A System to Measure Maximal Isometric Torque of the Mouse Quadriceps to Assess Functional Recovery of Skeletal Muscle in a Volumetric Muscle Loss Injury

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Abstract

Volumetric muscle loss (VML) is an injury characterized by the traumatic or surgical loss of skeletal muscle and a severe loss of function to the affected limb. Due to the injury's size and complexity, the body is unable to regenerate sufficient muscle tissue after a VML injury, causing a functional deficit to the affected limb. To better analyze functionality of limb recovery after VML, we have created a protocol and custom built apparatus to measure peak isometric torque conducted by the quadriceps muscle of our mouse model. To complete this process and analyze the data, we have also created a custom *in situ* apparatus and novel MATLAB code for data analysis of the functional force data.

One mouse underwent isometric torque testing immediately after receiving a surgically induced volumetric muscle loss injury. Maximum and average torque values of the injured leg saw a respective 53.2% and 55.6% drop when compared to the uninjured leg. After this preliminary testing, three mice received VML injuries and were allowed to heal for a 28-day period before isometric torque testing was conducted. All three mice showed decreases in both maximum and average torque readings across the injured legs, with maximum and average torque values of the uninjured leg being significantly greater than the injured leg (p values of 0.0325 and 0.0264 respectively). Our findings are promising and indicate that our testing method can quantify isometric torque readings and detect functional differences between injured and uninjured muscles. In the future we will apply this technique to studies in which regenerative therapies targeting muscle recovery, or even alternative injury models are explored.

Introduction

Volumetric muscle loss (VML) is a debilitating injury characterized by the traumatic or surgical loss of skeletal muscle and a loss of function to the affected limb. While skeletal muscle typically has the capacity to regenerate, muscle is incapable of recovery following a VML injury. This is partially due to the significant loss of extracellular matrix and muscle stem cells that provide both structure and regenerative capacities to the injury site[9]. Additionally, innervation and vascularization of the muscle are important for functional muscle recovery and are often damaged in VML[9]. Muscle trauma is common in the active duty military population and accounts for 50- 70%[6] of total military injuries. Complications in healing from these VML injuries accounts for 80% of delayed amputations,[3,12,14] and it is shown that greater than 90% of patients that experience muscle trauma involving VML have been medically retired due to long term disability[5].

A common standard of care for VML injuries requires an autologous muscle flap surgery[5]. While these procedures are often successful in limb salvage, they fail to successfully regenerate new functional muscle. Furthermore, muscle flaps do not provide functional strength to the traumatized musculature and can result in donor site morbidity[5]. The aim of this study is to assess the functionality of regenerated muscle after VML using a novel testing procedure and apparatus. To address the question of functional recovery of skeletal muscle in a VML injury, this study measures isometric torque produced by the tested muscle group as a measurement of functional output to quantify the regeneration of functional muscle tissue. Because functional recovery of muscle is an important metric in quantifying muscle regeneration, our goal was to build an apparatus and develop a protocol with validated metrics so that regeneration of healthy

muscle can be more easily quantified. In the future, this testing procedure could be adapted and used to measure muscle function of various different disease and injury models across various muscle groups and animal types.

The objective of this study was to develop an apparatus and a protocol to accurately measure peak isometric torque of the mouse quadriceps. It was hypothesized that the protocol and apparatus will accurately measure isometric torque and detect differences in VML injury models both before and during healing, providing a metric of functional recovery.

Literature Review

Current clinical methods for skeletal muscle tissue repair and regeneration following VML are very limited. Currently, treatment options for patients with VML injury include a combination of surgical techniques and physical therapy[9]. In treating VML, the gold standard of surgical strategies is an autologous muscle flap transfer, a surgery where a physician takes muscle from a different part of the patient's body and transfers it to the site of VML injury. While this method remains a successful surgery for limb salvage, it is largely unsuccessful in promoting a pro-regenerative environment for new muscle tissue to grow[1]. Autologous muscle flap surgeries are not only unable to regenerate functional muscle at the site of the injury, they can also cause additional problems, including donor site complications after surgery[5]. Due to the lack of treatment options for VML, research is being conducted in biomaterials, tissue engineering, and cell therapies to find ways in which muscle tissue's innate regenerative capacity can be utilized to enhance functional recovery following VML injury.

The use of biomaterials in preclinical VML models has been studied frequently within the past decade[6]. To improve the regeneration of skeletal muscle in VML, a biomaterial's microenvironment ideally would copy the natural structure of native tissue[6]. Biomimetic strategies, or strategies that mimic the natural structure of the body, can be achieved with biomaterials that have been designed to mimic structure and function of the tissue being healed. There are a variety of both natural and synthetic biomaterials that can be seeded with mesenchymal stem cells or laboratory derived pluripotent cell lines that attach, proliferate, and differentiate into the material (reviewed in *Beldjilali-Labro et. al (2018)*)[2]. Cells seeded on these biomaterials create a mechanical and biochemical environment advantageous to regeneration through cell signaling and physical factors that promote functional tissue growth into the scaffold[2].

After a skeletal muscle injury, the body naturally has a cascade of events that help initiate wound healing and recovery. Environmental factors initiate Notch and IGF-1 signaling, stimulating the proliferation of MuSCs[13] (satellite cells). Additionally, Wnt signaling assists in differentiating the MuSCs into muscle cells, allowing the process of new muscle fibers to form as muscle stem cells undergo myogensis[13]. After a VML injury, it is particularly challenging to regenerate skeletal muscle due to the disruptive nature of the injury that causes loss of specialized cells and complex muscle fiber orientation. In a VML injury localized MuSCs are absent with the loss of muscle and the local stem cell niche becomes disturbed. Additionally, a large portion of the muscle has been removed and the injury site is lacking the appropriate structure to rebuild. Cell therapy is a potential treatment inVML models that is being researched . The use biomimetic scaffolding seeded with MuSCs to assist the wound healing response and

promote functional recovery of the injury site could potentially help to replenish the localized MuSC population and aid the body's natural regenerative response, utilizing the complex signaling systems in place to regenerate muscle tissue. In future directions, functional recovery of muscle tissue assisted by cell therapy would be interesting to study using the proposed novel apparatus and protocol to test isometric torque. Using this device, functional outcomes and a quantitative metric of functional muscle regeneration could be established, helping to better understand the benefits of cell therapy.

Functional analysis of limb recovery can be accomplished through various methods. Some examples include gait analysis of the affected animal, magnetic resonance imaging of the VML injury site, and measurements of peak isometric contraction of the injured muscle[15]. The most direct measure of muscle function is through measuring peak isometric contraction. We proposed to use this method for our testing protocol of the quadriceps muscle, measured as peak isometric torque about the knee. This can be accomplished by using an electrical stimulus generator to stimulate the motor neuron innervating the muscle of interest and a force transducer to measure maximum torque about the connected joint of the animal model[15].

In this study, an *in situ* system was custom built for assessing maximal torque (Figure 2 A-B). For a VML model of an injured quadriceps muscle (Figure 1), high torque production about the knee shows functional capacity of the quadriceps muscle injured, indicating regeneration of the muscle. Conversely, low torque production shows minimal functional capacity of the quadriceps indicating that the injury site is fibrotic with little functional recovery. Optimal stimulation parameters are found by experimentally establishing a force/frequency relationship taking into account duty cycle, frequency, and total time stimulated[11]. A fatigue

protocol can also be established as another indicator of functional muscle recovery[4]. Through measuring peak isometric strength of both injured and uninjured muscle, the overall objective of this research was to show functional output of the quadriceps muscle at different stages of the injury process. We hypothesized that the designed protocol and apparatus would accurately measure isometric torque and detect differences in injury models both before and during healing. In future work we plan to use this testing method to validate various tissue engineering approaches, such as the use of biomaterials and cell therapy, and determine their success at promoting functional muscle recovery.

Materials and Methods

1.1 Animal Surgeries

C57Bl6/J mice were used to study and quantify the functional isometric torque output of the quadriceps muscle tissue after a VML injury. A novel testing protocol, apparatus, and MATLAB code were used to study functional deficits in mice with a VML injury to the quadriceps muscle compared to an uninjured control. To create the injury model, mice received a 3mm biopsy punch in the anterior left quadriceps. As shown in Figure 1, the 3mm diameter muscle segment that was removed extends from the surface of the muscle to the femur bone. The 3mm biopsy punch size was determined by the lab to be the threshold for critically sized VML defects[1]. This means that this injury is a critical size where the tissue will not spontaneously heal the injury and there is a suspected functional loss in muscle due to scar tissue infiltration during the healing process. The right quadriceps of each animal was tested with our isometric torque process as an uninjured control without the surgery. The other leg received the surgery to simulate the injury model. Testing was conducted immediately after surgery on one mouse acting

as a proof of concept for our testing apparatus. In a larger study, three mice with surgically induced 3mm VML injuries were allowed to heal for 28 days before isometric torque testing was completed. Test data from the injured leg was compared to the contralateral control leg to analyze the functional torque differences between the uninjured and injured models. All animals were used according to the protocols approved by the Georgia Institute of Technology Institutional Animal Care and Use Committee (IACUC).

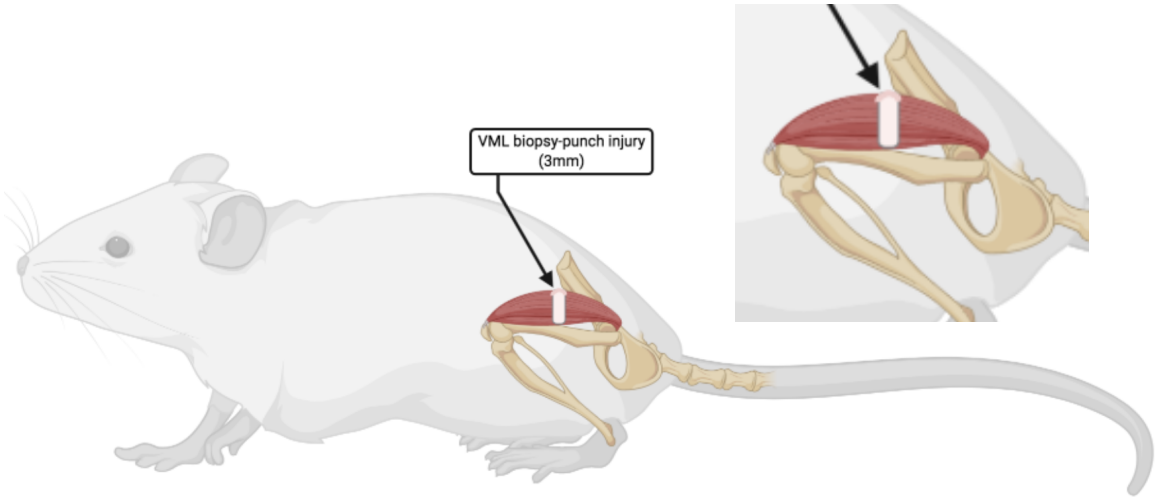

Figure 1: Anatomical placement of VML injury on left quadriceps. Surgery conducted with a 3mm biopsy punch

1.2 Creation of the Testing Apparatus

To complete this isometric torque testing, an in vivo system was custom built for assessing maximal torque (Fig.2 A-B). To secure the mouse in place and provide proper orientation of the animal, the top of the custom apparatus was modeled after a cross-slide vice. Sides of the apparatus were designed so that the force transducer could be adjusted on rails closer or farther away from the animal, adjusting for tension of the string attaching the animal to the

force transducer. The apparatus was designed in Adobe Illustrator and laser cut in clear acrylic with the built dimensions of 4'' high x 12'' long x 6'' wide. It was assembled using Gorilla-glue epoxy, and bolts and hex nuts were used to fasten the acrylic vice panels. In performing the isometric torque tests, the animal was secured between the vice-like panels, and centered over the apparatus. Its ankle was then secured to the force transducer, which hung on rails underneath the device.

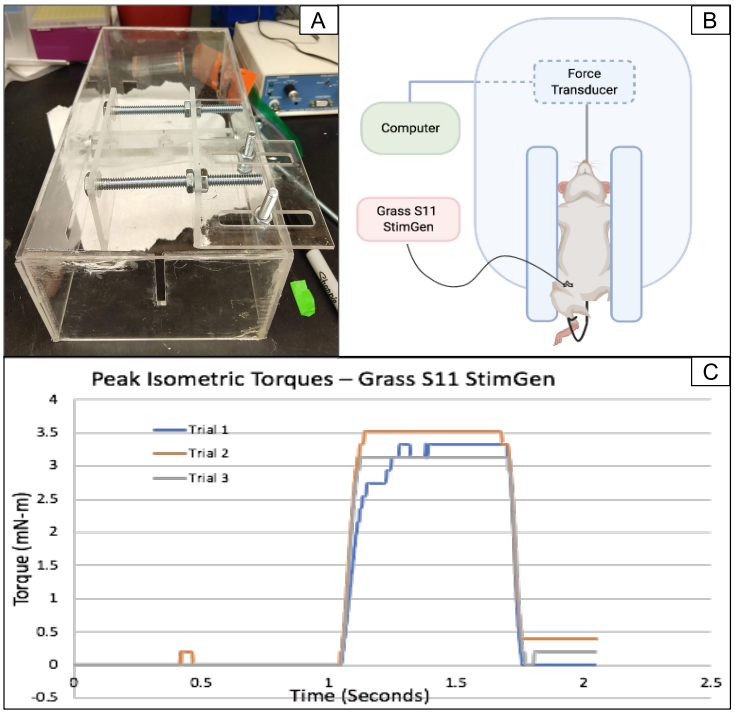

Figure 2: A. *Image of custom build testing apparatus.* **B.** Schematic of isometric torque testing procedure. **C.** Peak isometric torque readings indicating functionality under given parameters.

1.3 Nerve cuff construction

Nerve cuffs were constructed to be placed on the femoral nerve of the animal and stimulate the quadriceps muscle (Fig. 3). These nerve cuffs were constructed using silicone tubing cut in half to create a cuff, Teflon coated wire to provide electrical stimulation, and a silicone clone to insulate and hold pieces together.

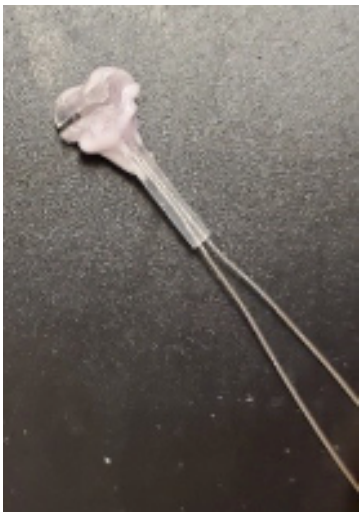

Figure 3: Custom constructed nerve cuff comprised of silicon tubing, wire, and silicon clone.

1.4 Isometric Torque Testing as a Metric of Functional Recovery

Using the custom-built apparatus described in section 1.2, isometric torque testing was conducted. Isometric torque measurements can be accomplished using an electrical stimulus generator to stimulate

the motor neuron innervating the muscle of interest and a force transducer to measure maximum torque about the knee of the animal model[15]. For this test a No. 60-2996 Harvard Apparatus force transducer, a GrassS11 Stimulus Generator, and a Grass SIU5 stimulus isolation unit were used. Optimal stimulus parameters were experimentally tested across a frequency range of 10-350 hz. From our testing, frequencies ranging between 120-200hz provided desired stimulus to the quadriceps muscle of the mice. Optimal stimulus parameters outputted by the GrassS11 stimulus generator were determined to be: 15-volt rectangle wave output, 175 Hz stimulus frequency, 0.02 ms pulse duration, and a stimulus time of 500ms. These stimulation parameters were found by empirically establishing a force/frequency relationship taking into account duty cycle, frequency, and total time stimulated[11]. Three trials were conducted on an uninjured animal acting as a scientific control to demonstrate and validate the testing method (Fig.1 C). To record the electrical stimulus, DASYLab Software was used on a laptop with a USB port and was connected to a data acquisition board (DAQ). Nerve cuffs were constructed and secured around the femoral nerve of the animal; stimulation by the electrical stimulus produced tetanic contraction of the quadriceps muscles. This contraction produced a measurable torque production about the knee which was calculated as the force output multiplied by the length between the generated force and the muscle of interest. To calculate the torque value, the mass value from the transducer was first calibrated from either the 50 or 500-gram setting. Then, because the force transducer measures in units of mass and not force, the transducer value was then multiplied by the gravitational acceleration constant of 9.81 m/s^2 to obtain a value in units of force. The equation to calculate Torque is $T = F\ x\ L$ where T, torque is measured by crossing F, force by L, the length of the lever arm. In this instance the lever arm was the length of a mouse tibia, which measures approximately 0.02 meters, or 2 cm, and rests perpendicular to the quadriceps muscle

where the force is being produced. Keeping mindful of units, the peak analyzing code was written so that torque values are outputted in millinewton – meters.

During the testing procedure, animals were fully unconscious under isoflurane gas. Legs were dissected to expose the femoral nerve for nerve cuff attachment. Animals were secured on our custom apparatus and a heat lamp was used to maintain the animals body temperature during testing. The ankle of the leg tested was attached to the force transducer, with the tibia at a 90-degree angle to the femur resting on the apparatus. The quadriceps muscle of each leg was stimulated three times, which was recorded by the DASYLab software and saved as a .csv file on the laptop used. Following testing of the right uninjured quadriceps, the left quadriceps of the mouse with the 3mm biopsy punch surgery simulating the VML injury model was tested. After data collection was complete, the animal was euthanized according to IACUC protocols without regaining consciousness using carbon dioxide gas. This is because no pain medication is administered to the animal and this is a terminal procedure.

1.5 Data Analysis of Isometric Torque Testing Results

Three MATLAB scripts were written for data analysis and statistical testing. Provided in Appendix A is the data analysis script that was written to read the saved .csv files from section 1.3 and isolate the tetanic muscle contraction peaks. This MATLAB code rank sorts the peaks of the force data using the FindPeaks MATLAB function and isolates the three trials conducted for each .csv file. Further, the code converts the units of force into units of torque using the method described in section 1.4. The MATLAB code then isolates each trial and graphs the output to show sustained tetanic curves for each trial. Lastly, the code outputs new .csv files which include

raw data from the three isolated trials along with maximum and average torque values for each trial.

Analyzed .csv files outputted from the first MATLAB code were then paired by animal. Right and left quadriceps values were compared in a second code provided in Appendix B. This code provides a graph of injured and uninjured tetanic curves as torque vs. time for each animal as well as a bar graph of max and average torque values for injured and uninjured legs (Figure 4,5). Additionally, this code outputs an averaged maximum and average torque values for the three trials conducted on each leg of the animal. This provides the user with an injured and uninjured maximum and average torque value per animal with a calculated standard deviation. Lastly, this code also provides a percent decrease value between injured and uninjured legs of each animal as a metric of percent functional recovery of quadriceps usage.

The third MATLAB code takes the original files saved from the first code referenced and conducts a pared right-tailed t-test on the maximum and average torque values for all animals tested (Appendix C). For this t-test, the null-hypothesis is that there will be no significant difference between injured and uninjured torque values. The code has factored in a 5% significance level, and outputs a p value and h value, indicating if the null hypothesis is rejected. If the p value is smaller than 0.05, h=1 which shows rejection of the null hypothesis. If the p value is greater than or equal to 0.05, the code outputs h=0, indicating the null hypothesis is not rejected. Statistical values of h and p are then saved in a separate .csv file. Lastly, this code outputs two bar graphs comparing maximum and average torque values across injured and uninjured legs on all animals tested (Figure 6).

Results

After preliminary validation of the device was conducted and optimal parameters were experimentally found, a pilot study was conducted with one mouse as a proof of concept to determine whether the device, protocol and data acquisition methods accurately measured isometric torque and could detect functional differences in the characterized 3mm VML injury model when compared to an uninjured control. One uninjured mouse underwent three isometric torque trials on its right quadriceps, producing isometric torque curves as seen in red in Figure 4. This data was analyzed in MATLAB across the three trials, and the uninjured quadriceps demonstrated a maximum torque of 9.21 ± 0.52 mN-m and an average torque output of 8.22 ± 0.36 mN-m. This mouse was then tested on its left quadriceps muscle and immediately before testing a surgically induced 3mm VML injury was given to the animal. As shown in Figure 4, the injured quadriceps experienced much lower and inconsistent isometric torque curves. The injured left quadriceps had a maximal torque output of 4.31 ± 0.34 mN-m, and an average torque of 3.65 ± 0.23 mN-m. When comparing the uninjured to the 3mm VML injured quadriceps, the leg of the mouse with the VML injury saw a 53.2% decrease in maximal isometric torque production and 55.6% decrease in average torque production when compared to the uninjured control (Figure 4).

After successful preliminary results, functional recovery of mice receiving 3mm VML injuries was further explored in a larger animal study. Three mice (referred to here as mice 4, 11, and 12) received a 3mm biopsy punch VML injury and were allowed to heal for 28 days. Animals were allowed to heal naturally without any additional treatments or therapies. After 28 days, isometric torque testing identical to the proof-of-concept study was conducted to determine if a loss in functional recovery of the injured quadriceps muscle was still present after a 28-day

recovery period. As shown in Figure 5, all three animals showed visible differences in the tetanic curves of their injured versus uninjured legs. Tables 1 and 2 summarize the maximum and average differences of the injured vs uninjured legs of each of the three mice. mouse 4 experienced a 41.6% drop in maximal torque values and 46.6% drop in average torque values when comparing the injured verses uninjured leg. Mouse 11 experienced a 86.2% drop in maximal torque values and 86.4% drop in average torque values and Mouse 12 saw a 60.8% and 59.8% drop in maximum and average torque values respectively. One uninjured trial from mouse 4 and mouse 12 were not considered in analysis as they did not properly capture a tetanic curve and therefore could not be analyzed.

For the maximum torque dataset, the uninjured leg showed average torque values of 18.13 mN-m, 21.36 mN-m, and 17.15 mN-m. The injured leg showed maximum torque values averaging 10.58 mN-m, 2.940 mN-m, and 6.729 mN-m for mice 4, 11 and 12 respecitvely. For the average torque dataset, the uninjured leg showed average torque values of 16.33 mN-m, 18.49 mN-m, and 15.00 mN-m. The injured leg showed average torque values averaging 8.722 mN-m, 2.506 mN-m, and 6.032 mN-m for mice 4, 11 and 12 respecitvely (Figure 6).

After data from all three mice were analyzed, a paired right-tailed t-test was conducted to determine if the uninjured right quadriceps produced significantly greater torque output than the injured left quadriceps after a 28-day recovery. The null hypothesis was that there will be no statistical difference between groups whereas the alternative hypothesis was that the uninjured leg would have greater torque output than the injured leg. Running this t-test, p-values were 0.0325 and 0.264 for maximum and average datasets respectively (Figure 6). For both cases we reject the null hypothesis. Our results are suggestive that across all three animals, torque

production in a recovered (28-day post injury) VML model is still significantly lower than in an uninjured quadriceps muscle.

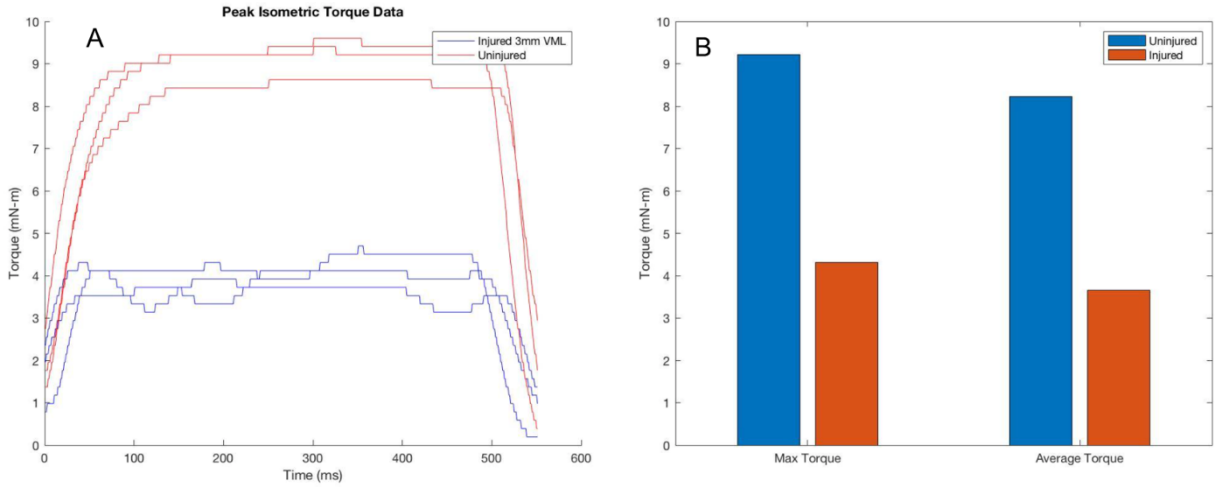

Figure 4: A. Isometric torque curves of mouse immediately after 3mm VML injury. Right quadriceps was uninjured, producing torque curves shown in red. Left quadriceps contains 3mm VML injury, shown in blue curves of smaller magnitude. Three trials were conducted on each leg of the mouse, producing a total of 6 torque curves. **B.** Bar graph illustrating average and maximum torque production for injured and uninjured legs of the mouse. Average torque uninjured was 8.22 ± 0.36 mN-m and injured was 3.65 ± 0.23 mN-m. Maximum torque uninjured was 9.21 ± 0.52 mN-m and injured was 4.31± 0.34 mN-m. Results show 55.6% decrease in average torque production and 53.2% decrease in maximum torque production when comparing uninjured to injured leg.

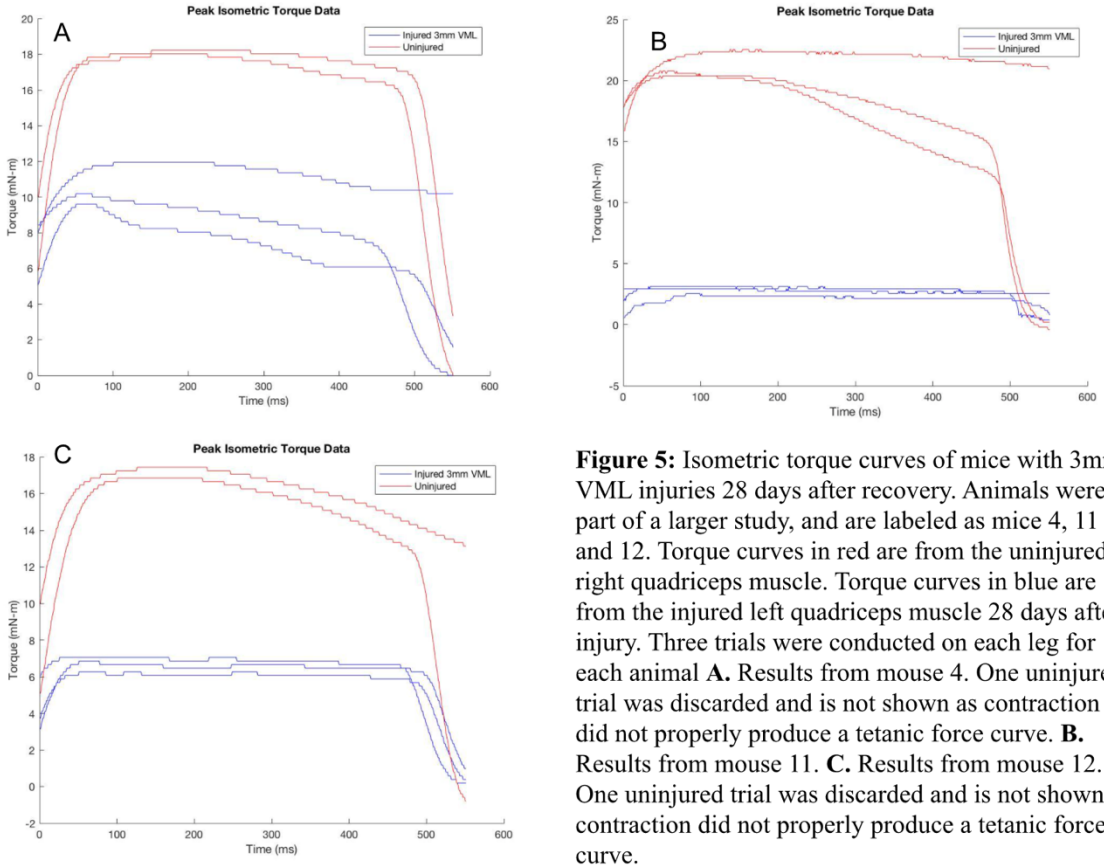

Figure 5: Isometric torque curves of mice with 3mm VML injuries 28 days after recovery. Animals were part of a larger study, and are labeled as mice 4, 11 and 12. Torque curves in red are from the uninjured right quadriceps muscle. Torque curves in blue are from the injured left quadriceps muscle 28 days after injury. Three trials were conducted on each leg for each animal **A.** Results from mouse 4. One uninjured trial was discarded and is not shown as contraction did not properly produce a tetanic force curve. **B.** Results from mouse 11. **C.** Results from mouse 12. One uninjured trial was discarded and is not shown as contraction did not properly produce a tetanic force curve.

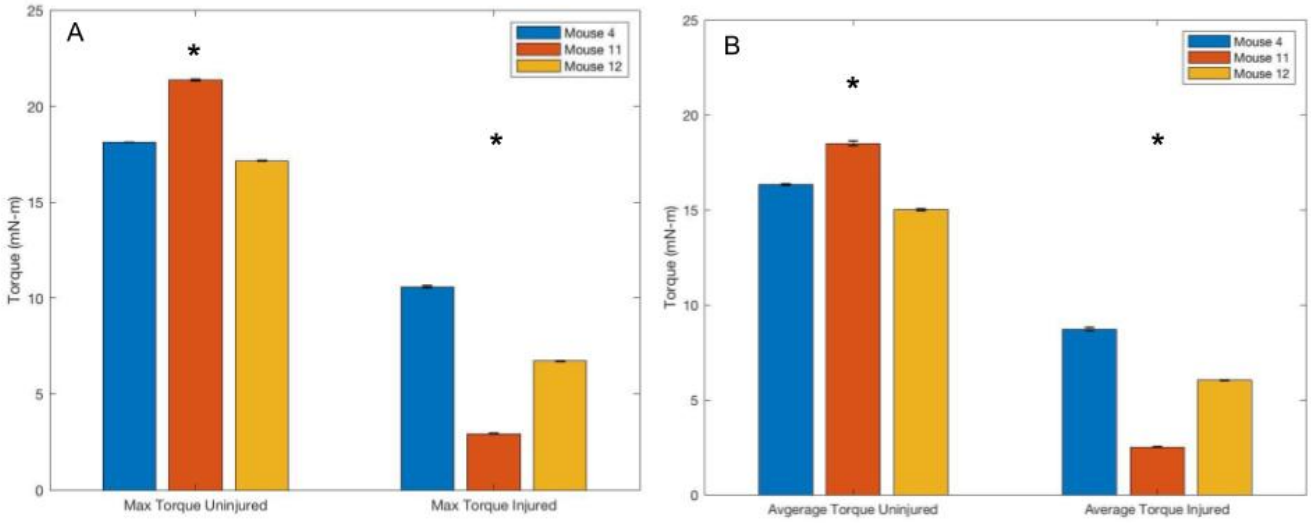

Figure 6: Bar graphs illustrating maximum and average torque values for mice 4 (blue) 11 (orange) and 12 (yellow) comparing injured and uninjured legs. A one sided (right tailed) paired t-test was conducted at the 5% significance level for both data sets. **A.** Maximum torque values rejected the null hypothesis with a **p-value of 0.0325,** showing uninjured max torque output was significantly greater than injured max torque output **B.** Average torque values rejected the null hypothesis with a **p-value of 0.0264,** showing uninjured average torque output was significantly greater than injured average torque output. In both graphs, * denote significance between injured and uninjured groups.

Mouse Number	Max Torque Uninjured (mN-m)	Max Torque Injured (mN-m)	% Difference
4	18.13 ± 0.14	10.58 ± 1.22	41.6%
11	21.36 ± 1.04	2.94 ± 0.39	86.2%
12	17.15 ± 0.42	6.73 ± 0.41	60.8%

Table 1: Maximum torque values from mice 4, 11, and 12 in millinewton - meters (mN-m). Percent difference between uninjured and injured torque values for each animal are also shown.

Mouse Number	Avg Torque Uninjured (mN-m)	Avg Torque Injured (mN-m)	% Difference
4	16.32 ± 0.58	8.72 ± 2.15	46.6%
11	18.50 ± 3.01	2.51 ± 0.41	86.4%
12	15.00 ± 1.36	6.03 ± 0.32	59.8%

Table 2: Average torque values from mice 4, 11, and 12 in millinewton - meters (mN-m). Percent difference between uninjured and injured torque values for each animal are also shown.

Discussion

While more animals will need to be tested to further understand functionality of this testing method and accuracy of torque output as a quantitative measurement, these results are promising. The use of the MATLAB code will be especially significant in increasing sample size and accurate data analysis because it allows for faster and more accurate data analysis of the testing, and also filters out noise and dead space in the data between trials conducted. By automating this process, it is easier to analyze more data and there is less error than manually searching through the .CSV files to calculate Max torque, average torque, and isolated peaks for each trial. Overall, the preliminary results show a significant decrease in both maximal and

average isometric torque in the injured quadriceps muscle across both our proof-of concept and three animal post-recovery study. This indicates that this method can correctly quantify functionality of the muscle tissue using isometric torque readings and future studies aim to support this trend.

While maximum and average percent drop across injured and uninjured legs was consistent across an individual animal, one unexpected finding is the variance in percent decreases in torque output across animals. For example, mouse 4 only saw a 46.6% decrease in average torque output of the injured leg where mouse 11 saw an 86.6% decrease in torque output. More experimentation will need to be done to see if this variation occurs across a larger set of animals. Additionally, future studies would aim to determine if these findings are naturally variable by animal or if there is an unknown source of human error that is not being controlled for. For example, an un-noticed inconsistency in animal testing, or slight differences in animal behavior or activity during the 28-day healing process could potentially be contributing to variable percent differences.

Conclusion

While more experimentation will need to be conducted to increase the power of this experiment and optimize parameters for maximal torque output, it has been shown that the current protocol and apparatus can be used to contract the quadriceps of mice and produce consistent isometric torque curves. These promising results are indicative that this testing method can be used as a metric for functional recovery of the quadriceps muscle when comparing torque values to an uninjured control. Aside from building on this work with more robust experimentation, it would be useful to study VML injuries that have been treated with cell

therapy, exercise therapy or another treatment intended to increase functional muscle recovery. Future work aims to compare 3mm VML injuries that are partially or fully healed at a 28-day time point with no treatment against animals that have received biomimetic scaffolds and cell treatment. We anticipate that this isometric torque testing protocol will be able to assess functionality of muscle and quantify differences after the injury has been healed with varies different treatment techniques. In addition, testing could also be experimented on other disease or injury models outside of VML, including ischemic muscle injury, cardiotoxin injury or cryogenic muscle injury.

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Appendix A

Code for Peak Isolation and Maximum and Average Torque Values

```
close all
clear all

% Under Signal copy pathname of the file you wish to analyze and put it in
the purple parenthasis

Signal = importdata('.csv')

%Under Filename write what you wish to save your new csv file as. Data for
%me is saved in a file on my desktop. Make sure to keep .csv in filename

Filename = '.csv'


data = Signal.data;
convfact = 10 * 0.0098 * 0.02 * 1000
data(:,2) = data(:,2) * convfact;
%time = data(:,1);
torque = data(:,2) ;
offset = torque(end);
data(:,2) = data(:,2) + (offset* -1);
torque = torque + (offset* -1);

x = 1:length(torque);

[psor,lsor] = findpeaks(torque,x,'SortStr','descend');

findpeaks(torque,x)

text(lsor+.02,psor,num2str((1:numel(psor))'))
legend('Raw Data (All Trials)')
% When code runs you can lengthen and shorten the window of the peak you
% are analyzing by adding or subtracting more miliseconds. It is important
% that the absolute value of miliseconds added and subtracted combined are
the SAME number for each peak.

a = lsor(1) - 100;
b = lsor(1) + 450;

peak1 = torque(a:b);
length1 = 1:length(peak1);


maxtorque1 = max(peak1)
averagetorque1 = mean(peak1)

%Graph of Trial 1
```

```
figure(2)
hold on
plot(length1,peak1)
title('Peak Isometric Torque Data')
xlabel('Time (ms)')
ylabel('Torque (mN-m)')
legend('Trial_1')
hold off

c = lsor(2) - 100;
d = lsor(2) + 450;

peak2 = torque(c:d);
length2 = 1:length(peak2);

maxtorque2 = max(peak2)
averagetorque2 = mean(peak2)


%Graph of trials 1 and 2
figure(3)
plot(length2,peak2)
hold on
plot(length1,peak1)
title('Peak Isometric Torque Data')
xlabel('Time (ms)')
ylabel('Torque (mN-m)')
legend('Trial_2','Trial_1')
hold off

e = lsor(3) - 100;
f = lsor(3) + 450;

peak3 = torque(e:f);
length3 = 1:length(peak3);

maxtorque3 = max(peak3)
averagetorque3 = mean(peak3)

%Graph of Trials 1,2, and 3
figure(4)
plot(length3,peak3)
hold on
plot(length1,peak1)
plot(length2,peak2)
title('Peak Isometric Torque Data')
xlabel('Time (ms)')
ylabel('Torque (mN-m)')
legend('Trial_3','Trial_1','Trial_2')
hold off

% Graphs can be saved individually or seperate by changing hold on and hold
% off parameters. Labels can be changed by changing
% the text in purple parenthesis
```

```
Peak1_in_mNxm = peak1;
Peak2_in_mNxm = peak2 ;
Peak3_in_mNxm = peak3;
sz = size(length1);
MaxTorque1_in_mNxm = zeros(sz)';
MaxTorque2_in_mNxm = zeros(sz)';
MaxTorque3_in_mNxm = zeros(sz)';
AverageTorque1_in_mNxm = zeros(sz)';
AverageTorque2_in_mNxm = zeros(sz)';
AverageTorque3_in_mNxm = zeros(sz)';
MaxTorque1_in_mNxm(1) = maxtorque1
MaxTorque2_in_mNxm(1) = maxtorque2
MaxTorque3_in_mNxm(1) = maxtorque3
AverageTorque1_in_mNxm(1) = averagetorque1;
AverageTorque2_in_mNxm(1) = averagetorque2;
AverageTorque3_in_mNxm(1) = averagetorque3;
Time_in_ms = length1'

T = table(Time_in_ms,Peak1_in_mNxm,MaxTorque1_in_mNxm,AverageTorque1_in_mNxm,
Peak2_in_mNxm, MaxTorque2_in_mNxm, AverageTorque2_in_mNxm, Peak3_in_mNxm,
MaxTorque3_in_mNxm,AverageTorque3_in_mNxm)

writetable(T,Filename)
```

Appendix B

Code for Comparing Uninjured and Injured Datasets

```
close all
clear all

injured = importdata('.csv')
uninjured = importdata('.csv')


FilenameMax = '.csv'
FilenameAvg = '.csv'

Injured = injured.data;
Uninjured = uninjured.data;

Time = Injured(:,1);
Injured1 = Injured(:,2);
Injured2 = Injured(:,5);
Injured3 = Injured(:,8);
Uninjured1 = Uninjured(:,2);
Uninjured2 = Uninjured(:,5);
Uninjured3 = Uninjured(:,8);

figure(1)
hold on
plot(Time,Injured1, 'b')
plot(Time, Uninjured1, 'r')
```

```
plot(Time,Injured2, 'b')
plot(Time,Injured3, 'b')
plot(Time, Uninjured2, 'r')
%plot(Time, Uninjured3, 'r')
title('Peak Isometric Torque Data')
xlabel('Time (ms)')
ylabel('Torque (mN-m)')
legend('Injured 3mm VML','Uninjured')
hold off

MaxTorqueInjured = (Injured(1,3) + Injured(1,6)+ Injured(1,9))/3
MaxTorqueUninjured = (Uninjured(1,3) + Uninjured(1,6)+ Uninjured(1,9))/3

PercentDiffMax = (MaxTorqueUninjured - MaxTorqueInjured)/ MaxTorqueUninjured

StdMaxInjured = std([Injured(1,3) Injured(1,6) Injured(1,9)])
StdMaxUninjured = std([Uninjured(1,3) Uninjured(1,6) Uninjured(1,9)])

AvgTorqueInjured = (Injured(1,4) + Injured(1,7)+ Injured(1,10))/3
AvgTorqueUninjured = (Uninjured(1,4) + Uninjured(1,7)+ Uninjured(1,10))/3
PercentDiffAvg = (AvgTorqueUninjured - AvgTorqueInjured)/ AvgTorqueUninjured

StdAvgInjured = std([Injured(1,4) Injured(1,7) Injured(1,10)])
StdAvgUninjured = std([Uninjured(1,4) Uninjured(1,7) Uninjured(1,10)])

figure(2)
vals = [MaxTorqueUninjured MaxTorqueInjured;AvgTorqueUninjured
AvgTorqueInjured];
b = bar(vals);

TMax = table(MaxTorqueUninjured,StdMaxUninjured,
MaxTorqueInjured,StdMaxInjured,PercentDiffMax)
TAvg = table(AvgTorqueUninjured,StdAvgUninjured,
AvgTorqueInjured,StdAvgInjured,PercentDiffAvg)
writetable(TMax,FilenameMax)
writetable(TAvg,FilenameAvg)
```

Appendix C

Code for Statistical Analysis and Comprehensive Bar Graph

```
Ms4Max = importdata('.csv')
Ms11Max = importdata('.csv')
Ms12Max = importdata('.csv')

Ms4Avg = importdata('.csv')
Ms11Avg = importdata('.csv')
Ms12Avg = importdata('.csv')

Ms4Max = Ms4Max.data;
```

```
Ms11Max = Ms11Max.data;
Ms12Max = Ms12Max.data;

Ms4Avg = Ms4Avg.data;
Ms11Avg = Ms11Avg.data;
Ms12Avg = Ms12Avg.data;


InjuredAvg = [ Ms4Avg(1,3) Ms11Avg(1,3) Ms12Avg(1,3)]
UninjuredAvg = [ Ms4Avg(1,1) Ms11Avg(1,1) Ms12Avg(1,1)]

[hAvg,pAvg] = ttest(UninjuredAvg,InjuredAvg,'Tail','right');



InjuredMax = [ Ms4Max(1,3) Ms11Max(1,3) Ms12Max(1,3)]
UninjuredMax = [ Ms4Max(1,1) Ms11Max(1,1) Ms12Max(1,1)]

[hMax,pMax] = ttest(UninjuredMax,InjuredMax,'Tail','right')

%Error bars
std_error_uninjuredAvg = [Ms4Avg(1,2),Ms11Avg(1,2),Ms12Avg(1,2)] / sqrt(500)
std_error_injuredAvg = [Ms4Avg(1,4),Ms11Avg(1,4),Ms12Avg(1,4)] / sqrt(500)
std_error_uninjuredMax = [Ms4Max(1,2),Ms11Max(1,2),Ms12Max(1,2)] / sqrt(500)
std_error_injuredMax = [Ms4Max(1,4),Ms11Max(1,4),Ms12Max(1,4)] / sqrt(500)
figure(1)


figure(1)
model_series = [UninjuredAvg; InjuredAvg];
model_error = [std_error_uninjuredAvg; std_error_injuredAvg];
b = bar(model_series, 'grouped');
hold on
% Find the number of groups and the number of bars in each group
[ngroups, nbars] = size(model_series);
% Calculate the width for each bar group
groupwidth = min(0.8, nbars/(nbars + 1.5));
% Set the position of each error bar in the centre of the main bar
% Based on barweb.m by Bolu Ajiboye from MATLAB File Exchange
for i = 1:nbars
    % Calculate center of each bar
    x = (1:ngroups) - groupwidth/2 + (2*i-1) * groupwidth / (2*nbars);
    errorbar(x, model_series(:,i), model_error(:,i), 'k', 'linestyle',
'none');
end


legend('Mouse 4', 'Mouse 11', 'Mouse 12')
 xticklabels({'Avgerage Torque Uninjured','Average Torque Injured'})
 ylabel('Torque (mN-m)')

hold off

figure(2)
model_series = [UninjuredMax; InjuredMax];
```

```
model_error = [std_error_uninjuredMax; std_error_injuredMax];
b = bar(model_series, 'grouped');
hold on
% Find the number of groups and the number of bars in each group
[ngroups, nbars] = size(model_series);
% Calculate the width for each bar group
groupwidth = min(0.8, nbars/(nbars + 1.5));
% Set the position of each error bar in the centre of the main bar
% Based on barweb.m by Bolu Ajiboye from MATLAB File Exchange
for i = 1:nbars
    % Calculate center of each bar
    x = (1:ngroups) - groupwidth/2 + (2*i-1) * groupwidth / (2*nbars);
    errorbar(x, model_series(:,i), model_error(:,i), 'k', 'linestyle',
'none');
end


legend('Mouse 4', 'Mouse 11', 'Mouse 12')
 xticklabels({'Max Torque Uninjured','Max Torque Injured'})
 ylabel('Torque (mN-m)')

hold off


Filename = 'ThesisStats.csv'

t = table(hMax,pMax,hAvg,pAvg)
writetable(t, Filename)
```