Mechanical Stimulation of Engineered Muscle

A Major Qualifying Project
Submitted to the Faculty of
Worcester Polytechnic Institute
in partial fulfillment of the requirements for the
Degree in Bachelor of Science
in
Biomedical Engineering
By

Stephanie Godding

Susannah Gray

Rebecca Hamilton

Date: 4/26/18

Project Advisors:

Professor Raymond Page, Advisor

Table of Contents

Table of Figures 5

Table of Tables 6

Acknowledgements 7

Abstract 8

Chapter 1: Introduction 9

1.1 Background 9

1.1 Project Goals 11

1.2 Project Approach 13

Chapter 2: Background Research 16

2.1 Clinical Significance 16

2.1.1 Diseases 16

2.1.2 Injuries 18

2.2 Skeletal Muscle Anatomy 20

2.2.1 Structure and Function 20

2.2.2 Skeletal Muscle Development 23

2.2.3 Mechanism of Action-Contraction 23

2.3 Tissue Regeneration 26

2.3.1 Function & Purpose 26

2.3.2 Regeneration in Tissue Engineering 28

2.4 In-vitro Engineered Muscle Tissue 28

2.4.1 Tissue Engineering Approaches 29

2.4.3 Limitations of Current Techniques 30

2.5 Strategies for Advancement: 31

2.5.1 Electrical Stimulation 31

2.5.2 Existing Methods for Electrical Stimulation of Engineered Muscle 32

2.5.3 Mechanical Stimulation 33

2.5.4 Existing Methods for Mechanical Stimulation of Engineered Muscle 33

Chapter 3: Project Strategy 37

3.1 Initial Client Statement 37

3.2 Revised Client Statement 37

3.3 Design Requirements 38

3.3.1 Design Objectives 38

3.3.2 Design Specifications and Constraints 40
3.3.3 Design Functions 42
Chapter 4: Design Process 46
4.1 Need Analysis 46
4.2 Concept Map, Design, Prototyping, Modeling, & Feasibility Studies 47
Chapter 5: Design Verification 55
5.1 Seeding System Verification 55
5.1.1 Adequacy of Seeding System 55
5.2 Mechanical Stimulation Verification 56
5.2.1 Function of Pegged Plate 56
5.2.2 Function of Arduino Uno, NEMA Stepper Motor, Table Jack Assembly 56
5.2.3 Function of 96-Well Plate Stand 57
5.3 Final Experiment 57
5.3.1 Final Experiment 57
Chapter 6: Final Design and Validation 62
6.1 Device Overview 62
6.2 Device Validation 63
6.2.1 Industry Standards 63
6.2.2 Economic Impact 63
6.2.3 Environmental Impact 64
6.2.4 Societal Impact 64
6.2.5 Political Impact 65
6.2.6 Ethical Concerns 65
6.2.7 Health and Safety Issues 66
Chapter 7: Discussion 67
7.1 General Discussion 67
7.1.1 Seeding System Verification 67
7.1.2 Mechanical Stimulation Verification 67
7.2 Reviewing Design Requirements 68
7.2.1 Fulfillment of Design Objectives 69
7.2.2 Fulfillment of Design Requirements 69
7.2.3 Fulfillment of Design Specifications 70
7.2.4 Compliance with Design Constraints 72

Chapter 8: Conclusions and Recommendations 73
8.1 Conclusions 73
8.2 Recommendations 73
References 75
Appendix 77
A: Arduino Code 77
B: PDMS Protocol 78
C: Budget Analysis 79

Table of Figures

Figure 1. The structure of skeletal muscle. 21
Figure 2. Anatomical structure of sarcomeres within myofibrils. 22
Figure 3. Illustration of the H zone. 22
Figure 4. The regulation of muscle contraction via ion channels. {1} 24
Figure 5. The regulation of muscle contraction via ion channels. {2} 25
Figure 6. Repair following volumetric muscle loss. 28
Figure 7. Schematic of Mechanical Cell Stimulator Model I 34
Figure 8. Schematic of Mechanical Cell Stimulator Model II 35
Figure 9. Design attributes are illustrated and prioritized. 48
Figure 10. Preliminary illustration of Design Concept 1. 49
Figure 11. Preliminary illustration of Design Concept 2. 49
Figure 12. Schematic of Alternative Design #1. 51
Figure 13. Schematic of Alternative Design #2. 51
Figure 14. Schematic of Alternative Design #3. 52
Figure 15. Schematic of Alternative Design #4. 52
Figure 16. SolidWorks schematic of pegged plate/96-well plate assembly. 53
Figure 17. Illustration of mechanical stimulation means. 54
Figure 18. Relaxed state of PDMS posts. 58
Figure 19. Flexed state of PDMS posts. 58
Figure 20. Demonstration of measurement in ImageJ. 59
Figure 21. Percent strain plotted as a function of z-displacement. 60
Figure 22. An overview of design components. 62

Table of Tables

Table 1: List of Requirements 38
Table 2: Design Specifications 40
Table 3: Design Constraints 42
Table 4: Function-Means Chart 43
Table 5: Industry Standards 44
Table 6: Functional Blocks of Conceptual Designs 49
Table 7: Empirical and Theoretical Results Relative to Design Condition 61
Table 8: Budget Analysis 79

Acknowledgements

The authors would like to formally acknowledge and give thanks to the following people, who made this project possible: Professor Raymond Page (MQP Advisor), Bryan Choate (WPI PhD Candidate), Lisa Wall (Laboratory Manager), Tom Partington (WPI Machinist). The team would also like to acknowledge Jason Forte for his consult.

Abstract

Engineered skeletal muscle tissue has the potential to aid in valuable research and treatment methods for a plethora of musculoskeletal disorders and injuries. While advances have been made in the past decade, diseases such as muscular dystrophy lack sufficient predictive disease models to facilitate trials of novel medicine. The main technique utilized by scientists and researchers are “lab grown” tissues, cultured from mouse or human myoblasts, in-vitro; these techniques allow for growth of muscle cells from their proliferative and differentiating stages to the formation of mature myofibers. However, these tissues tend to lack vital characteristics of native tissue. This project thus focuses on one mechanism which has the potential to improve the traits of myoblasts cultured ex-vivo; mechanical stimulation. In the absence of a streamlined mechanical stimulation device, the goal of this MQP project was to design, prototype, and test such a device.

Chapter 1: Introduction

1.1 Background

The effects of musculoskeletal disorders and conditions are widespread amongst world populations. Musculoskeletal conditions impact up to 1.7 billion people worldwide, being the second greatest cause of disability, and the fourth greatest impact on the world's population health overall [1]. Specifically in the United States, musculoskeletal conditions are reported by 1 of every 2 Americans, and were estimated at costing $213 billion [1]. These conditions and disorders cause impairment of performing daily activities, heightened visits to the physician's office, and high costs and spending for the individuals and families afflicted with such conditions.

Musculoskeletal conditions are a result of a series of causes such as genetic inheritance, age, or injury. One major field of musculoskeletal conditions are sports-related injuries. Skeletal muscle injuries account for 55% of all sports related injuries [2]. Injuries to the skeletal muscle tissue vary in size and severity. Therefore, the most important factor in finding a treatment is understanding the mechanisms of the muscle. Mechanisms such as contraction and flexion. Muscle strains and contusions are the most common injury among athletes. A muscle strain occurs when a heavy, compressive force is applied to the muscle and a strain occurs when excessive tensile force is applied to the muscle. The excessive force leads to the overstraining of the myofibers leading to a rupture near the myotendinous junction [2]. Put simply, skeletal muscle is composed of two main components: the myofibers and the connective tissue [2].

In addition to physical injuries, genetic disorders, such as muscular dystrophy can lead to the degradation of muscle tissue. In patients with muscular dystrophy, gene mutations, such as missense mutation, interrupt the production of protein that forms healthy muscle tissue [3].

Different types of muscular dystrophy affect different muscle groups and different ages of individuals. The most common types of muscle dystrophy are Duchenne and Becker Muscle Dystrophy [3]. In the United States, about 1 out of every 7,250 males from age 5-24 were diagnosed with DMD or BMD in 2007. Duchenne Muscle Dystrophy is about three times as prevalent as Becker Muscle Dystrophy [3]. DMD is earlier onset and diagnosed than BMD but both conditions still result in devastating effects on afflicted individuals such as loss of muscle mass and function, loss of independence, and premature death. Currently on the market, there is no treatment to cure or reverse the damage done by muscular dystrophy.

Engineered skeletal muscle tissue has potential to further *in vitro* and *in vivo* research for the treatment of such muscular diseases and injuries. Currently, there is no technology that can accurately mimic *in-vivo* skeletal muscle tissue outside the body. With the lack of a proper model, the medical industry is struggling to develop treatments for muscular diseases and injuries. Currently on the market, there are 2D in vitro models that utilize mouse cells. These models do not accurately represent native tissue. In order to properly represent native tissue, the tissue must be able to recapitulate the communication of muscle cells to the extracellular matrix. Drugs must go through rigorous testing before being released.

An in-vitro skeletal muscle sample can help expedite the process of pharmaceutical drug testing. Drug testing begins on a large scale. Once the compound shows promise, in-vitro testing on cell compounds is typically performed first. Next, testing is performed on an animal disease model. After those tests bring a positive result, in-vivo animal disease model is performed [4]. In-vitro skeletal muscle tissue model is essential for treating and curing muscle injuries and diseases.

Mechanical and electrical stimulation is the most effective way to mature in vitro skeletal muscle tissue. The current gold standard for in-vitro skeletal muscle tissue engineering is designed to mechanically stimulate or electrically stimulate large tissues of muscle. Therefore, many of the systems are not capable of high-throughput applications [4]. Two systems on the market that are able to do so are MagneTissue Bioreactor and IonOptix C-Pace EP. MagenTissue Bioreactor These two systems are made to be used on larger tissue so they are not suited for high-throughput applications. Although these are the gold standard they lack the ability to form in-vitro muscle tissue that mimics the native tissue.

1.1 Project Goals

Researchers currently lack any true in-vitro model of human muscle cell. This has led to a gap in the understanding and treatment of the many musculoskeletal disorders that affect a portion of the human population. It has also led to a demand and high cost of five billion dollars per viable drug in the pharmaceutical drug market. In order to make the necessary advancements for the benefit of both the industry and those afflicted individuals it is vital to produce an accurate human model. In order to accomplish this, there are a series of considerations and goals which must be made and met. In the design of an in-vitro skeletal muscle tissue system that will mimic the traits of normal in-vivo human muscle cells many considerations arise in regards to both the technical and physiological aspects. Some of the major aspects of this model which must be considered and met in the design include; dimensions, stage of differentiation, maturation, fiber alignment, stimuli, materials, and anchorage. Accurately doing these things will allow for the model to succeed i.e the muscle cells will mature and function like those would in the human body. Considerations must also be made in regards to the user, federal regulations, as well as funding.

The first major considerations involve the proper tissue dimensions, fiber alignment, and mechanical and electrical stimuli. These aspects directly correlate to the survival of the muscle cells. The cells must be accommodated in a properly sized structure, guided to the right alignment as they grow to differentiate into muscle fibers, as well as exercising the cells with electrical and mechanical apparatus. The muscle cell model needs to mimic the in-vivo conditions and functions of native human muscle cells. In-vitro human cells must be cultured to differentiate, mature, and linearly align as they would in the human body to ensure they become strong and well-formed muscle fibers.

Another goal which is central to the project's success is the determination of the optimal stage of myoblast differentiation. Some considerations will include the initial seeding of the system and the amount of time for the cells to mature. Maturation is extremely important for the viability and proliferation of the human muscle cells. It will ensure they mature to the point of contractile function which is something human muscle cells do in the body for survival. Optimal cell culture conditions are not exact but differing approaches will be analyzed for their effect on the differentiation and survival of the muscle cells.

An additional aspect would be addressing material selection issues. This includes regard to the extracellular matrix or scaffold, natural polymers utilized, and other artificial materials which may be used. The selected materials and scaffold directly affect the desired cell tissue and also affect cell growth, differentiation, and cell organization during formation. For example, the use of hydrogels which can be natural or artificial polymers can assist the cells in growth and alignment into muscle fibers. Also the use of PDMS as apparatus in contact with the cells will allow the cells adhere and grow due to its biocompatibility. Various materials such as the

hydrogel or PDMS apparatus, etc. will be explored for their effects on the growth and survival of human muscle cells.

Also considering the methods of applying and measuring contraction to the muscle cells is an aim of the model. This aspect will be indicted by the mechanisms of electrical and mechanical stimulation. Directly connected to the stimulation and contraction of the human muscle cells is their fiber strength. Ensuring that the muscle cells in the model are exercised and maintain sufficient strength will allow the model to be representative of the dense, long muscle fibers in human skeletal muscle. Some important kinetics of muscle cells include force, extension, and relaxation. Ensuring muscle cells are stimulated and exercised in a series of ways is important for developing complex muscle cells which can regrow and grow stronger and communicate with their surrounding matrix and cells.

One of the additional but equally important goals is to address the potential requirement for supporting the cells. As mentioned briefly before, human muscle cells are anchorage dependent for survival. Muscle cell cultures adhere to the surface of their environment. For example, muscle cells grown in a petri dish will adhere to the bottom of the plate. When the cells contract they will lift off the petri dish and will no longer be viable. For this reason, the muscle cells require another source of anchorage to ensure that they mature and differentiate.

1.2 Project Approach

The general approach of the project will cater to the overall need, which is to recapitulate the interactions of muscle cells with their extracellular matrix in order to create mature muscle tissue for research purposes. Our basic approach to this need is outlined by the following goals: design a system to create skeletal muscle in vitro, determine the optimal stage of myoblast differentiation, address material selection requirements, as well as maintenance of the mature

cells. When we state the phrase “optimal stage of myoblast differentiation”, we are referring to the many characteristics that should be exhibited in order to ensure that we have produced accurate, mature skeletal muscle tissue, that which can be manipulated for research purposes. The optimal stage of myoblast differentiation will be the point at which our engineered tissue most closely replicates the properties of native tissue, and depends on multiple factors, including choice of culture media, i.e. the composition of our extracellular matrix, as well as our means of applying the appropriate stimuli.

During and after the induction of differentiation, the cell needs to receive nutrients from our in-vitro extracellular matrix (ECM). In-vivo, the extracellular matrix consists of collagen, other glycoproteins, hyaluronic acid, proteoglycans and elastins, and these molecules are responsible for the harboring and delivery of nutrients [5]. The interactions between the ECM and cells have a direct influence on their adhesion, migration, growth, differentiation, and finally, maintenance. Collagen provides structural support for muscle tissue cells and is crucial for the conversion of the myoblasts.

In order to produce optimal myoblast differentiation the cells must assemble properly. Amateur skeletal muscle cells, or myoblasts, need cell-to-cell contact in order to fuse with each other and form multinucleated myotubes. This is one known limitation of current in-vitro models. Alternately, magnetic field guided-assembly for the design of cell environment will produce better cell alignment [6].

The cells must be stimulated and contractions measured. Past approaches use flexible PDMS posts as anchorage points within a 96-well plate. With this technique, the PDMS posts are manipulated to exercise the muscle. One alternate consideration for mobilization of PDMS posts is an air-pocket design that can change the volume of a chamber beneath the matrix with the

addition or withdrawal of air. Another consideration is the use of magnets for PDMS post mobilization. Regarding electrical stimulation, stipulations must be made concerning voltage, frequency, amplitude, and pulse width. All of these variables will have a bearing on the resulting force production of the mature muscle [7].

With these approaches to engineering the human skeletal muscle tissue, it is ultimately our hope that this model fosters clinical applications within the pharmaceutical industry as well as other research responsible for creating more efficient predictive disease models.

Chapter 2: Background Research

2.1 Clinical Significance

Human muscles aid in the function and motion of the body as a whole. When there is a defect or injury to them, muscles cannot function properly. Muscle disorders, known as myopathies, in addition to muscle injuries account for a huge world-wide impact. There is a large range of conditions, each with varying causes and symptoms. One of the things that most have in common however, is lack of an effective cure. As a whole, musculoskeletal conditions impact the quality of life of individuals greatly.

To specify, musculoskeletal diseases impact about one out of every two people in the United States over the age of 18, with about three out of four individuals over the age of 65 being impacted [8]. The rate of these diseases and conditions outweigh that of other significant afflictions, such as circulatory and respiratory disorders [8]. One huge impact these afflictions have is the rate of visits to the physician's office, emergency room, and hospital thus amounting to large costs on both the individuals and the various medical institutions. Another aspect often inflicted by various musculoskeletal disorders is the cost of major diseases or significant injury which require long term care for the pain or disability that may affect the individual [8].

2.1.1 Diseases

As mentioned before, there is a wide range of skeletal muscle conditions and disorders. Some major types of diseases include metabolic diseases, genetic disorders, inflammatory diseases, and neuromuscular disorders.

Diagnosis is key in proper management and treatment of the various conditions. Some major diagnostic procedures include; history and clinical exams, blood biochemistry,

electromyography, muscle biopsy, nuclear magnetic resonance, measuring the muscular cross-sectional area, functional tests, provocation tests, and studies on protein turnover [9]. Typically, one or all of these tests are crucial in comprehensive diagnosis and treatment plans. However, there is the drawback that identification of the exact disorder may not be possible [9]. All in all, these procedures are necessary at providing relevant information to both the patient and the doctors.

One of the most prevalent musculoskeletal disorders is muscle dystrophy. The two most common types of muscular dystrophies are Duchenne and Becker's muscular dystrophy, both of which the muscle protein dystrophin is either present in lesser amounts or completely lacking [11]. The function of the dystrophin protein is to transfer force of the muscle contraction from the inside of the muscle cell to the cell membrane and beyond [10]. The protein is extremely long because it physically connects the center of the muscle cell to its peripheral anatomy; with the central region, known as the rod domain consisting of spectrin repeats [10]. These units are extremely important in linking the interior to the exterior of the muscle cell, and in the communication between the cell interior and exterior proteins that work together to initiate muscle contraction [10]. In these genetic disorders the proteins lose their function as a result of fewer spectrin repeats, which contributes to an overall muscle weakness.

Both of these dystrophies, amongst the nine other types, are both degenerative and genetically inherited that greatly impact the function and health of voluntary muscle groups [10]. With Duchenne muscular dystrophy, there is no functional dystrophin produced, and with Becker Muscular Dystrophy dystrophin created is partially functional [10]. The direct implication of this is muscles in patients with BMD degrading slower and to less of an extent than patients with DMD.

Duchenne muscular dystrophy (DMD) and Becker Muscular Dystrophy (BMD) are X-linked disorders, meaning they can only impact male populations. DMD is most common in children, characterized by proximal muscle weakness as well as hypertrophy in early childhood development [11]. The affected children are typically wheelchair bound by age 12, and die in late teens or early twenties due to cardiorespiratory complications [11]. With BMD, the onset occurs in late childhood or adolescence, with a slower and less predictable course in comparison to DMD [10]. A general weakness occurs at first specifically in the hip, pelvic, thigh, and shoulder areas, followed by calf enlargement and significant cardiovascular issues later down the road [10]. Unlike DMD, individuals with BMD can survive into mid-late adulthood. While there are various breakthroughs with identifying the protein dystrophin, no treatments have progressed to the experimental stage [11]. In addition, there is no real cure for any form of muscular dystrophy.

2.1.2 Injuries

Another relevant area in musculoskeletal conditions are injuries to the muscle. While set specifications for muscle injuries lack, the main differentiating factor is how the injury occurs and to what extent the trauma is [12]. In a broad sense, skeletal muscle injuries are classified as either acute/traumatic injuries or chronic/overuse injuries [12]. Acute injuries are characterized by a singular traumatic event that results in a large injury to the muscle, while overuse injuries are typically exercise induced and result from a build-up over time and tend to be more subtle [12]. Acute injuries are easier to diagnose because the link between the cause and symptoms is made immediately and easily after injury. Since chronic muscle injuries occur over time, little by little, diagnosis is a little more challenging because the link between the symptoms and cause is less immediate [12].

Another type of muscle injury are muscle strains, which are contraction induced, and result in the tearing of muscle fibers due to an excessive mechanical loads or stresses [12]. There are three categories of muscle strains based on the severity of the injury. Grade I, or mild strains, are when a limited number or muscle fibers are impacted, there is little to no decrease in strength or range of motion, and resultant pains are delayed [12]. Grade II, or moderate muscle strains, are defined as injuries that impact nearly half of the muscle fibers, pain and swelling occurs with a minor decrease in strength and function [12]. Lastly, grade III, or severe muscle injuries are constituted by a complete rupture of the muscle, accompanied by severe swelling and pain and loss of function at the musculotendinous junction [12].

With these types of injuries the usual repair process follows a typical pattern of two phases; destruction phase and repair and remodeling phase [12]. The first phase is constituted by the initial trauma and the muscle fiber tear. Necrosis of myofibers occurs due to the injury, followed by an inflammatory process and the removal of the necrotic tissue elements by specialized cells [12]. In the repair and remodeling phase satellite cells begin the myofiber regeneration and a connective tissue scar attempts to fill the gap created by the tear. In the first 10 days, the scar tissue represents the weakest aspect of the injury, after 10 days the surrounding muscle tissue is impacted, while full functional and strength recovery times are hard to quantify [12]. The most important processes in recovery include vascularization and nerve to muscle contact [12].

As with musculoskeletal diseases, proper diagnosis and treatment are crucial to patient recovery from muscle injuries. Diagnosis tools for muscle injuries include a thorough physical examination, factoring in patient history of injury, testing functionality, MRI, X-ray, Ultrasound, and CT scan [12]. For acute injuries the best treatment includes a short period of immobilization,

elevation, compression, elevation, followed by proper mobilization and rehabilitation exercises [12]. Traumatic muscle injuries typically require surgical intervention, healing time, and proper rehabilitation and conditioning [12]. Overall, with the current treatments for muscle injuries, outlook is positive. However, knowledge on the proper function and healing process will only aid the recovery process.

2.2 Skeletal Muscle Anatomy

This section outlines the structure, function and anatomy of skeletal muscle tissue.

2.2.1 Structure and Function

Skeletal muscles are responsible for the movement and structure of the human body. An individual skeletal muscle are made up of hundreds of skeletal muscle fibers wrapped in a protective connective tissue. Skeletal muscle fibers are made up of a single cylindrical muscle cell. The muscle is surrounded by epimysium, a connective tissue sheath. Fascia is located outside the epimysium and separates the muscle. The epimysium creates compartments in the muscle by projecting inward and dividing the muscle [13]. Those compartments hold a bundle of muscle fibers called a fasciculus. The perimysium is a layer of connective tissue that surrounds each fasciculus. The endomysium surrounds the each muscle fiber within the fasciculus. The connective tissue covering the muscle fibers act as support and protection from contraction forces.

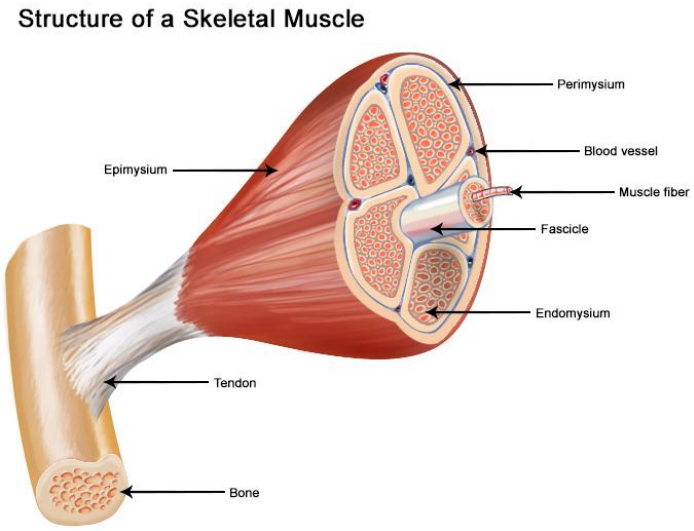


Figure 1. The structure of skeletal muscle. [13]

The sarcomeres are the contractile backbone of the myofiber. They are cylindrical bundles composed of actin, a thin filament about 7 nm, and myosin, a thick filament about 15 nm. The actin filaments are contained in the I bands and the myosin is found in the A bands. Tropomyosin and troponin are also found inside the cylindrical bundles. The Z disc is where the sarcomeres end. Figure 3 below shows the H zone, an area where only myosin is observed. The M line is the anchor of the myosin filament.

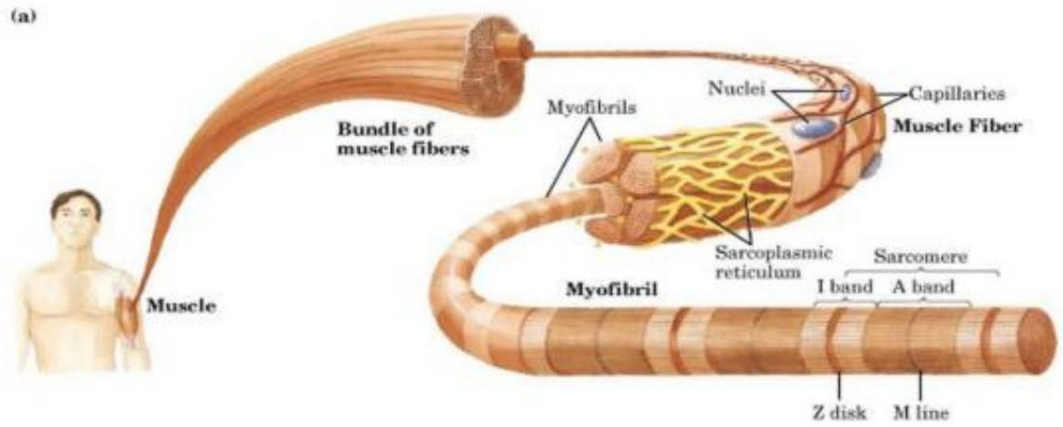


Figure 2. Anatomical structure of sarcomeres within myofibrils. [13]

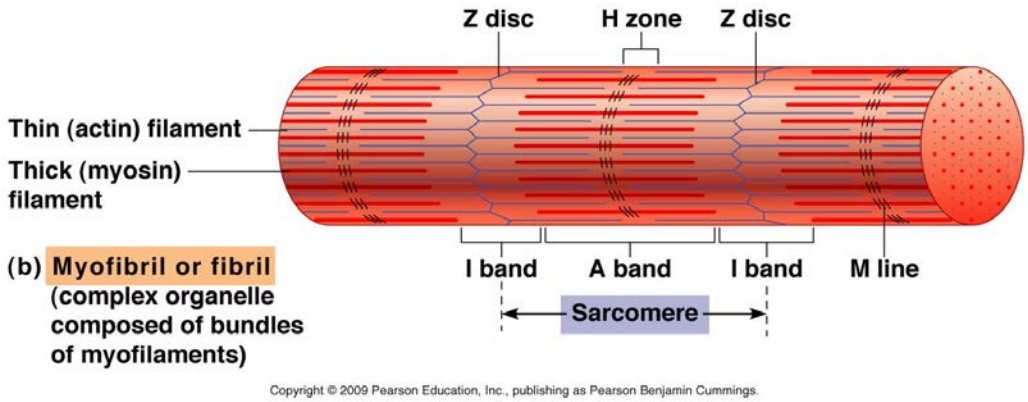


Figure 3. Illustration of the H zone. [13]

Skeletal muscle is responsible for many functions of the body, both mechanically and metabolically. Mechanically, skeletal muscle controls the conversion of chemical energy to mechanical energy. This creates power, helps maintain posture, and generates movement [13]. Metabolically, skeletal muscle acts as a storage unit for important substrates for basal energy metabolism [13]. This substrates include amino acids and carbohydrates. These aid in the body's

ability to produce heat and maintain core temperature, and the consumption of oxygen during physical activities. Amino acids are used by other tissues like skin, brain, and heart for protein synthesis [13]. Additionally, muscle mass is important in maintaining health. A lower muscle mass decreased the body's ability to respond to stress and illness.

2.2.2 Skeletal Muscle Development

Myogenesis is the formation of skeletal muscle tissue. Myoblasts proliferate in the presence of fibroblast growth factor. The fusion of the myoblasts forms muscle fibers [13]. When there is no fibroblast growth factor present, the myoblasts stop dividing and form fibronectin in the extracellular matrix. Next, the myoblasts align and form myotubes. There are multiple muscle-specific genes that are expressed during myogenesis. These include the myocyte enhancer factors, serum response factor, and myogenic regulatory factors [13]. The serum response factors express the striated alpha-actin genes and regulate myogenesis. The myogenic regulatory factors are muscle-specific and include myogenin. Myogenin is involved in the alignment of cells during myogenesis.

2.2.3 Mechanism of Action-Contraction

Contraction is the forceful shortening or tightening of a muscle that pulls on a joint as a result and causes a movement [14]. Contraction is initiated by the brain sending signals to the muscle and as a result the muscle will contract and move. Another big factor in the contraction of a muscle is the muscle tone. The tone of a muscle speaks to its readability to contract and the tendency of relaxed muscle to resist stretching [14]. A muscle with good tone will hold its shape and elastic properties and respond promptly to signals of contraction. Both contraction and tone of muscles are very important for the health and function of the human body impacting muscle

strength, body posture, and coordination. The tone and contraction of the muscles rely heavily on their ability to respond to stimulation signals nerve cells relay from the brain [14].

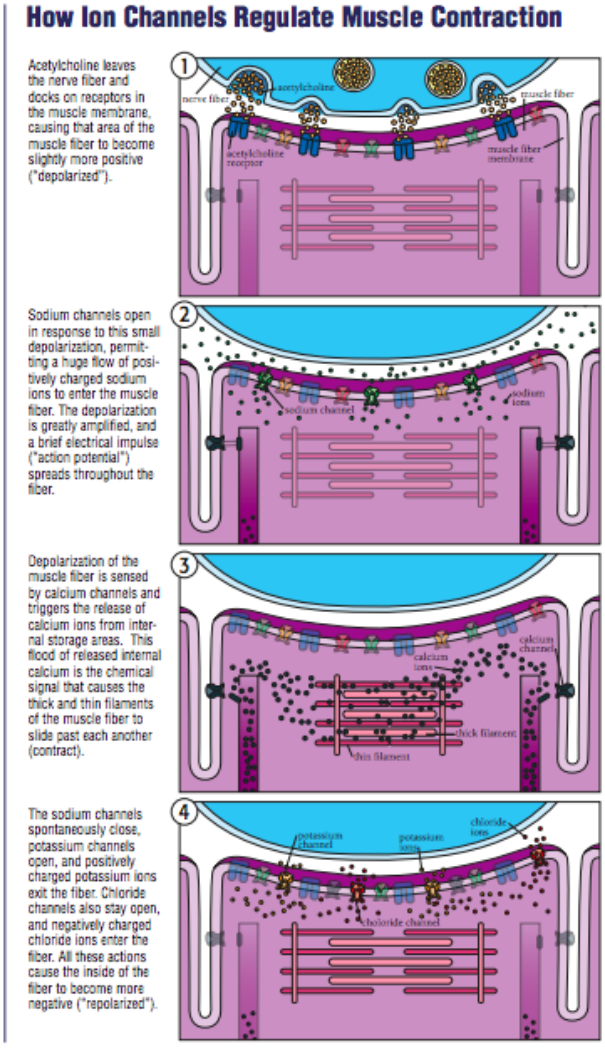


Figure 4. The regulation of muscle contraction via ion channels. [15]

The sliding filament theory is the accepted explanation for how muscles contract to produce a force [15]. Each muscle is made up of small fibers called myofibrils. The myofibrils have actin and myosin which slide in and out of each other, generating a contraction. There are

four major steps in the process. The first step is muscle activation where the motor nerve sends an impulse that arrives at the neuromuscular junction. The sarcoplasmic reticulum is stimulated and releases calcium into the muscle cell. Next, the calcium binds with troponin which binds the actin and myosin together. ATP energy is used to bind and contract the actin and myosin together. After, actin and myosin remain strongly bonded because ATP is synthesized again. The final step is relaxation. During this step stimulation to the nerves is stopped and calcium is pumped back into the sarcoplasmic reticulum. This breaks the bond between the actin and myosin. Once the actin and myosin are separated, the muscle can relax. Additionally, relaxation also occurs when there is no more ATP [15].

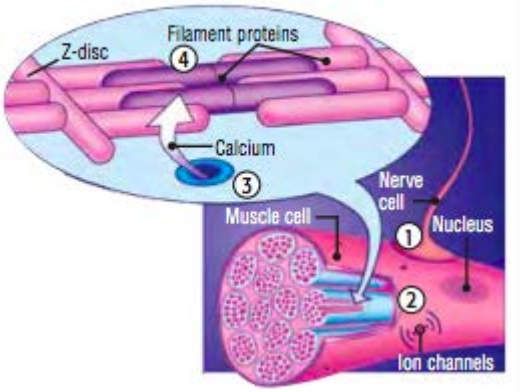


Figure 5. The regulation of muscle contraction via ion channels. [16]

There are three main “rules” for there to be a muscle contraction. The first is that there must be a neural stimulus. The second, there must be calcium inside the muscle cells. Finally, ATP must be available for the bonding of actin and myosin to occur. There are four main ways to stop a contraction. The first is fatigue of the energy system. This occurs when there is no more ATP left so the cells stop contracting. Next, the nervous system is unable to produce enough

impulses to release the calcium into the muscle cells. Voluntary nervous system control can send signals to stop the release of calcium ions, stopping the contractions. Finally, a sensory neuron will tell the brain that the muscle is being injured while lifting something heavy, and stop the contraction.

If the process described above is interrupted at any stage from the reception of the nerve signal and the action of the filament proteins then the muscle itself will lose the normal capacity it has for contraction and tone [16]. As an extreme the muscle could be limp and weak as a result or the muscle can be involuntarily active and not able to relax. Both of these extremes or any variation in between would definitely affect the physical capabilities of an individual who requires constant and consistent muscle stimulation and strength.

2.3 Tissue Regeneration

Tissue regeneration is the regrowth of new tissue to a damaged or injured site. In the case of severe damage, there is typically volumetric muscle loss. In the case of severe injuries the tissue will scar over, the tissue will lose physiological function. This section outlines the regeneration process and its impact in tissue engineering.

2.3.1 Function & Purpose

Injuries to the skeletal muscle system are common. The ability of the muscle to repair itself after damage is largely dependent upon the type and severity of the injury. If an injury diminishes the muscle by more than 20%, the muscle will form scar tissue and the natural repair process will not occur [17]. If the damage is not as severe, the muscle will undergo the regeneration process. The repair process can be divided into three main phases [17]:

1. The first phase is the destruction and inflammatory phase. During this phase myofibers experience rupture and necrosis and experience a cellular inflammatory response.
2. The second phase is focused on the phagocytosis of the necrotic muscle fibers. This phase includes the generation of muscle fibers and formation of scar tissue.
3. The third phase is the remodeling phase. The muscle fibers reorganize and restore function to the muscle.

Repair to a muscle with volumetric loss occurs differently. Figure 6, below, demonstrates the repair process. Step A in the figure below shows the myofibers being degraded by the invading inflammatory cells. This removes cellular debris and attracts satellite cells. Step B shows the satellite cells differentiating into myoblasts. Next, step C, is where fibroblasts deposit scar tissue to the impacted area. New muscle tissue is formed in step D by forming myotubes that fuse with existing myofibers. This process is known as myogenesis. In injuries with large volumetric loss, scar tissue begins forming faster than the myogenesis process, seen in step E. The scar tissue prevents the myofibers from closing the wound. This results in the lack of neuromuscular junctions in the damaged tissue of step F.

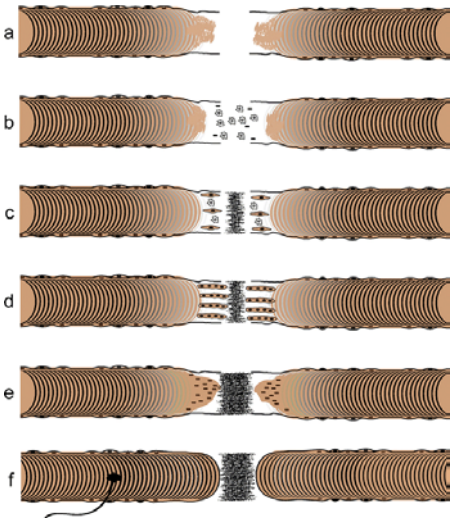


Figure 6. Repair following volumetric muscle loss. [17]

2.3.2 Regeneration in Tissue Engineering

Tissue engineering aims to create a model that accurately mimics native tissue. In-vivo, tissue engineering aims to replace loss or damaged tissue by sending out progenitor cells or scaffolds to regenerate the tissue [18]. The aim of regeneration in tissue engineering is to replace loss or damaged tissue. Tissue engineering implements biomaterials that mimic the extracellular matrix in order to promote the growth, differentiation, and alignment of cells [19]. Regeneration of tissue via engineering can repair tissue that would otherwise lose function. Implantation of engineered skeletal muscle tissue has been shown to accelerate functional recovery [19].

2.4 In-vitro Engineered Muscle Tissue

Developing an in-vitro engineered skeletal muscle model poses a challenge due to the complex nature of native tissue. Recreating similar maturation and alignment of cells found in-vivo is difficult. Currently, there is no technology that creates an engineered muscle tissue that mimics the packed, uniformly aligned, and differentiated myofibers that make up native tissue [20]. However, there are techniques that can aid in the maturation process. Electrical and

mechanical stimulation have been shown to increase the biomimetic properties. The following section outlines current tissue engineering approaches in the industry.

2.4.1 Tissue Engineering Approaches

There are multiple techniques for tissue repair and regeneration of skeletal muscle tissue. One strategy is to use biomaterials to rebuild tissue [21]. New materials such as tetrafluoroethylene and silicone are used to provide structure to the muscle, but lack the functional component that is found in native tissue. A better understanding of the extracellular matrix and the role it plays in the body, provides new biomaterials that have been designed to better mimic in vivo tissue. There are three main types of biomaterials that are used in tissue engineering [21]. The first is naturally derived materials such as collagen and alginate. Next is acellular tissue matrices like bladder submucosa. Lastly, is synthetic polymers such as PGA, PLA, and PLGA. The first two classes, naturally derived materials and acellular tissue matrices, have been shown to display regeneration in muscle tissue. Synthetic polymers are able to be produced on a large scale and have controllable properties like strength and degradation rate. In all three classes, the biomaterials aim to replicate the mechanical and biological function of native tissue. The aim is to have the cells adhere and grow around and on the biomaterial in order to form new tissue. Muscle cells are anchorage dependent, meaning they will die if they do not have a substrate to attach to. In this strategy, it is important for the biomaterial to be compatible with the body. An incompatible material will cause an inflammatory response and be rejected.

In addition to biomaterials, another tissue engineering approach is the implementation of scaffolds. The scaffolds are used to mimic the structure of in-vivo conditions, including the extracellular matrix to allow for cells to adhere, differentiate and mature in the same manner found in-vivo [22]. Two techniques are typically used: top-down and bottom-up approaches. In

the top-down approach, the scaffold the cells are cultured on is designed to mimic the structure, composition, and mechanical properties of the tissue [22]. In the bottom-up approach, the aim is to create a scaffold that is more biomimetic by replicating the functional unit of the tissue [22]. The scaffolds should be designed to be porous. The pores allow the cells to migrate throughout the scaffold and allows nutrients and oxygen to diffuse. Cells showed better maturation and differentiation on a 3D scaffold when compared to cells cultured on a 2D surface [22]. Scaffolds function as a microstructure for the development of engineered tissue.

The cell selection process is an important factor in tissue engineering. Growth of in-vitro tissue largely depends on the accessibility of tissue-specific cellular phenotypes [22]. The cells must be able to recapitulate the characteristics of native tissue. Additionally, the number of cells being cultured should represent the physiologically demand. When testing drugs in pre-clinical and clinical trials, it is important to use human cells, as other types may not illicit the same drug response. Cells that are cultured from humans are adult primary cells, but are hard to isolate and remove. Adult primary cells have a short life same and slow proliferation rate [22]. Stem cells have been used as an alternative. Stem cells are undifferentiated and are able to differentiate into multiple different specialized cells. The main concern when using stem cells is controlling the desired lineage and the immature phenotype with a similar expression profile to fetal cells [22].

2.4.3 Limitations of Current Techniques

Currently on the market there is no set standard for how to properly engineer muscle tissue. There is no accurate in-vitro model that has been developed, therefore there is limitations to every technique. In native tissue, cells are able to differentiate and expand as long as they have an anchor point. In-vitro cells are limited to the anchorage site and are not able to proliferate as freely [23]. In-vitro, the tissue often lacks the same density and alignment as native tissue. The

extracellular matrix found in-vivo is difficult to replicate, usually utilizing proteins that have been left over from the hydrogel assembly. In some cases, the use of a 2-dimensional surface has been utilized. The 2D surface is non-ideal as it resulted in the cells lifting from the surface and consequently dying. Additionally, native tissue experiences regular exercise and stimulation in order to increase alignment and strength. It is difficult to exercise the muscle in the same manner ex-vivo. Matching the oxygen and nutrient supply of native tissue is another obstacle [24]. There is a limited amount of nutrient and oxygen that can be supplied to in-vitro muscle tissue. Matching the mechanics of the vascular system in native tissue is difficult, as there is no predetermined amount.

2.5 Strategies for Advancement:

In an ex-vivo or in-vitro muscular environment there are many crucial characteristics from native tissue which must be mimicked. In order for a good predictive disease model the cells and tissue must mature and function properly. That way, they possess the same traits as native tissue and can be used to accurately learn about the potential treatments and cures for diseases related to skeletal muscle function. Some vital characteristics for the tissue to obtain include; fiber alignment, fiber strength, cell density, and vascularization.

2.5.1 Electrical Stimulation

Electrical pulse stimulation is necessary for the development of skeletal muscle. In the body, the central nervous system sends electrical impulses from the motor neurons. This is an important step in muscle development and maturation [25]. Mimicking of these crucial impulses is a core requirement for in vitro engineered skeletal muscle tissues. The main struggles of simulating electrical impulses ex vivo is however is controlling/perfecting the various

parameters; voltage, amplitude, pulse width, and pulse frequency so that the regimen properly induces maturation and functionality while avoiding electrochemical damage to the tissue [25].

2.5.2 Existing Methods for Electrical Stimulation of Engineered Muscle

Due to the importance of electrical stimulation to biomimetic tissue constructs in vitro, researchers have attempted to perfect a regimen. Considering the difficulty of perfecting all the parameters of electrical stimulation, there is no set standard in the industry.

A few methods which exist include the Myoforce Analysis Device (MAD) created and tested by Herman Vandenburgh, electrical pulse stimulation (EPS) outlined by Akira Ito et. al [25][26]. The MAD is an automated system that inserts electrodes into each well of a 96-well plate, implements a set stimulation regimen, and captures images of the contracting cells. This electrical stimulation device is encased and insulated for incubation of cell cultures. The specifications include a heated stage to keep the cells warm and lifts the plate up to electrodes which applied a 13V voltage, 40Hz frequency, over the course of 2 seconds with pulse widths of 4ms[26]. In addition to the stimulation 40-60 images were captured of contractions and automatically processed by a MatLab software algorithm to measure force [26]. In the Akira Ito model, the electrical pulse stimulation was applied to mouse myoblasts cultured and differentiated for four days. The cells were housed in 4 and 6 well plates in a chamber, and stimulated by two carbon electrodes at voltage rates of 0.1,0.3, and 0.5V/mm and pulse widths of 2,4, and 10 ms [25]. The analysis of twitch peak forces allowed for the researchers to conclude that EPS aided in proper functional development of engineered in vitro skeletal muscle. A continuous electrical regimen of 0.3V/mm amplitude, 4ms pulse width, and 1Hz frequency showed the most promising results of a 4.5 fold increase in muscle force after 14 days [25].

2.5.3 Mechanical Stimulation

Cells, such as muscular cells, are aware and sensitive to the changes which occur in their environment, including certain physical and mechanical stimuli. A specific cellular response occurs as a result of these stimuli, which has been modeled and analyzed in in vitro studies for better knowledge on how muscles grow and function outside of the body [27]. There is an inherent and vital relationship between the cytoskeletal architecture of skeletal muscle and their proper maturation and functionality [27].

Specific physical training such as endurance and resistance directly impacts crucial characteristics of muscle fiber development and function [28]. Endurance training reduces the susceptibility of muscle fibers to fatigue by increasing aerobic capacity, while resistance training correlates to an increase in the number and size of myofibrils called hypertrophy [28]. These mechanisms are important to both native and engineered muscle tissues. In the case of engineered muscle, recreating these mechanisms are crucial to biomimetic tissues ex vivo.

2.5.4 Existing Methods for Mechanical Stimulation of Engineered Muscle

Model systems utilize tensile forces for mechanical stimulation in vitro to simulate in vivo conditions. External mechanical forces applied to muscle tissues correlate to growth rates and metabolic adaptations [29]. Models created and tested with muscle tissues represent key research and findings about the relationship between mechanical transduction processes and cell and molecular interactions [29].

Over the years there have been many devices and studies performed to physically stimulate muscle cells in vitro and analyze the effects. One of the first researchers to make headway was Vandenberg et al, who experimented with bioreactors with mechanical stimulation. The first two which were developed by Vandenberg researchers include a Mechanical Cell

Stimulator Model I and Model II [29]. The first model is pictured below and the basic concept is sandwiching of circular tissue constructs with Teflon, and collagen coated Silastic membranes; while a motor pushes up a stainless steel membrane with prongs to push up on the constructs [29]. Mechanical stimulation was applied over the course of days to help the tissues develop structural strength without rupturing.

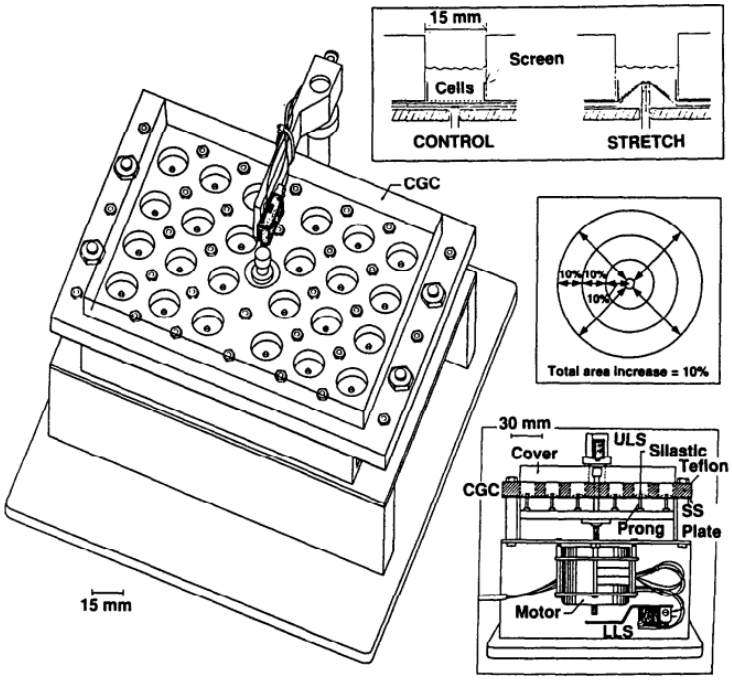


Figure 7. Schematic of Mechanical Cell Stimulator Model I [29]

The second model is pictured below and utilized a similar culturing methods however the elastic wells were rectangular and mechanical stimuli were applied in one dimension horizontally [25]. This regimen allowed for myoblast orientation, proliferation, fusion, and growth rates [25].

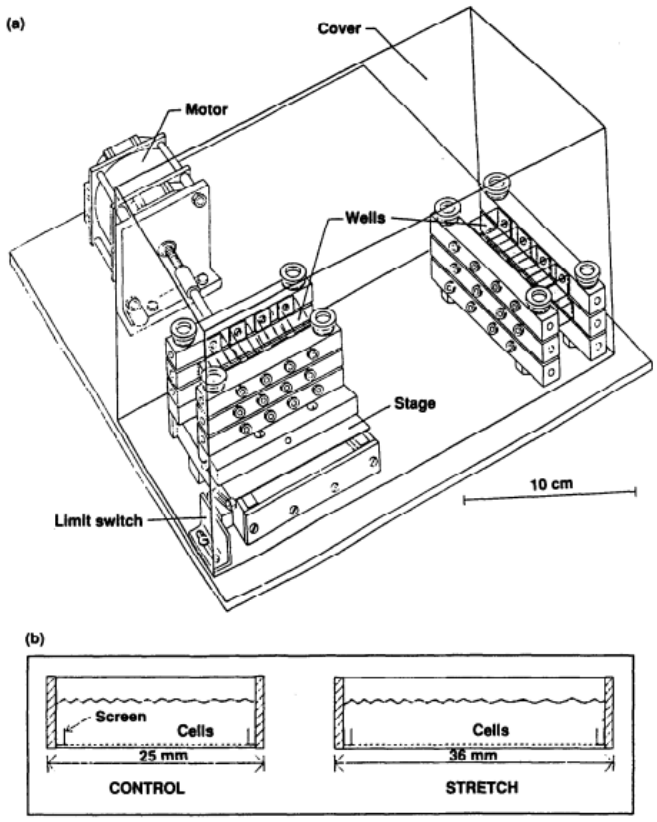


Figure 8. Schematic of Mechanical Cell Stimulator Model II [29]

Other notable studies continued to adapt elements of the design and process including construct size and shape, stimulus regimen, and means of achieving mechanical conditioning. In a study conducted by Candiani et al, a biomimetic stretch regimen over 10 days of 3.3% steady ramp stretch every 2 days with a 0.5Hz cyclic 5-pulse which increased protein expression of mouse myoblasts [25]. Another study conducted by Machingal et al conditioned muscle progenitor cells over a week with a 10% stretch 3 times per minute for 5 minutes every hour [25]. These constructs showed promising functional recovery and biomechanical properties [25].

Researchers with Ito et al developed a magnetic force-based tissue engineering technique that utilized nanoparticles for magnetic labeling of mouse myoblasts which were seeded as a ring and stimulated using a round magnet [25]. While some or many of these current methods show promise at creating biomimetic muscle tissue constructs for knowledge on myoblast differentiation and function, and for use in predictive disease modeling, new methods are continually being explored.

Chapter 3: Project Strategy

3.1 Initial Client Statement

The initial client statement is to design a system to create skeletal muscle in vitro using cultured myoblasts that is capable of facilitating both fiber alignment and mechanical or electrical conditioning. Additionally, the appropriate overall tissue dimensions must be determined as well as the optimal stage of myoblast differentiation with which to seed the system and the time required to achieve contractile function. The material selection issues with respect to provisional extracellular matrix, natural polymers such as fibrin/collagen, or artificials must be addressed and selected. Methods to measure contraction due to electrical stimulation and fiber strength must be designed and implemented. A requirement for supporting cells such as fibroblasts must be addressed. Finally, we must develop a process engineering strategy to include kinetics of muscle cell differentiation and maturation.

3.2 Revised Client Statement

Upon further discussion and review, the client statement was narrowed down and the goals were further specified. The revised client statement became:

“Design an in-vitro system to mechanically stimulate engineered muscle in order enhance maturation. The system should be conducive to producing biomimetic tissue to be used with drug-screening technology.”

3.3 Design Requirements

This section outlines the objectives and requirements of our project and device.

3.3.1 Design Objectives

The overall design objective is to enhance the maturation of in-vitro, engineered skeletal muscle tissue via mechanical stimulation.

After reviewing the revised client statement, the main design objectives were listed in Table # 1, below.

Table 1: List of Requirements

Design Requirements
Tissue anchorage
Interface with high throughput format
Method of measuring strain
Non-cytotoxic
Sterilizable
Repeatable, reproducible
Ability for 96-well culture plate removal

The first requirement is to have an anchorage system for the tissue to adhere to. Tissue requires an anchoring system in order to grow and will die without a system. PDMS posts are typically used as an anchoring system. The device will have to be compatible with a high throughput format. This means that the system should be able to be used with drug screening

technology. A 96-well plate can be used in order to achieve high throughput. In order to ensure that the tissue is being exercised, the design must include a method of measuring strain. Typically, imaging can be used to measure the strain.

The first objective speaks to the ability of our device in regard to its biomimicry. This means that the device should have good tissue maturity and density. In the examination of the Myomic drug screening platform the group discovered that this was one of the major limiting characteristics.

In order for the in-vitro system to properly mimic native tissue, it must form densely packed, uniformly aligned, and differentiated myofibers [13]. Thus, it is crucial that this model address the past issues with dense, mature tissue formation. The in-vitro skeletal muscle tissue developed needs to mimic native tissue formation.

The final model must be reproducible. This means that the contractile function, fiber length, diameter, and density must be able to be produced repeatedly. A standard must designed to achieve repeatable results.

The third design objective aims to build off the positive, functioning aspects of the Myomics drug-screening platform. This means factoring in the well-plate for growing the tissues as well as posts which can anchor the cells. The device should also incorporate the assessment mechanism of contractile function utilized in the Myomics platform. Building off the aspects of this platform which work well as a skeletal muscle model will allow the group to focus on other aspects which can be improved for the new model. A removable 96-well plate can be used for culturing in an incubator.

3.3.2 Design Specifications and Constraints

The design constraints we have developed from the aforementioned design requirements are as follows:

Table 2: Design Specifications

Design Specification	Technical Specification
Mechanical stimulation	0-23% strain of original tissue length
Operative under physiological conditions (incubator environment)	37 °C 5-7% CO_2 95% humidity
Anchorage system	PDMS posts
Frequency of strain application	0.5-1.5Hz

- Culture and maintain in physiological conditions (incubator environment):
    - 37 °C
    - 5—7% CO_2
    - 95% humidity

Because we are attempting to produce the most accurate human model of skeletal muscle tissue to-date, we must ensure that our system operates well in typical human physiological

conditions, or in an incubator environment. This environment is comprised of the above attributes.

Specific to engineering tissues for use as in-vitro models, we will be utilizing a 3D tissue engineering technique. The determination of which method creates the most accurate environment biomimetic to the extracellular matrix in which native muscle tissue exists is crucial.

In order to create an accurate model, we must develop a standard for skeletal tissue engineering that is reproducible, meaning, with our methods and procedure, the ability to engineer skeletal muscle tissue with consistency in the above technical areas. This is a crucial step if we are to improve on current research methods of in-vitro skeletal muscle. Further constraints of this project include the size.

The anchorage system are necessary for the viability of the cells. PDMS posts are used in the WPI labs and create an environment for the cells to be seeded to. Additionally, the frequency of the applied strain should be between 0.5-1.5 Hz. This mimics what is found in-vivo. Table 3 lists the design constraints.

Table 3: Design Constraints

Constraint	Specification of Constraint
Compatible with a standard culture plate	128x85x14mm
Materials must be appropriate for use with live tissue constructs in the future	Biocompatible with cells
Budget	$750
Timeline	< 9 months timeline

3.3.3 Design Functions

Given the stipulated design constraints above, we are able to create a more comprehensive list of necessary design functions. These are as follows:

- Nutrient perfusion
- Fiber anchorage
- Mechanical stimulation
    - Application of strain (anchorage prevents collapse)
    - Stimulation
- Measurement of force (to ensure accurate biomimicry)
    - Imaging

Below is the function-means table for aforementioned design functions.

Table 4: Function-Means Chart

Function	Means	Means	Means
Culture Environment	96-well plate	Commercial “enhanced” culture flasks	Petri dish
Nutrient Perfusion			
Fiber Anchorage	PDMS posts	Metallic alloy posts	Hydrogel posts
Mechanical Stimulation	Magnets	Sub-matricial air-pocket	Hydraulic piston
Measurement of Stress	Force transducer (utilize LabView)		
Measurement of Strain	ImageJ	Calipers	

Part 3.3 Design Standards

The design of a human skeletal muscle tissue model requires attention to be paid in regard to industry and ethical standards. The adherence to such standards will ensure the safety, effectiveness, and legality of the system. Below is a table of the relevant industry standards to the design and testing of a human skeletal muscle tissue model:

Table 5: Industry Standards

ISO 22442-2:2007	Sourcing, collection, handling of animals and tissues
ISO 13022:2012	Management of viable human cells
ISO 10993-1:2009	Biological evaluation of biocompatibility of medical devices
ISO 17422:2002	Plastic environment impact
ISO 7712:1985	Use of disposable micropipettes
ISO 7550:1985	Use of disposable serological pipettes

These various standards apply to the tissue and cell cultures which will be central to the design of the skeletal muscle tissue system. There are many standards associated with the use of tissue cultures. Adhering to such standards will overall ensure that improper or poor handling of living tissues will not impact the accuracy of the results acquired. The standards which directly relate to biocompatibility are also essential to the operation of this project. These standards will ensure a good relationship between the living cells and the device materials, meaning that the materials used in the design will not harm the muscle cells or affect their growth and maturation. As a result of the materials negatively impacting the cells, the results of the project would be deemed inaccurate. As a result of the adherence to these standards, all materials considered and used in the design process and final design will be biocompatible. Another important aspect is the prevention of contamination or toxification of the muscle cells. The industry standards for sterility will play a major role in protecting the viability of the cells. The environmental standards also play a role in this project as they will serve to protect the environment from

potential threats or damage. These standards give a way for the potential impact on the environment of the device. The avoidance of accumulating material waste and the safe use of hazardous materials are also ensured by following these industry standards. Lastly the standards which apply to the laboratory equipment will be important in assessing the efficacy of the device created as well as ensuring the device utilizes common laboratory equipment and standards.

Financial Statement

The financial considerations for the group involve the total budget of $750. In regards to design materials, such as well plates and supplies, the team must remain within this budget throughout the course of the project.

Chapter 4: Design Process

4.1 Need Analysis

In order to create a skeletal muscle tissue model there are a series of criteria which need to be considered and included in the design. When considering parameters which may not fall on the technical side there are a series of desires which arise in the design and creation of such a device. Outlining the needs and wants of this design project will highlight what will be accomplished. Below is a table of design requirements.

One of the primary goals is the growth of viable skeletal muscle tissue. Characteristics that will contribute to viable tissue include muscle fibers which can metabolize, proliferate, and contract. The application of stimuli both of mechanical and electrical origin will contribute to the ability of the skeletal muscle cells to differentiate, grow, proliferate, and exercise. In the human body, both electrical and mechanical impulses force muscle cells to grow and strengthen. Thus in an in-vitro model, the cells must be subject to the same impulses in order for them to mimic the in-vivo cells. Muscles subject to shear stress and cyclic strain are as a result much denser and thicker. The recommended mechanical stimulation falls between 10 and 15 percent stress and relaxation from the initial tissue length. Anchorage and materials such as scaffolds are crucial when working with muscle cells due to the need for muscle cells to grow on biocompatible, dimensionally appropriate scaffolds that also provide a mechanism of stabilization. Myofibers tend to have a diameter of 10-100μm, and thus this must be addressed by the design size. This project works with living sensitive cells, meaning that contamination and toxicity must be avoided in order to prevent slow growth, adverse effects, or cell death.

The project wants encompass additional desires in order to elevate the overall design. One of the desires is to achieve biomimicry. While this achievement would produce an accurate

and comprehensive model for study, it is known that in-vitro muscle cell biomimicry is extremely difficult. Currently there is a lack in high-throughput technology as well as a true and accurate skeletal muscle tissue model. For these reasons it seems that great usability and reproducibility could be sacrificed in order to focus on other vital characteristics.

4.2 Concept Map, Design, Prototyping, Modeling, & Feasibility Studies

The design functions mapped out in chapter three are comprehensive concepts, each with sets of sub-functions. Each function will be mapped out and analyzed as a functional block. The design process will be applied to each functional block starting with supporting and growing the cells, mechanically stimulating the cells, and measuring the displacement of the posts when mechanically stimulated.

After brainstorming various design concepts, we are considering the design of two possible systems in which to stimulate, strengthen and maintain our cells. The chart below maps out various design requirements that must be incorporated into the system we are developing, as well as various means of accomplishing or meeting those requirements. The design components circled in red are those which pertain to our two preliminary concepts and are explained below the diagram.

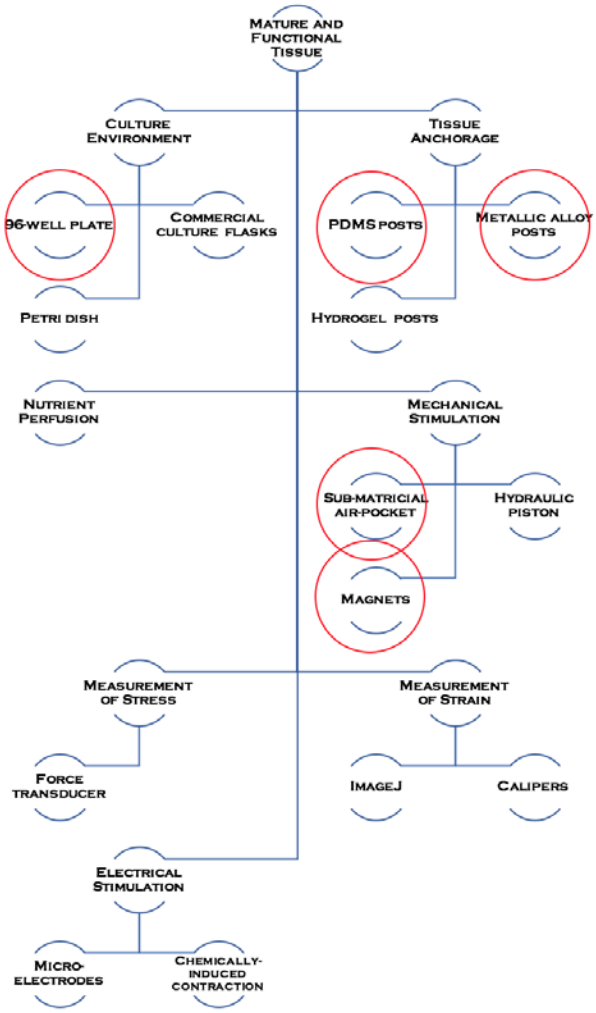
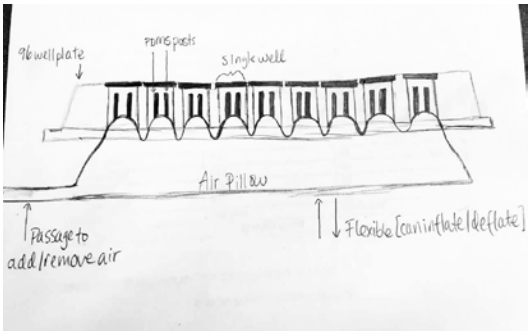


Figure 9. Design attributes are illustrated and prioritized.

Table 6: Functional Blocks of Conceptual Designs

96-well Plate	*Compatible with lab equipment and living cells, will provide housing/culturing environment for muscle cells*
PDMS Posts	*Anchorage mechanism, biocompatibility, allow for physical manipulation and cellular exercise*
Metallic Alloy Posts	*Anchorage mechanism, physical magnetic manipulation and cellular exercise*
Submatricial Air-pocket	*Manipulation of PDMS posts for mechanical stimulation- allows for indirect manipulation (i.e. use of air to change volume of pocket, which will in turn cause movement of PDMS posts)*
Magnets	*Manipulation of metallic alloy posts for mechanical stimulation- allows for indirect manipulation (i.e. use of magnetic field to influence movement of metal posts)*



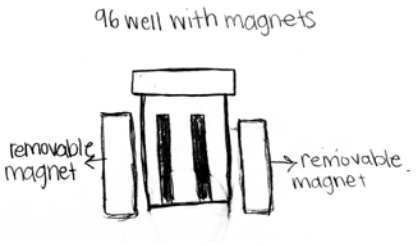


Figure 10. Preliminary illustration of Design Concept 1. *Figure 11. Preliminary illustration of Design Concept 2.*

1. Use of PDMS Posts and Manipulation by Submatricial Air-Pocket

The design of a system that utilizes flexible PDMS posts in conjunction with Myomics drug-screening platform was used by the 2016 WPI design team. With the development of their Sliding Comb Rack, the team was able to develop a system which applies a stress and

electrically stimulates muscle cells. This design, however, only manipulated the PDMS posts in one direction. In a new approach, we will attempt to modify and improve upon this past design. Consistent with our needs, the implementation of a submatricial air pocket (see diagram below) would allow us to manipulate the posts in an orientation which stretches the muscle cell. This is crucial for the development of fibers which resemble those of native muscle tissue. In addition to this, withdrawal of air from the submatricial air-pocket would shorten the muscle cells and simulate atrophy, or the degeneration of the cells.

2. Use of Metallic-Alloy Posts and Manipulation by Magnet

In an alternative approach to apply a mechanical stimulus to the cells, we are considering the use of metallic posts within our culture system. This development entertains the same logic as the aforementioned design concept; designing a system in which we can both apply and relieve stress from the muscle fibers. With careful determination of magnetic poles and fields within the system, there is potential for the application of a magnetic field to cause movement of the metallic posts, either toward each other, to relieve stress, or away from each other, to apply a stress. Factors to consider moving forward with this design include the biocompatibility of the alloy used, direction of magnetic fields, potential interference of opposing magnetic fields, potential adverse effects of a relatively strong magnetic field on the muscle fibers, the shielding of test environment from outside magnetic fields, among others.

After reviewing the preliminary designs, the team came up with 4 other alternative designs:

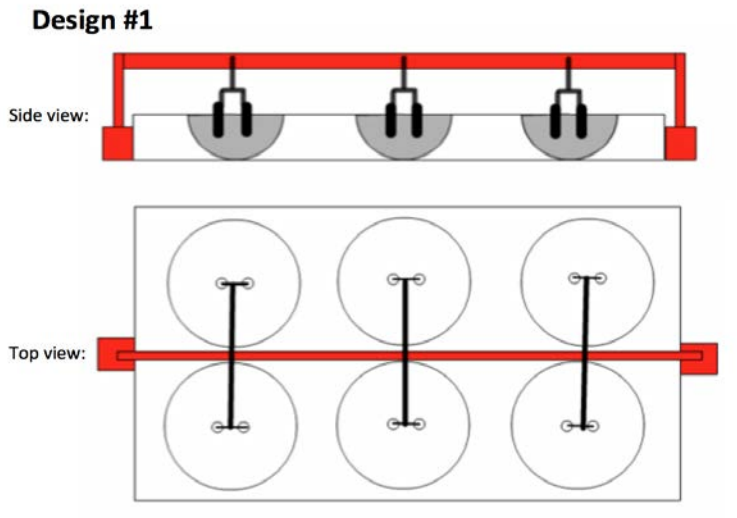


Figure 12. Schematic of Alternative Design #1.

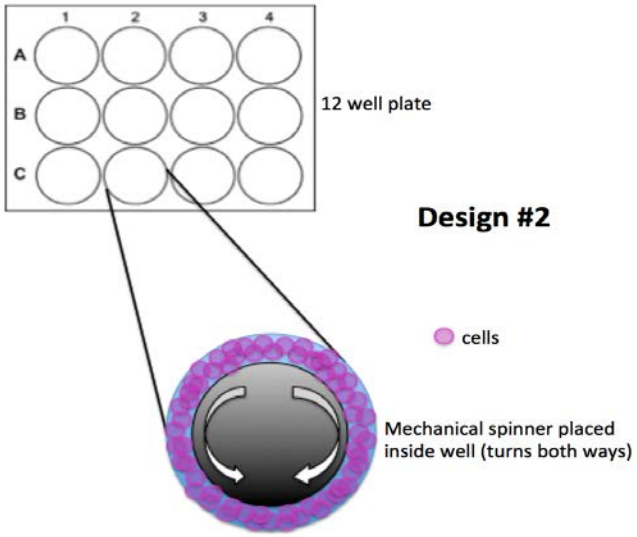


Figure 13. Schematic of Alternative Design #2.

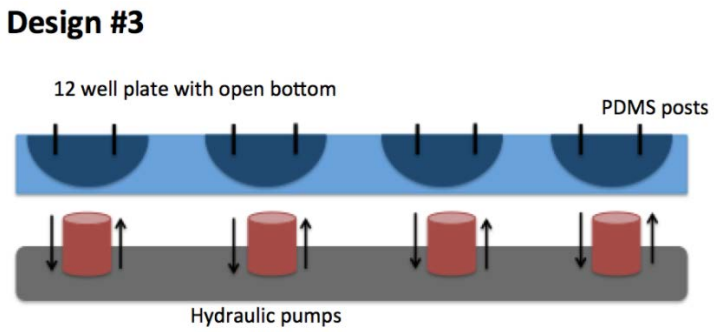


Figure 14. Schematic of Alternative Design #3.

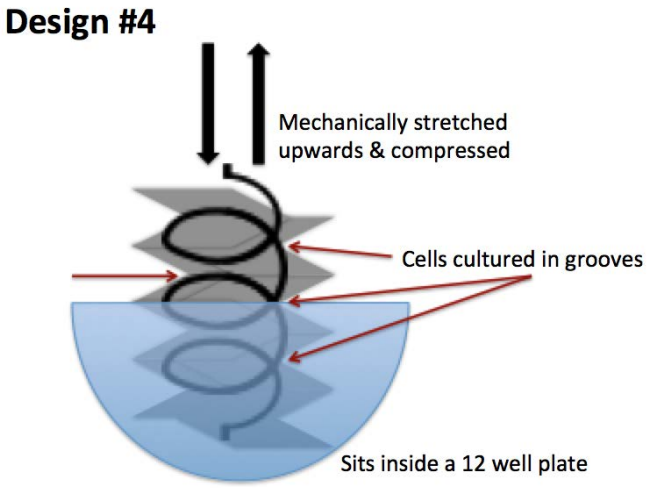


Figure 15. Schematic of Alternative Design #4.

The first design would use mechanical actuators to move the PDMS posts and mechanically stimulate the tissue. The second alternative design would use a mechanical spinner placed inside the well. Cells would be seeded between the spinner and side of the well. The

spinning action would stretch and exercise the cells. Alternative design 3 would use a hydraulic pump and a flexible bottom on a well plate to deflect the PDMS posts. Alternative design 4 would use a spring system inside a well plate to mechanically stretch and compress the tissue. The cells would be seeded in the grooves of the spring and attach to the top and bottom parts of the grooves.

After discussing each design with our client, design number 3 was further expanded. The same concept was used in the creation of the final design. A pegged plate placed on a jack was used in place of the hydraulic lift. The pegged plate would push up into the bottom of the 96-well plate with a flexible PDMS bottom. The jack would act as a lift system to push the pegged plate up into the well, causing the PDMS posts to deflect. Below is the schematic for the pegged plate fitting into the bottom of the 96-well plate:

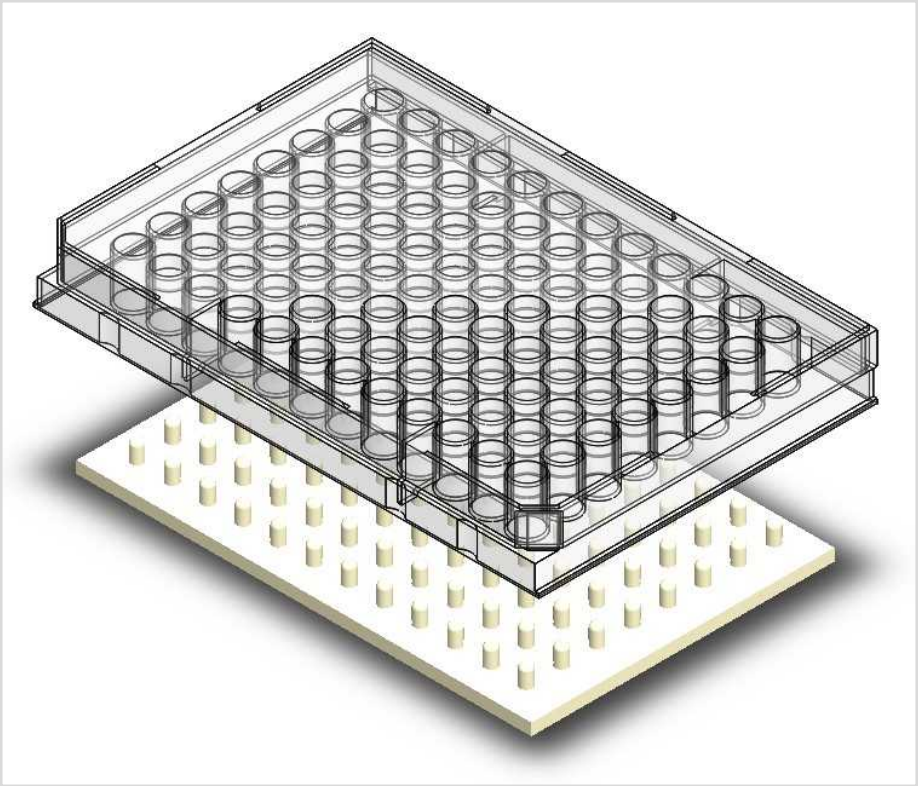
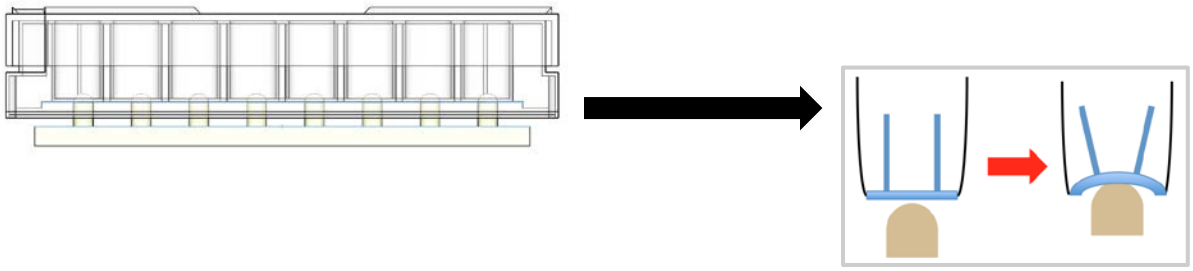


Figure 16. SolidWorks schematic of pegged plate/96-well plate assembly.

The pegged plate is attached to a jack that elevates the pegs to apply an upward force on the bottom of the wells. The PDMS posts are placed inside the well and deflect as shown in Figure 17, below:

Figure 17. Illustration of mechanical stimulation means.



Chapter 5: Design Verification

This chapter outlines the facts considered and experiments conducted in order to confirm that the decided-upon design meets its stipulated design objectives, requirements and specifications. The actions taken during the design verification process ensure that the final design proposes an adequate seeding system, and means of maintaining sterility, and method of mechanical stimulation.

5.1 Seeding System Verification

The following section details and verifies how the chosen design offers a suitable seeding system for the intended use of the system.

5.1.1 Adequacy of Seeding System

As stated in the Background Research section, polydimethylsiloxane (PDMS), a silicon-based organic polymer, has been incorporated into past and current research experiments for the mechanical stimulation of engineered muscle. For this project, the team effectively redesigned the standard 96-well plate, curing flexible PDMS post inserts into a PDMS layer with the dimensions of the bottom of a 96-well plate. In order to ensure that the fabricated PDMS layer would remain intact, and that the PDMS post inserts would remain sealed within the PDMS layer, the team produced three bottom layers with the post inserts. The same production protocol was used for each, and each of the three layers were subject to “destructive” testing; after subjecting the PDMS layers to multiaxial stretch with forces greater than that with which the PDMS posts would be flexed by the pegged plate, the team was able to verify that the PDMS-bottom/PDMS-post assembly would stand as an effective choice of seeding system.

5.2 Mechanical Stimulation Verification

The following section describes the ability of the system to achieve adequate flexion of the PDMS posts.

5.2.1 Function of Pegged Plate

In order to verify that the pegged plate and 96-well plate assembly was effectively flexing the PDMS bottom, the team first constructed a modified SolidWorks model of the original pegged plate, featuring more pointed pegs than the flat-topped pegs the concept originated with. The computer-aided design files were converted into the appropriate 3D-printing file format, and the plate was made with PLA plastic. Upon completion of printing, the team fixed the pegged plate to the table jack purchased for the design assembly. The 96-well plate assembly, with its flexible PDMS bottom and posts, was placed directly above the plate, and pressure was applied in the downward direction. The observance of posts flexion allowed the team to verify the pegged plate as an adequate means of mechanical stimulation.

5.2.2 Function of Arduino Uno, NEMA Stepper Motor, Table Jack Assembly

To verify the functional capability of the Arduino Uno to operate the NEMA Stepper Motor, and the capability of the motor to control the jack, simple tests were conducted. After assembling all three components, the Arduino Uno was connected to a continuous power source, such as a computer or 120V wall outlet. The team then continuously altered the Arduino code to produce varied responses from the motor, and observed whether the jack demonstrated the desired mechanical response. The assembly proved to function most efficiently when the motor steps taken remained below 200 steps; the motor exhibited inadequate strength when the motor

step input was too high. However, due to time constraints, the team was unable to modify both the motor and jack components of the final assembly.

5.2.3 Function of 96-Well Plate Stand

The provision of the 96-well plate stand allows for removal of the 96-well culture plate, and its rigid nature facilitates the acquisition of repeatable and reproducible strain regimens.

5.3 Final Experiment

The following section explains the final experiment that was carried out once the various capabilities of the design were verified. The experiment aimed to review the theoretical ability of the device to mechanically stimulate mature muscle tissue.

5.3.1 Final Experiment

Two crucial variables were defined before the final experiment was initiated. *Percent strain* was defined as the percent increase in distance between the two PDMS posts from the relaxed state to the flexed state. The *z-displacement* was defined as the upward vertical distance traveled by the pegged plate from a constant starting position, which was consistent for each trial.

A total of four experimental conditions were stipulated. For each separate condition, the Arduino Uno code was altered in order to program the NEMA Stepper Motor to perform various steps in the backward direction; the motor executed 300 steps, 100 steps, 50 steps, and 30 steps. The backward steps resulted in upward movement of the jack and thus, pegged plate. For three of these conditions, 100 steps, 50 steps, and 30 steps, the motor was programmed to perform an equal number of steps in the opposite direction so that constant z-displacement could be maintained for each respective test. Due to limitations posed by motor strength, the execution of

300 steps in the upward direction was belabored, and the team found that alternatively programming the motor to perform 150 steps for downward movement resulted in the maintenance of z-displacement throughout the trial. The the top surface of the jack was manually positioned 3.23mm below the PDMS bottom before the commencement of the trials. In order to maintain this starting distance for each trial, the NEMA Stepper Motor was always deactivated upon completion of downward movement, or upon its return to the initial starting position.

To begin each trial, the tops of all PDMS posts were marked in black, to provide fiducial points for data analysis. Then, the system was allowed to run for a prolonged period of time, estimated to be > 10 minutes for each trial. After doing so, confirming the proper operation of all components, visual data was logged with video capture. Videos were taken directly above the wells, so that, in their relaxed state, the PDMS posts appeared as singular points. A total of four videos were recorded above the 96-well plate. Figure 18 and 19 demonstrate the camera positioning above the device.

Figure 18. Relaxed state of PDMS posts.

Figure 19. Flexed state of PDMS posts.

For the express purpose of providing a proof-of-concept, three wells containing the PDMS post inserts were tested at different locations on the plate, under the four aforementioned conditions. The team then uploaded the visual data files into a video editing software to capture freeze-frame images of the PDMS posts at relaxation and at flexion. These freeze-frames were captured for every relaxation-flexion interval for the extent of each video, and organized into condition-specific files. Thereafter, the team uploaded each image file to ImageJ, an image processing program, to measure distance between PDMS posts. In order to calibrate the software, a known distance was incorporated into the video frames during recording. This allowed for the establishment of a universal pixel/millimeter ratio, which could be used for better accuracy across all images. Figure 20 shows the ImageJ interface.

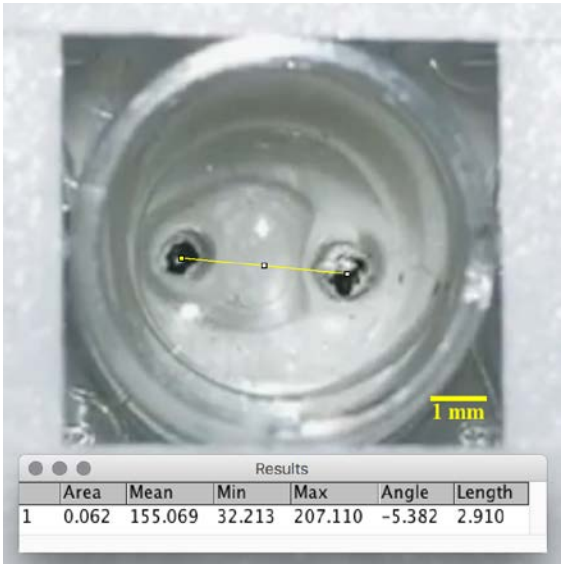


Figure 20. Demonstration of measurement in ImageJ.

Once measurements of relaxed and flexed states for each condition were documented in a spreadsheet, the team proceeded to calculate the percent strain using the following equation:

$$percent\ strain = \frac{Distance\ Between\ PDMS\ Posts_{flexed}}{Distance\ Between\ PDMS\ Posts_{relaxed}} - 1$$

. Because the team's stipulated experimental

conditions were in terms of motor steps rather than z-displacement, an empirically derived proportion was used to calculate the z-displacement per motor step condition. The resulting z-displacements found were as follows: 4158µm (300 steps); 1386µm (100 steps); 693µm (50 steps); 415.8µm (30 steps). The average percent strain for each condition is plotted as a function of z-displacement in Figure 21.

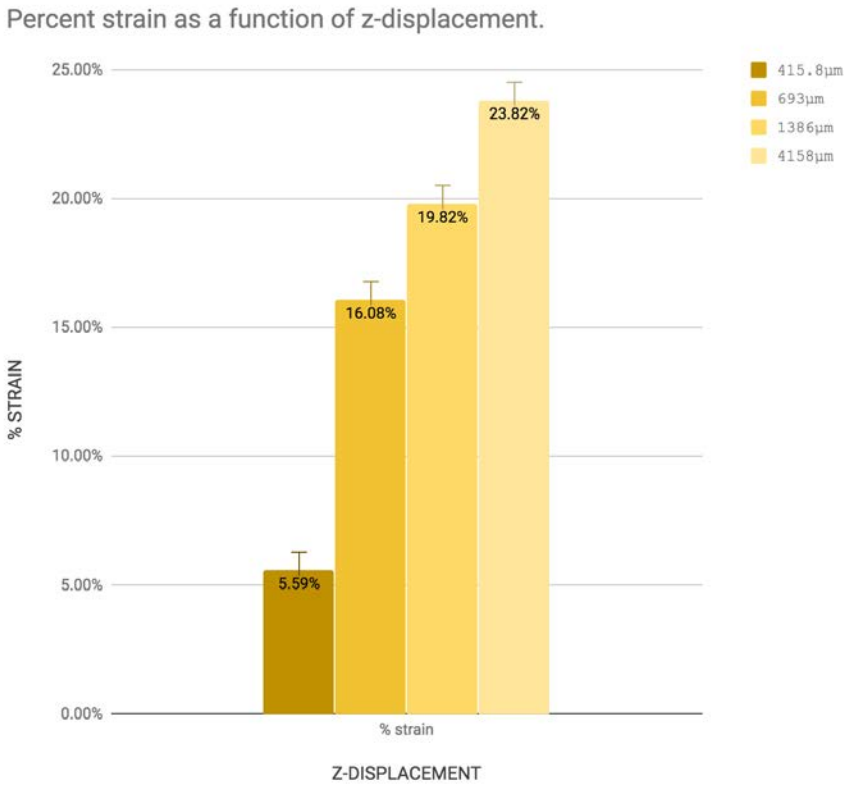


Figure 21. Percent strain plotted as a function of z-displacement.

The frequency at which various strains were applied was also measured upon completion of the trials. The team again utilized video editing software, and was able to distinguish singular intervals of flexion. The duration of one interval per condition was documented in seconds. The

reciprocal of these times, $(frequency\ (Hz) = \frac{1}{Duration\ of\ Interval\ (s)})$, were taken to attain flexion frequency. The various stipulated, theoretical and empirical values are quantified in Table 7.

Table 7: Empirical and Theoretical Results Relative to Design Condition

Motor steps (upward direction)	Motor steps (downward direction)	Resulting z-displacement of pegged plate (μm)	Average strain of PDMS posts (%)	Standard deviation	Frequency (Hz)	Samples per trial
30	30	415.8	5.59	0.710	0.6	10
50	50	693	16.08	0.804	0.45	10
100	100	1386	19.82	0.704	0.3	10
300	150	4158	23.82	0.577	0.2	10

Chapter 6: Final Design and Validation

This chapter outlines and demonstrates the incorporation of each design component within the follow design. The chapter also discusses the industry standards and potential social impact of the project.

6.1 Device Overview

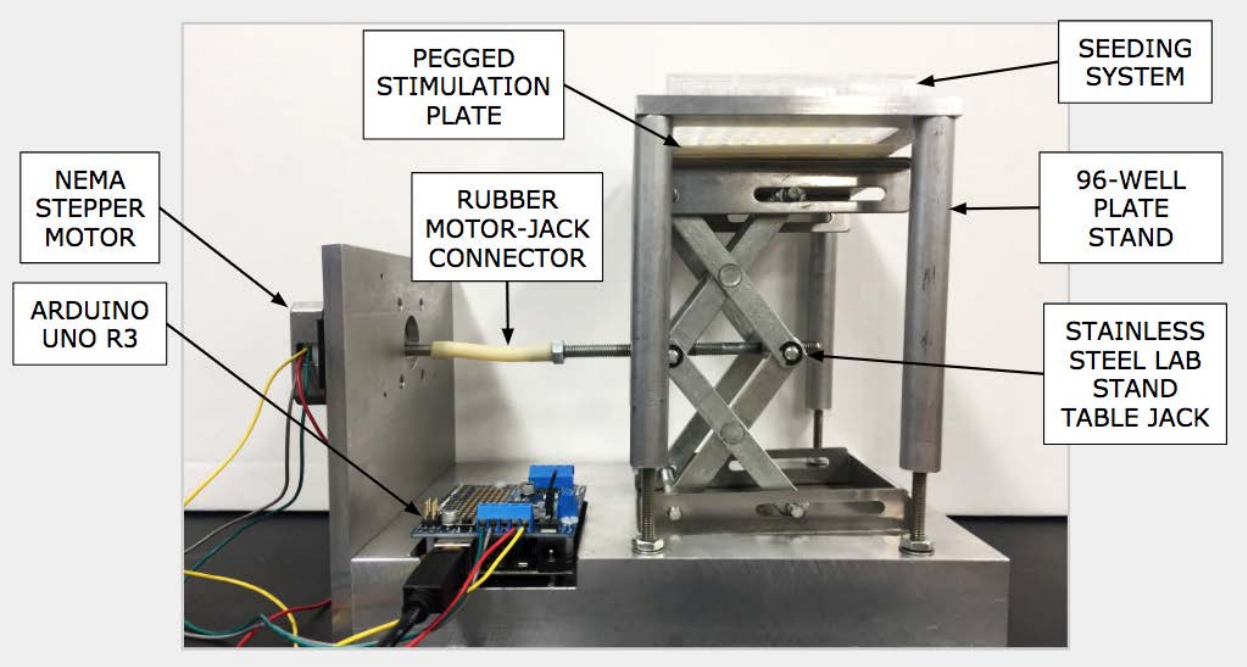


Figure 22. An overview of design components.

The system is powered using a *NEMA stepper motor* that is hooked up to an *Arduino UNO R3*. The Arduino UNO R3 can be programmed to change the number of rotations the stepper motor turns the jack. An increased number of steps will move the jack further up, increasing the deflection of the PDMS posts. The *rubber motor-jack connector* connects the motor to the jack and is designed to be lightweight so it does not interfere with the up and down

movement of the jack. The *96-well plate stand* holds the bottomless 96-well plate above the jack. The *seeding system* consists of a flexible PDMS bottom (placed on the underside of the 96-well plate) with PDMS posts placed in the center of the well. On the top of the jack there is a *pegged stimulation plate* designed so each of the individual pegs can enter the center of each well. All components are placed in the *stainless steel lab stand table jack* with cutouts to accommodate each of the different components.

6.2 Device Validation

This section outlines the validity of the design in a social context.

6.2.1 Industry Standards

Industry standards are put in place in order to ensure safety of the user when designing and working with biological substances. These standards come from the International Organization of Standardization (ISO) and set national standards for industrial and commercial use. ISO 18458:2015 and ISO 18459:2015 are standards on how to produce the constructs when creating biomimetic tissue. The biocompatibility of a medical device with biologics is ISO 10993-1. The in-vitro cytotoxicity of medical devices is standardized and sterilization protocol is in ISO 10993-5:2009 and ISP 11737-2:2009, respectively. ISO 17422:2002 ensures the device has no negative environmental impact.

6.2.2 Economic Impact

Currently, the device would have little impact on the overall economy. In the future, this could expedite the drug development process by creating a biomimetic tissue muscle. This would help those living with muscular disorder have a better quality of life. This would also decrease the cost for the development of treatments and therapies. Rapid testing of drugs could further

develop the economy, allowing for new products to be brought to the market more often. This could impact patients by lowering the costs of drugs do to the increase in competition. The market could be better balanced, with no single company holding stake over the entire drug and market.

6.2.3 Environmental Impact

The device itself would have little to no environmental impact. Should the scope of environmental impact be broadened, it’s conceivable that each of the design components and the materials used to produce them could have an environmental impact.

6.2.4 Societal Impact

Engineered skeletal muscle tissue can be used to develop treatments and cures for musculoskeletal disorders. A device that can properly maturate in-vitro skeletal muscle tissue can be used in pharmaceuticals. With further development, the device could possibly maturate tissue to mimic native tissue. If this occurs, society would see an improvement in the speed and distribution of curative medicine for disorders such as DMD and atrophy. The device could also be used to culture different types of cells, resulting in cures for other tissue or physiological issues. Additionally, an accurate in-vitro model could eliminate the need for animal testing during the drug testing process. Although, testing on animals does not always provide an accurate response in the human body. An accurate model will be able to better predict than methods that are currently being used. This will speed up the process, allowing for more time and money to be put into the development of other drugs for different ailments. Society would be positively impacted.

6.2.5 Political Impact

Many political parties and politicians are funded by major pharmaceutical companies. Therefore, there are politicians that benefit from the way pharmaceutical companies are being ran now. If the device is able to further develop the drug creation process, this could change the way the industry is set up. Therefore, the unpredictable nature of politics could be impacted. Although not a direct correlation, the financial impact a device like this could bring to the pharmaceutical industry could impact the political climate.

6.2.6 Ethical Concerns

Currently, there are ethical concerns surrounding the drug creation process and testing protocols. Drugs have to go through extensive testing before being put on the market. This includes animal testing and human trials. Animal testing is used to see an in-vivo response of the drug. There is controversy about the ethical treatment of the animals. There are many organizations and groups who protest animal testing and try to stop the process. An accurate in-vitro tissue model could eliminate the need for animal testing. Additionally, human trials are a part of the drug development model. Patients are able to sign up for trials, but the side effects of the drug are often not known. Also during human trials there is a control group that receives a placebo drug. Ethically, it is difficult because there is no way of choosing who receives the drug verse who receives the placebo. There is also no way of knowing the body's reaction to a foreign compound prior to testing. An in-vitro skeletal muscle model could eliminate the need for animal and human testing. This would improve the ethical implications of the present drug testing methods.

6.2.7 Health and Safety Issues

The current model of the device does not present any health and safety issues. Upon further development, the device would have to be compatible with an incubator in order to properly culture cells. There is no foreseeable safety or health issue to the user of the system. Since there is an electrical component, the device should not come in contact with water.

Chapter 7: Discussion

This chapter will discuss the results from experiments outlined in Chapter 5, and the ways in which the findings support the team's proof-on-concept. It will also be discussed whether the final design was able to meet or fell short of the various design objectives, requirements and specifications.

7.1 General Discussion

This section elaborates on the results of verification experiments outlined in Chapter 5.

7.1.1 Seeding System Verification

As outlined in Section 5.1, various tests were conducted to verify the seeding system decided upon for the final design. The first component selected by the team was the PDMS-posts. Having been proven as an effective anchorage mechanism for engineered muscle tissue, the team decided to use this pre-existing component. While the posts are typically used within standard 96-well plates, the team decided to modify the standard plate so that it would be compatible with a means of mechanical stimulation which resides outside of the culture system. The multiaxial stretch testing of the PDMS layer/PDMS post assembly proved that it could withstand substantial forces due to stretch, and therefore was a viable design component for the final design system.

7.1.2 Mechanical Stimulation Verification

As outlined in Section 5.2, further tests were conducted to verify the means of mechanical stimulation decided upon for the final design. As previously described, the pegged plate was 3D-printed with PLA plastic. Tests to verify the ability of the pegged plate to flex the PDMS bottom layer, and thus flexion of the PDMS posts, proved successful.

The second test, as described in Section 5.2, was conducted to verify the functional capability of the Arduino/NEMA Stepper Motor/table jack assembly to achieve repeatable and reproducible agency. It was stated that the assembly proved to function most efficiently when the motor steps taken remained below 200 steps, because the motor exhibited inadequate strength when the motor step input was too high. This complication was both due to the nature of the jack as well as the physical capabilities of the NEMA Stepper Motor. When the jack operated at too low a depth, the amount of torque it required for elevation increased. The amount of torque necessary for all ranges of elevation for the jack was not achievable by the NEMA Stepper Motor; the assembly operated most efficiently when movement of the jack was initiated at higher elevations. To address this, the team made a point to operate the jack and conduct experiments at its maximum allowable height. This allowed for the experiments conducted at 100 steps, 50 steps and 30 steps to proceed unhindered, and the steps taken by the motor in the upward and downward directions were equal and opposite. The experiment conducted at 300 motor steps in elevation was modified so that the motor would take 150 steps in depression; it was found that these motor steps allowed for an equal and opposite elevation and depression interval for the jack, and data was collected as normal.

7.2 Reviewing Design Requirements

This section will detail where the final design met or fell short of the original design objectives, requirements and specifications.

7.2.1 Fulfillment of Design Objectives

The design objective stated at the outset of the project is as follows:
To enhance the maturation and function of in-vitro, engineered skeletal muscle tissue via mechanical stimulation.

While the final design verification did not include cell culturing, this is attributed solely to time constraints faced by the group. The design shows significant focus on mechanical stimulation, with the sole intention of repeatedly conditioning muscle tissues in vitro for biomimetic enhancements. Initial verifications of the mechanical stimulation mechanism provided a proof of the concept. As is, the device shows potential for adjustable stimulation regimens to enhance the maturation of in vitro-engineered muscle tissues.

7.2.2 Fulfillment of Design Requirements

The design requirements stated at the outset of the project are as follows:

1. Tissue anchorage
2. Interface with high-throughput format
3. Method of measuring strain
4. Non-cytotoxic
5. Sterilizable
6. Repeatable and reproducible

The several design requirements reiterated above were addressed in the completion of this design project. Tissue anchorage was an important requirement for the survival of the muscle cells ex vivo. This was addressed in the device design by the use of PDMS posts which are flexible and biocompatible. The posts have been used in past and present models, and work well with cell adhesion of the myoblasts as well as with the various electrical and mechanical

stimuli.

The second requirement was the compatibility with high-throughput format due to the intended clinical use of the device in predictive disease modeling. The way in which the team addressed this requirement was the use of a 96-well plate due to its compatibility with current drug screening protocols. This ensures that when the time comes, this device will have the ability to be used in conjunction with high-throughput technologies.

A method of measuring strain was a crucial requirement to quantify the mechanical stimulation capabilities of the device. The strain capabilities of the device needed to be monitored, especially for the purpose of creating similar regimens as in vivo. The team addressed this requirement by utilizing an interface with image capture and processing software. The requirements for non-cytotoxicity and sterility were crucial to protecting the viability of the cells in culture. The team addressed these concerns by selecting materials such as the standard 96-well plate and PDMS material, which are known to be sterile/sterilizable and also pose no threat to cell viability. In addition, the design allows for removal of the well plates for use in culture and incubation, and for the ability of well plates to be interchanged. The last requirements included repeatability and reproducibility. Both of these aspects are crucial for the verification and validation of any device. These requirements were addressed by the programmable motor through Arduino coding which allows for trials to run automatically over any amount of time.

7.2.3 Fulfillment of Design Specifications

The design specifications stated at the outset of the project are as follows:

1. Mechanical stimulation
    a. 0-23% strain of original tissue length
2. Operative under physiological conditions (incubator environment)

    a. 37°C
    b. 5-7% CO_2
    c. 95% humidity
3. Anchorage system
4. Frequency of strain application
    a. 0.5-1.5Hz

The design specifications outlined above were all considered during the project design process. Final experimentation validated the capability of the system to achieve strains between 0 and 23% of original tissue length. For the purpose of this project, muscle tissue was not seeded into the system, and therefore the percent strains are purely theoretical. Because results of the cyclic straining regimens demonstrated a proportionality between displacement of the pegged plate and percent strain between posts, it can be concluded that in order to achieve a desired strain, reference can be made to the results of the final experiment to identify an appropriate z-displacement value. Following suit, in future experiments, the percent strain achieved by the system can be tuned by alteration and updating of the Arduino programming.

The final design is not capable of operating under physiological conditions. The team addresses this design specification in the Recommendations section. As previously described, the team decided to use PDMS post inserts as the fiber anchorage mechanism. Although no tissues were seeded to evaluate this method, the Background Research section outlines their capability in experiments conducted by Vandenburgh et al. Finally, he team achieved frequencies of strain between 0.2 and 0.6 Hz across the four trials. Although these frequency values lie largely outside of the stipulated 0.5-1.5Hz within the design specifications, the frequency of strain can be tuned via alteration of the Arduino code.

7.2.4 Compliance with Design Constraints

The design constraints stated at the outset of project are as follows:

1. Compatible with a standard culture plate
    a. 128x85x14mm
2. Materials must be appropriate for use with live tissue constructs
3. Budget
    a. $250/student; total budget of $750
4. Timeline
    a. < 9 months

As discussed throughout this report, a standard 96-well plate was used to fabricate a novel seeding system for the mechanical stimulation of engineered muscle. Because the first design constraint requested compatibility with a standard culture plate, a aluminum stand was machined according to the dimensions of a 96-well plate, therefore, the final design complied with that constraint. As discussed in section 7.2.2, the team selected prototyping materials such as the standard 96-well plate and PDMS layer, which are both known to be sterile/sterilizable and also pose no threat to cell viability.

The team encountered no issues regarding exceeding the maximum allowed budget for the project. A table outlining the project budget is presented in the Appendix section; Part D. In addition, while the presence of a < 9 month time constraint put pressure on the team to efficiently identify and execute the given problem within the revised client statement, the team still provided a sufficient proof-of-concept.

Chapter 8: Conclusions and Recommendations

8.1 Conclusions

In conclusion, the final proof-of-concept demonstrated by the team was successful in meeting the objectives, requirements and specifications as derived from the revised client statement. The final experiment proved the ability of the final design to yield a correlation between percent strain and z-displacement. The device exhibited the ability to apply theoretical tissue strains between 0 and 24%. Furthermore, variable experimental conditions can be achieved by alteration of the Arduino code; the frequency of stretching is tunable by the increasing the temporal delay between downward and upward movement, and the strain rate is tunable by altering the speed at which the motor operates. All of this can be done with the simple exchange of numbers within the Arduino code.

8.2 Recommendations

Due to time and resource constraints, at the termination of the project, the team noted several improvements which could be made. The first takeaway from the testing results and function of the device was improved precision, namely the accuracy of the jack component in addition to motor strength. A more level jack would directly correlate to more precise linear actuation and attribute to an overall more repeatable and reproducible procedure. In addition, a stronger motor would allow actuation to occur more smoothly and without risk of wearing out the motor. The inclusion of electrical stimuli, in addition to the mechanical stimuli, would further improve the biomimetic properties of the engineered muscle tissues. If not for the time constraint, the group would have included both means of stimulation, knowing that in conjunction with each other, robust in-vitro muscle tissue can be generated. The next crucial

improvement would be waterproofing the electrical components of the design, if not the whole device, in order for compatibility with incubator conditions. This would make the system more efficient, while catering to maintenance of sterility.

Improvements led the group to also consider recommendations in regard to future testing and work. The main recommendation is to perform further validation testing, mainly for consistency and abundance in the data collected. Important future work is actually seeding cells into the system the team designed and testing the maturation and function of the tissue constructs grown and conditioned. This can include the use of mouse and human muscle cells, as well as a mechanism to measure force production. These studies and the data collected will speak to the capabilities of the device created at engineering biomimetic muscle tissue. After the device is validated for this purpose it can then be considered for its intended use with drug screening technology.

References

[1] “What is the Impact of Burden of Musculoskeletal Disorders and Why is the U.S. Bone and Joint Initiative Important?,” Bone and Joint Initiative USA.

[2] Longo, U., Loppini, M., Berton, A., Spiezia, F., Maffulli, N. and Denaro, V. (2012). Tissue Engineered Strategies for Skeletal Muscle Injury. [online] Stem Cells International. Available at: https://www.hindawi.com/journals/sci/2012/175038/cta/

[3] Centers for Disease Control and Prevention. (2018). Facts | Muscular Dystrophy | NCBDDD | CDC. [online] Available at: https://www.cdc.gov/ncbddd/musculardystrophy/facts.html

[4] Bach, A. D., Beier, J. P., Stern-Staeter, J. and Horch, R. E. (2004), Skeletal muscle tissue engineering. Journal of Cellular and Molecular Medicine, 8: 413–422. doi:10.1111/j.1582-4934.2004.tb00466.x

[5]Principles of Tissue Engineering, edited by Robert Lanza, et al., Elsevier Science, 2007. ProQuest Ebook Central, https://ebookcentral-proquest-com.ezproxy.wpi.edu/lib/wpi/detail.action?docID=305644.

[6] Guven, Sinan et al. “Multiscale Assembly for Tissue Engineering and Regenerative Medicine.” *Trends in biotechnology* 33.5 (2015): 269–279. *PMC*. Web. 16 Sept. 2017.

[7] Koning, M., Harmsen, M. C., van Luyn, M. J. A. and Werker, P. M. N. (2009), Current opportunities and challenges in skeletal muscle tissue engineering. J Tissue Eng Regen Med, 3: 407–415. doi:10.1002/term.190

[8] BMUS: The Burden of Musculoskeletal Diseases in the United States. (2016). Musculoskeletal Diseases. [online] Available at: http://www.boneandjointburden.org/

[9] A. M. Pearson and R. B. Young, “Diseases and disorders of muscle.,” *PubMed*. [Online]. Available: https://www.ncbi.nlm.nih.gov/pubmed/8398047.

[10] “Becker Muscular Dystrophy (BMD),” Muscular Dystrophy Association, 31-Jan-2018. [Online]. Available: https://www.mda.org/disease/becker-muscular-dystrophy.

[11] Vandenburgh, H., Shansky, J., Benesch-Lee, F., Skelly, K., Spinazzola, J., Saponjian, Y. and Tseng, B. (2018). Automated drug screening with contractile muscle tissue engineered from dystrophic myoblasts. NCBI.

[12]Physiopedia. (2017). Muscle Injuries. [online] Available at: https://www.physio-pedia.com/Muscle_Injuries

[13] SEER Training Modules, *Module Name*. U. S. National Institutes of Health, National Cancer Institute. Day Month Year (of access) <*https://training.seer.cancer.gov/*>.

[14]Gilbert SF. Developmental Biology. 6th edition. Sunderland (MA): Sinauer Associates; 2000. Myogenesis: The Development of Muscle.Available from: https://www.ncbi.nlm.nih.gov/books/NBK10006/

[15] “Facts About Myopathies,” Dec-2009. [Online]. Available: https://www.mda.org/sites/default/files/publications/Facts_Myopathies_P-208.pdf.

[16]“The Physiology of Skeletal Muscle Contraction,” *The Physiology of Skeletal Muscle Contraction*, 17-Dec-2015. [Online]. Available: https://www.ptdirect.com/training-design/anatomy-and-physiology/skeletal-muscle-the-physiology-of-contraction.

[17] Turner, N.J. & Badylak, S.F. Cell Tissue Res (2012) 347: 759. https://doi.org/10.1007/s00441-011-1185-7

[18] Grounds, Miranda D(Aug 2011) Regeneration of Muscle. In: eLS. John Wiley & Sons Ltd, Chichester. http://www.els.net [doi: 10.1002/9780470015902.a0001106.pub2]

[19] Rizzi, R., Bearzi, C., Mauretti, A., Bernardini, S., Cannata, S., & Gargioli, C. (2012). Tissue engineering for skeletal muscle regeneration. *Muscles, Ligaments and Tendons Journal*, *2*(3), 230–234.

[20] Bian W, Bursac N. Engineered skeletal muscle tissue networks with controllable architecture. *Biomaterials*. 2009;30(7):1401-1412. doi:10.1016/j.biomaterials.2008.11.015.

[21] Olson JL, Atala A, Yoo JJ. Tissue Engineering: Current Strategies and Future Directions. *Chonnam Medical Journal*. 2011;47(1):1-13. doi:10.4068/cmj.2011.47.1.1.

[22] Caddeo S, Boffito M, Sartori S. Tissue Engineering Approaches in the Design of Healthy and Pathological *In Vitro* Tissue Models. *Frontiers in Bioengineering and Biotechnology*. 2017;5:40. doi:10.3389/fbioe.2017.00040.

[23] Ikada Y. Challenges in tissue engineering. *Journal of the Royal Society Interface*. 2006;3(10):589-601. doi:10.1098/rsif.2006.0124.

[24] Caddeo S, Boffito M, Sartori S. Tissue Engineering Approaches in the Design of Healthy and Pathological *In Vitro* Tissue Models. *Frontiers in Bioengineering and Biotechnology*. 2017;5:40. doi:10.3389/fbioe.2017.00040.

[25] Rangarajan, S., Madden, L. & Bursac, N. Ann Biomed Eng (2014) 42: 1391. https://doi-org.ezproxy.wpi.edu/10.1007/s10439-013-0966-4

[26] Vandenburgh H, Shansky J, Benesch-Lee F, et al. Automated drug screening with contractile muscle tissue engineered from dystrophic myoblasts. *The FASEB Journal*. 2009;23(10):3325-3334. doi:10.1096/fj.09-134411.

[27] Cittadella Vigodarzere G, Mantero S. Skeletal muscle tissue engineering: strategies for volumetric constructs. *Frontiers in Physiology*. 2014;5:362. doi:10.3389/fphys.2014.00362.

[28] "Chapter Summary," *Human Physiology | Chapter Summary*. [Online]. Available: https://highered.mheducation.com/sites/0070272352/student_view0/chapter12/chapter_summary.html.

[29] H.H. Vandenburgh, S. Hatfaludy, P. Karlisch, J. Shansky, Mechanically induced alterations in cultured skeletal muscle growth, Journal of Biomechanics,Volume 24, 1991, Pages 91-99, ISSN 0021-9290, https://doi.org/10.1016/0021-9290(91)90380-6.

Appendix

A: Arduino Code

The Arduino code to program the stepper motor was obtained from the Arduino library and adapted for the desired physical parameters.

```
#include <Adafruit_MotorShield.h>
#include <Wire.h>
#include <Adafruit_MotorShield.h>
#include "utility/Adafruit_MS_PWMServoDriver.h"

// Create the motor shield object with the default I2C address
Adafruit_MotorShield AFMS = Adafruit_MotorShield();
// Or, create it with a different I2C address (say for stacking)
// Adafruit_MotorShield AFMS = Adafruit_MotorShield(0x61);

// Connect a stepper motor with 200 steps per revolution (1.8 degree)
// to motor port #2 (M3 and M4)
Adafruit_StepperMotor *myMotor = AFMS.getStepper(200, 1);

void setup() {
  Serial.begin(9600);           // set up Serial library at 9600 bps
  Serial.println("Stepper test!");

  AFMS.begin();  // create with the default frequency 1.6KHz
  //AFMS.begin(1000);  // OR with a different frequency, say 1KHz

  myMotor->setSpeed(40);  // 10 rpm
}

void loop() {
  Serial.println("Single coil steps");
  myMotor->step(100, BACKWARD, SINGLE);
  myMotor->step(100, FORWARD, SINGLE);

delay(1000);

  }
```

B: PDMS Protocol

The protocol used for fabrication of the PDMS layer with incorporated PDMS inserts was taken from a database of WPI's undergraduate Biomedical Engineering laboratories.

Protocol for Making Polydimethylsiloxane (PDMS)

Materials:

- Sylgard Silicone Elastomer base (Ellsworth Adhesive #184 SYL ELAST)
- Sylgard Silicone Elastomer curing agent (Ellsworth Adhesive #184 SYL ELAST)
- Gloves (The elastomer reagents are sticky and may be difficult to wash off)

Procedure:

1. Weigh 10 parts Sylgard silicone elastomer base and 1 part Sylgard silicone elastomer curing agent. Note: DO NOT MIX THE STOCK SOLUTIONS!!! Use separate weighing materials for each reagent.
2. Pour reagents together and thoroughly mix the elastomer base and curing agent.
3. Pour the well mixed solution into your mold.
4. Degas the PDMS by putting it into a vacuum chamber for at least 1 hour (larger/thicker volumes of PDMS may require more time).
5. After degassing, visually inspect the PDMS to ensure that there are no more bubbles. If there are, repeat steps 4 and 5.
6. Cure the PDMS by placing the mold into an oven set for 60 °C for at least 1 hour (larger samples may require more time).

One important thing to keep in mind is that the uncured reagents are very tacky and can make a big mess of anything they contact (the degassing chamber, the scale used to weigh reagents). Students should wear gloves when handling PDMS, be careful not to spill, and make sure they clean up the space and equipment they use when preparing PDMS. We keep "Goo Gone" in the lab for this reason…

Students using PDMS will need access to the desiccating/vacuum chambers, a vacuum source, a scale (~ grams), weigh boats and an oven for curing.

C: Budget Analysis

Table 8: Budget Analysis

Item	Date of Purchase	Price Estimate	Total Budget Left
RoboGets Arduino Uno R3 Compatible Electronic ATmega328P Microcontroller Card & USB Cable	February 6, 2018	$17.90	$732.10
Adafruit (PID 324) Stepper motor - NEMA-17 size - 200 steps/rev, 12V 350mA	February 6, 2018	$25.62	$706.48
Adafruit Motor/Stepper/Servo Shield for Arduino v2.3 Kit	February 6, 2018	$21.18	$685.30
Aluminum 6061 block	March 23, 2018	$70	$615.30
96-well plates (bottomless)	January 31, 2018	$136	$479.30
Adherent, clamps	March 17, 2018	$25	$454.30