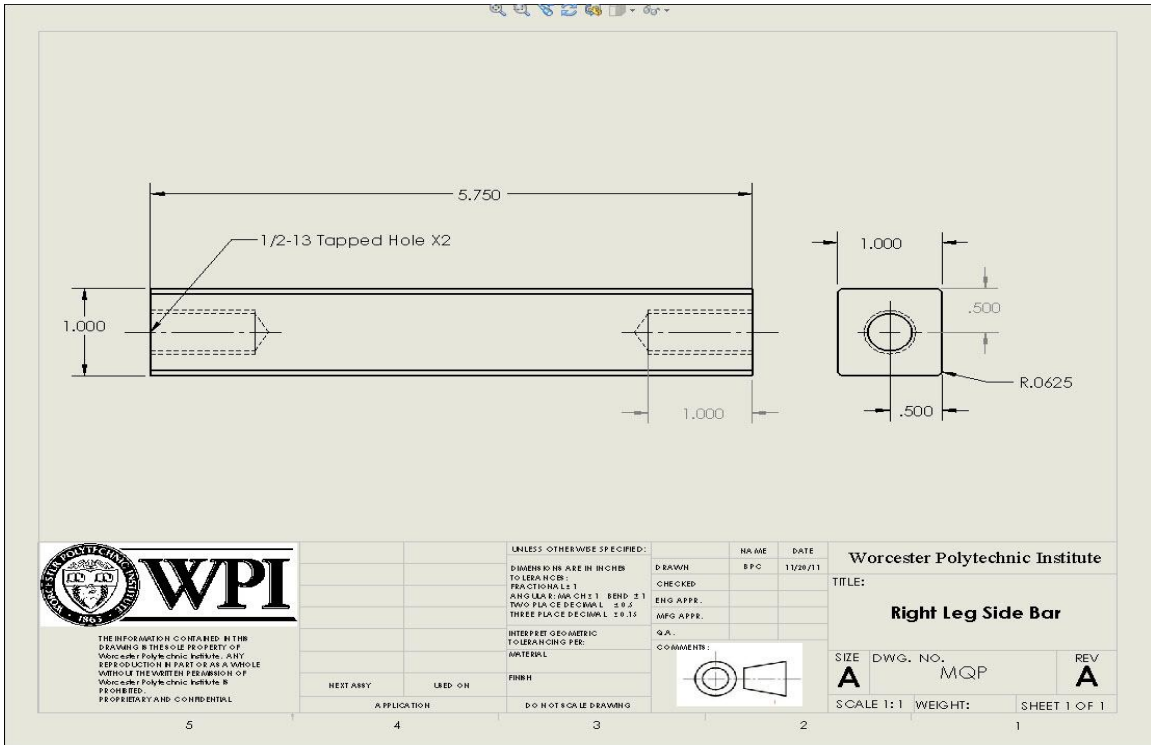
Figure 30: Final drawing of right leg side bar design
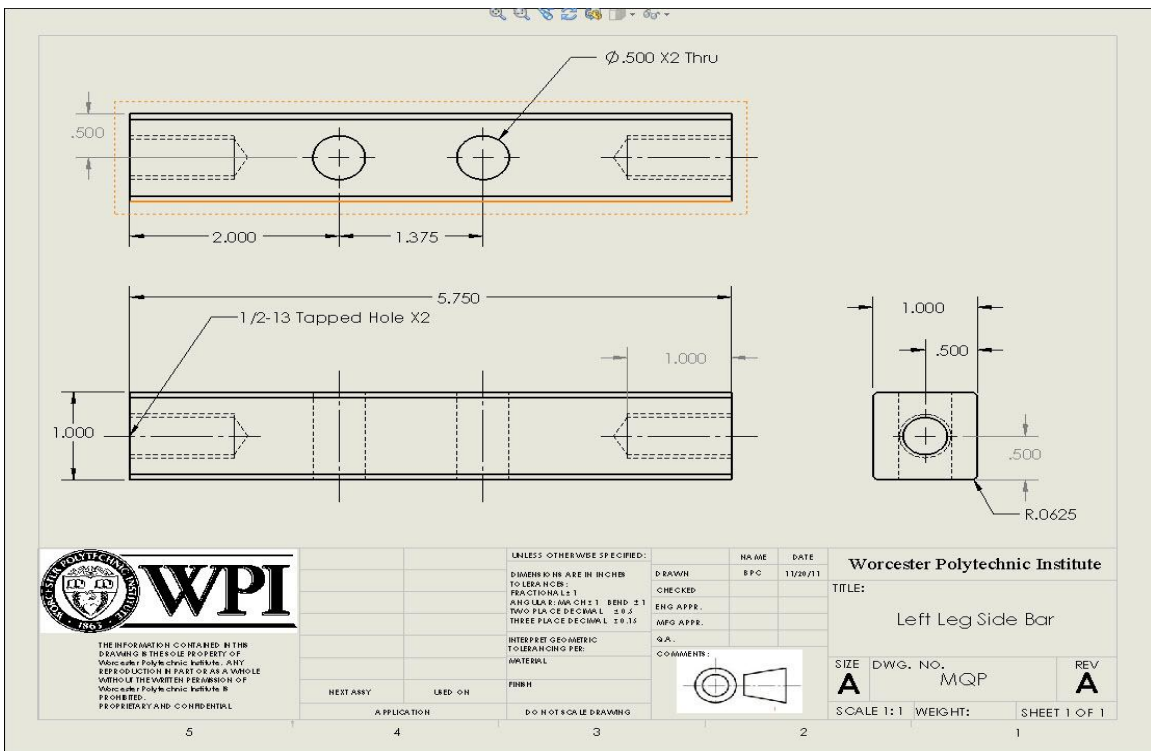
Figure 31: Final drawing of left leg side bar design
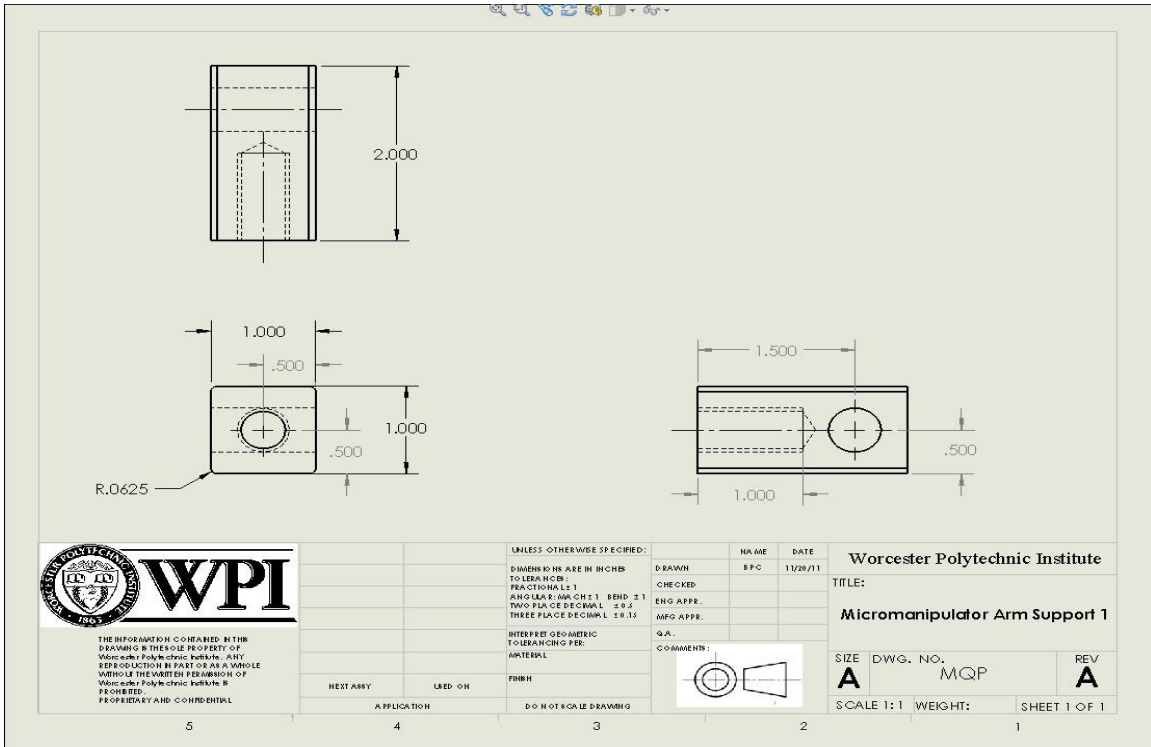
Figure 32: Final drawing of micromanipulator arm support one design
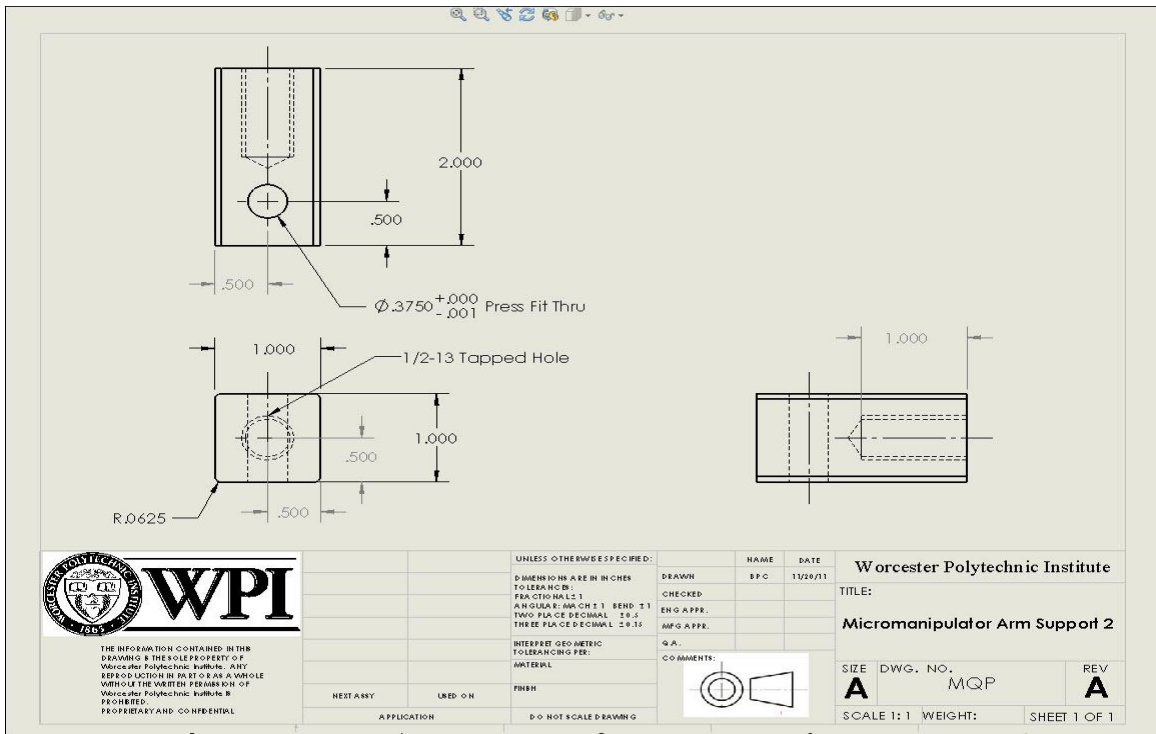
Figure 33: Final drawing of micromanipulator arm support two design
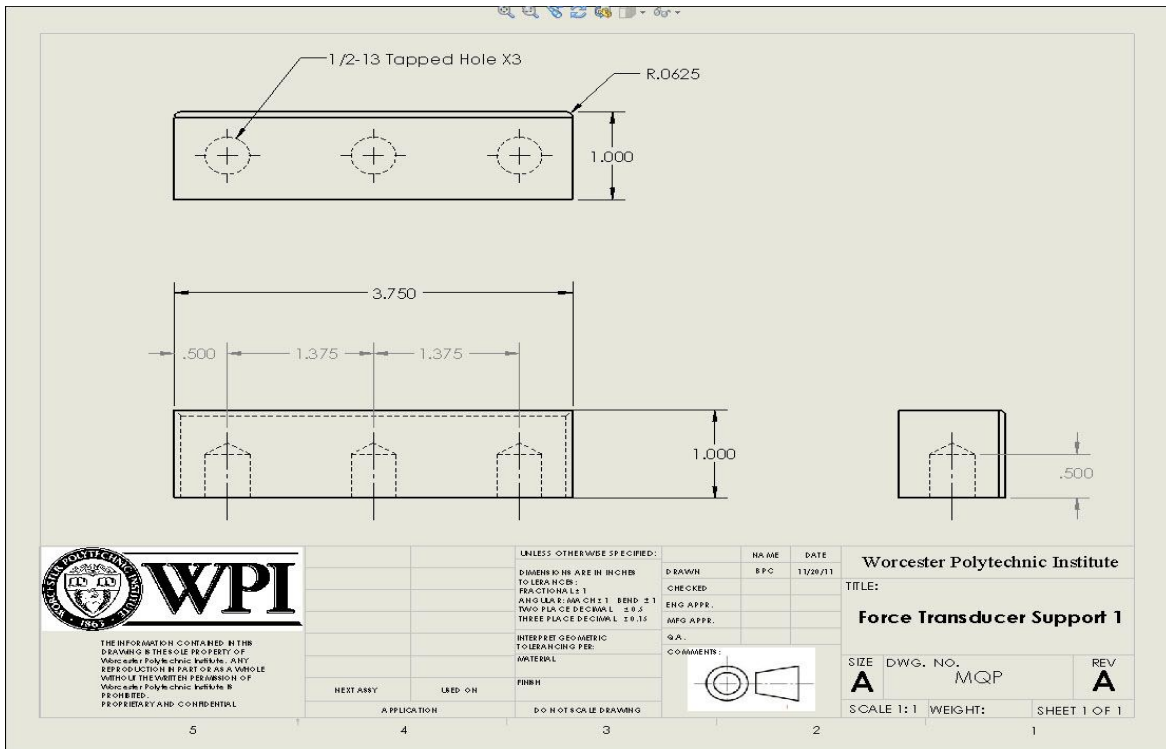
73
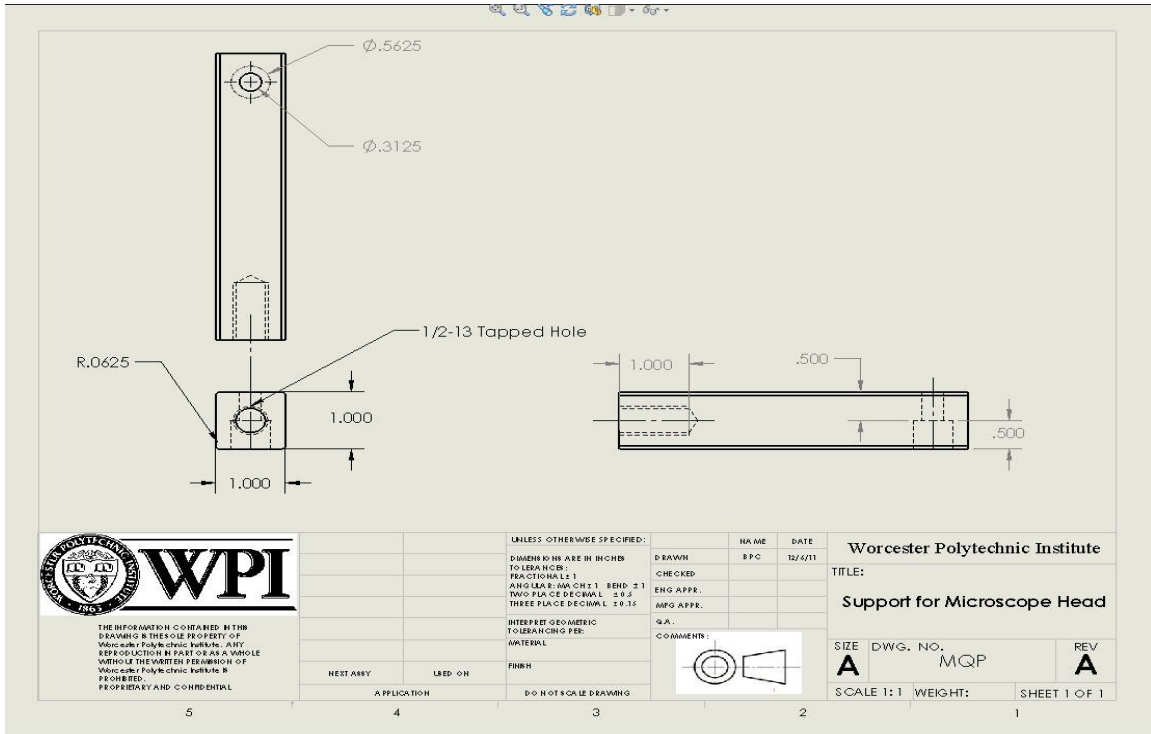
Figure 34: Final drawing of stereomicroscope support design

Figure 35: Final drawing of force transducer one design
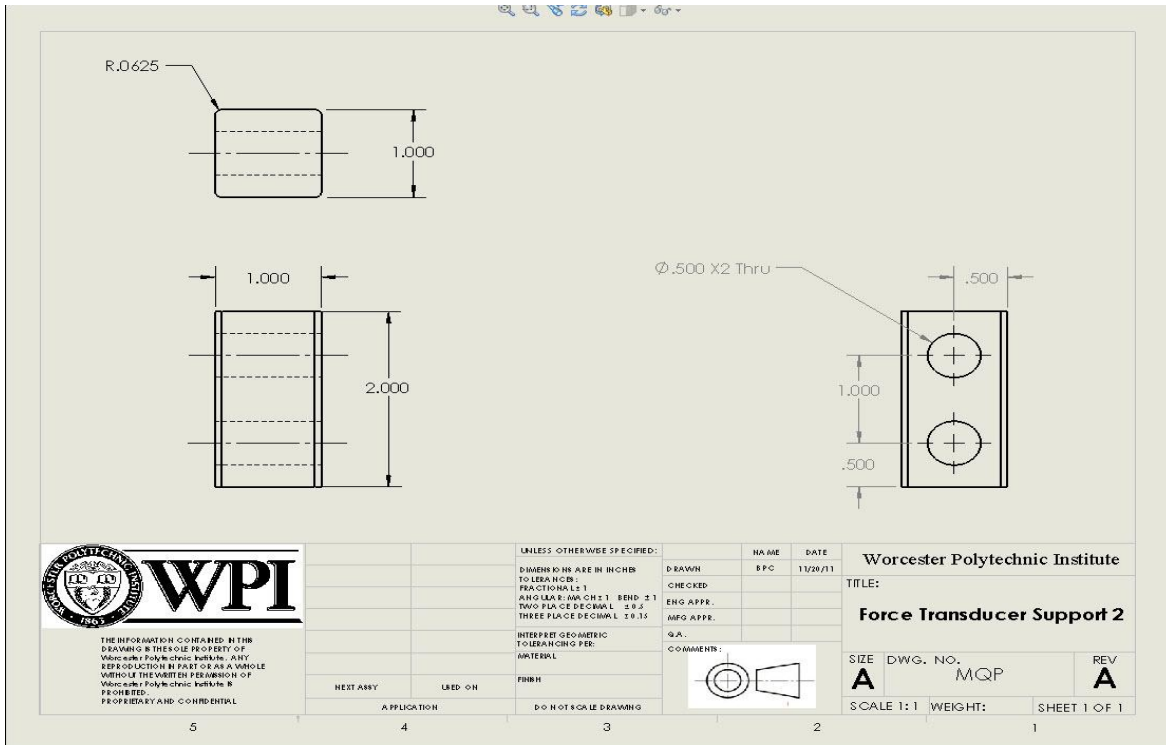
Figure 36: Final drawing of force transducer support two design
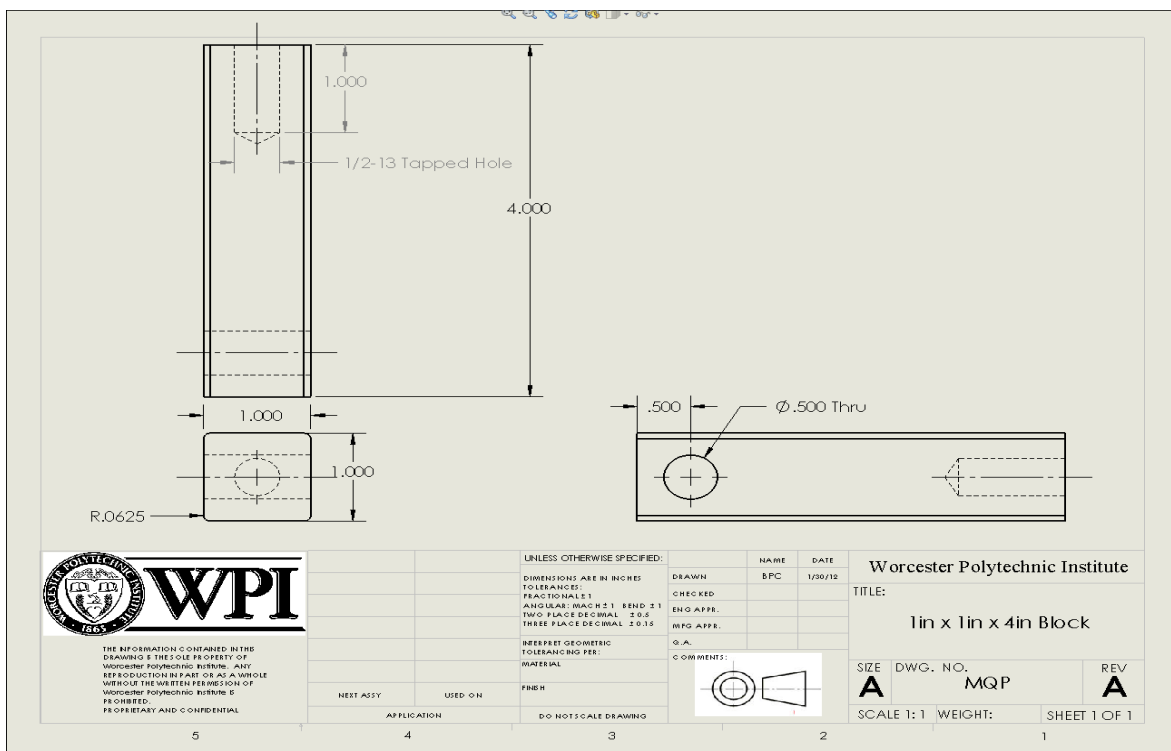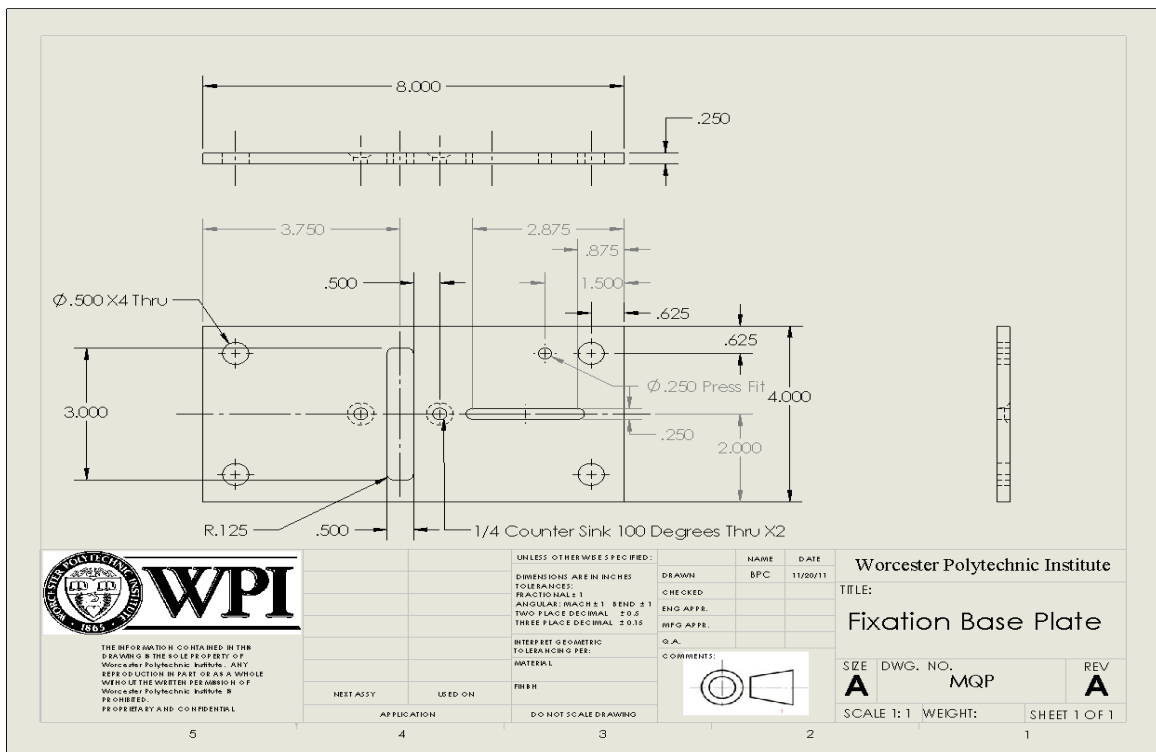

Figure 37: Final drawing of force transducer support one design
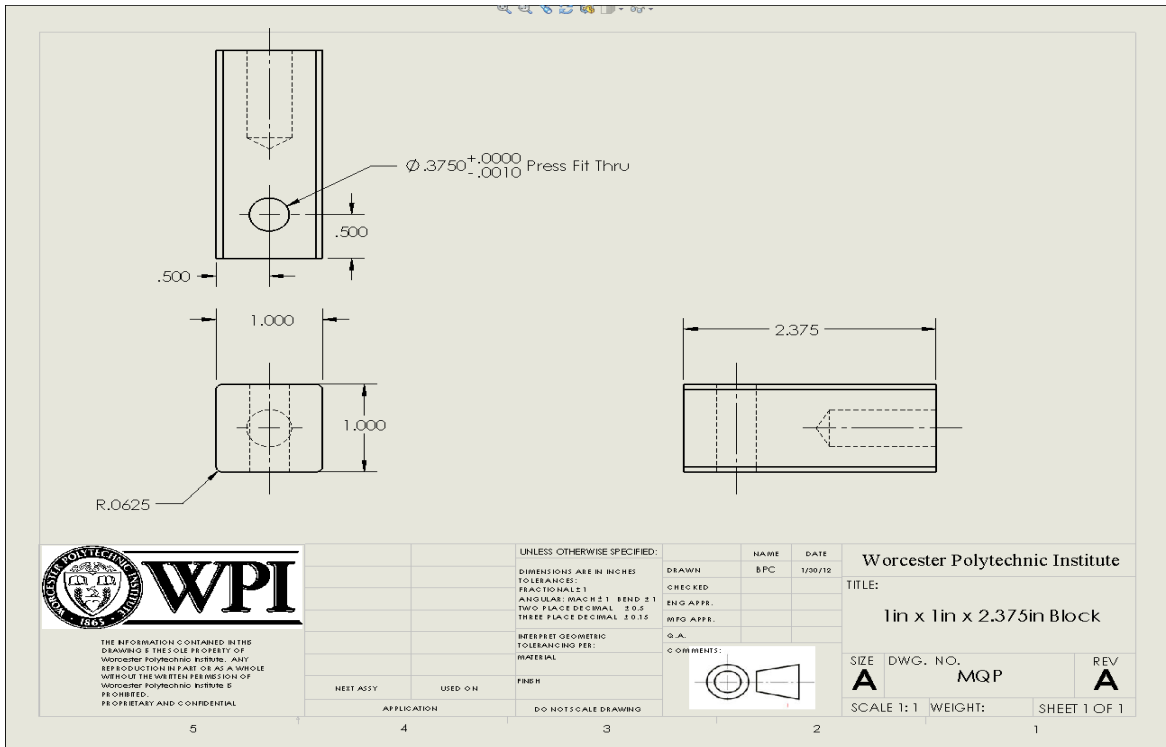
Figure 38: Final drawing of force transducer shaft support design

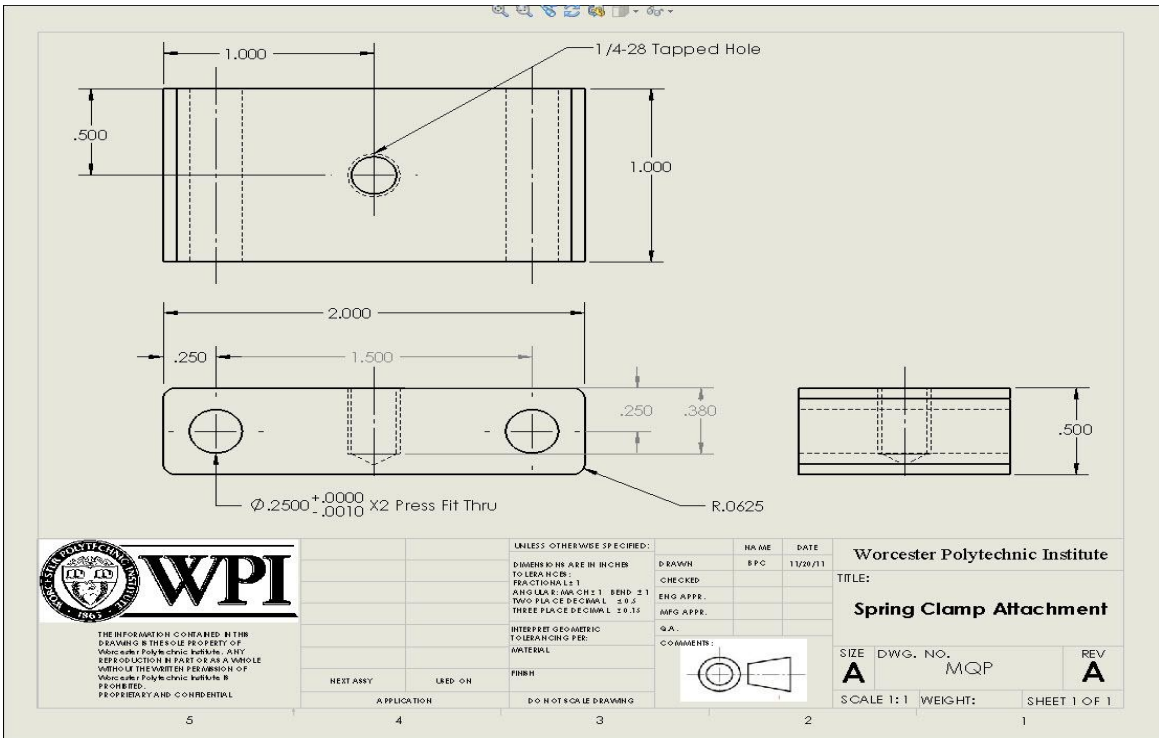**Figure 39: Final drawing of fixation base plate design**

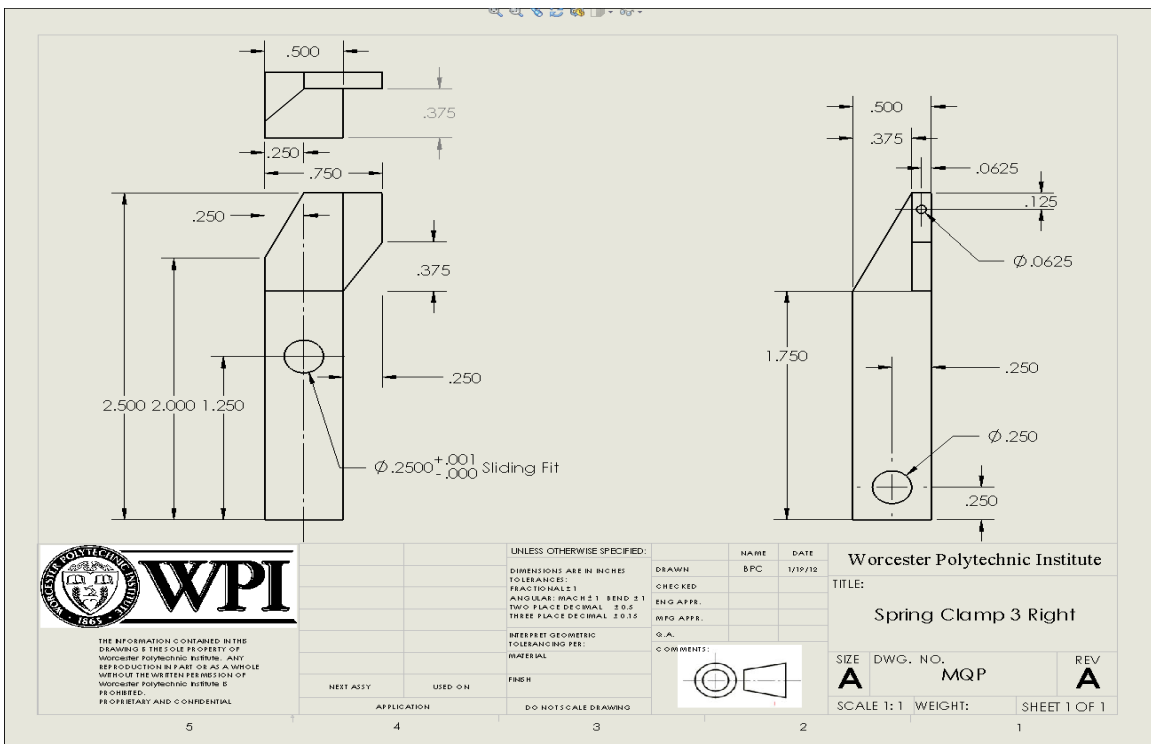**Figure 40: Final drawing of spring clamp attachment design**

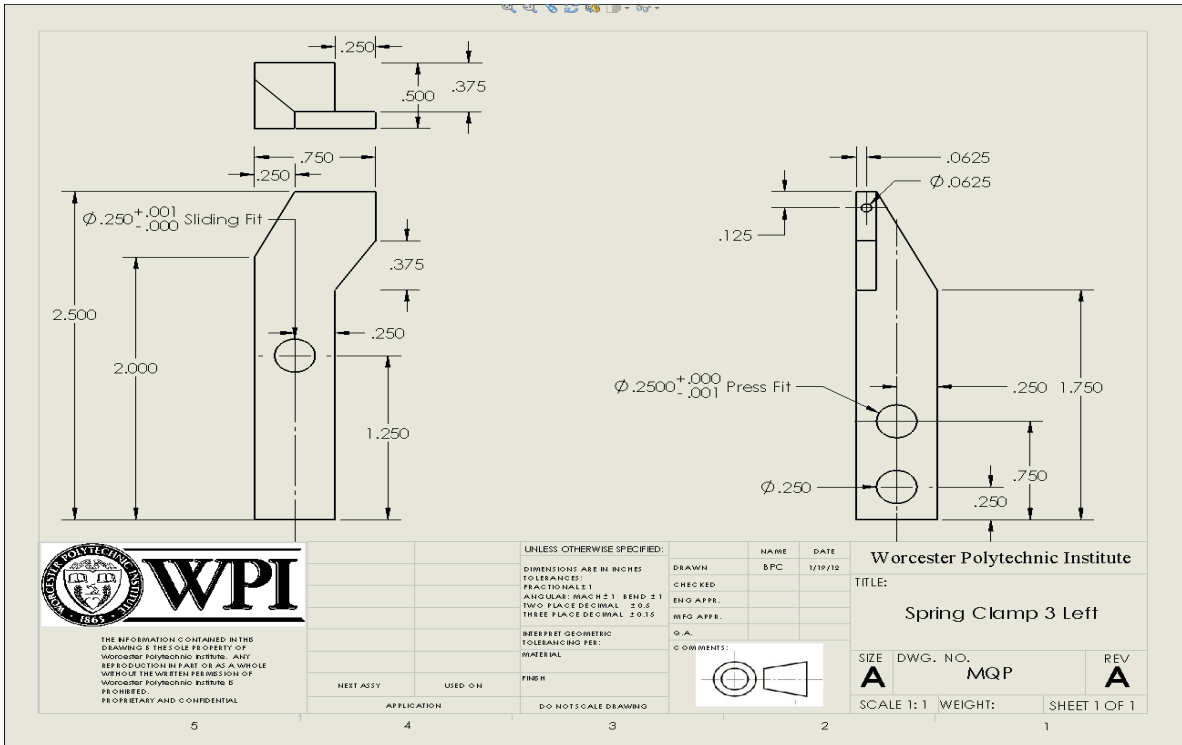**Figure 41: Final drawing of spring-loaded modular clamp design (Right)**

Figure 42: Final drawing of spring-loaded modular clamp design (Left)