# Isolation of Single Muscle Fibers in Preparation for Loading into Microfluidic Devices


## Summary

Modeling and simulation of microfluidic devices to capture single muscle fibers has led to several design possibilities. Before testing the fabricated devices, however, a procedure for the isolation of single muscle fibers from muscle tissue must be developed. The aim is to optimize a collagenase treatment that can yield consistent fibers of large quantity and dimensions that correspond to device tapering regions. Optimization of collagenase concentration and trituration showed that a 0.6% (w/v) solution produced the most fibers and the wide bore pipette tip method of trituration produced the longest fibers. Further analysis of centrifugation reinforced the theory of fiber separation and clean up based upon length. Future work includes testing of a larger sample size of muscle tissue/type, testing fiber functionality, preparing new methods of collagenase preparation and treatment, and seeing fiber response/analysis in the fabricated devices.

## Introduction

Skeletal muscle in organisms, under the control of the somatic nervous system, provides the external skeleton and body the ability to generate force and movement. The force output depends upon the molecular mechanism of muscle contraction. Skeletal muscle is made up of large bundles of muscle cells or fibers surrounded by connective tissue. Each fiber lies parallel with each other in the bundle and contains contractile elements called myofibrils. Figure 1 shows the basic diagram of a muscle fiber.

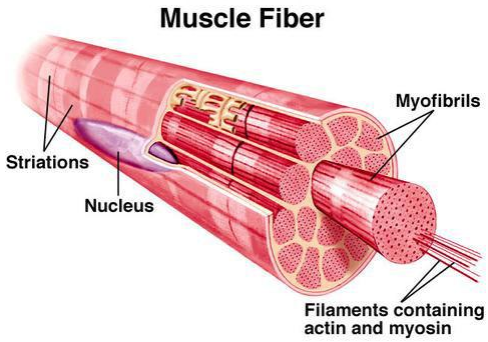


**Figure 1 - Muscle Fiber Diagram**[1]

The myofibrils have similar repeating patterns due to the end to end connection of the contractile elements of sarcomeres. These sarcomeres are made up on thick and thin myosin and actin filaments and bounded by Z-lines. Skeletal muscle contraction occurs through the cross bridge cycle, which is activated through the energy consumption of ATP (Figure 2). First, ADP binds at the cross bridge section of the myosin filament, which is opened up by the binding of calcium to troponin and the movement of tropomyosin to block the cross bridge. ADP binding causes the myosin cross bridge segment to attach to actin and cause a sliding movement. The detachment of the actin bound to the myosin is triggered by binding of ATP to the cross bridge section of the myosin. Hydrolysis of the ATP reenergizes the cross bridge to cause ADP binding the contraction to ensue yet again.[3]

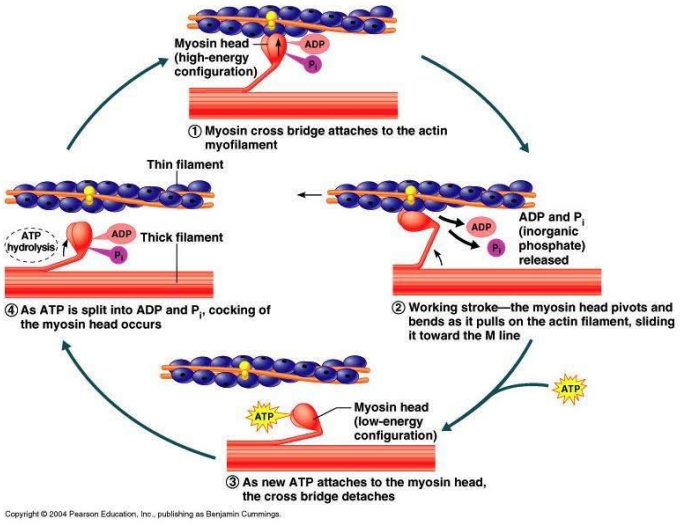


**Figure 2 - Cross-Bridge Cycle of Muscle Contraction**[2]

There are numerous instances where muscle contractility diminishes and fails. Muscular atrophy is the general tendency for a decrease in muscle mass, which in turn causes a drop in muscle force that can be exerted. Muscle weakness is often caused by aging and has been linked to diseases such as congestive heart failure, cancer, AIDS, and renal failure.[4] The cause of muscle weakness is an active area of research, and studies have shown that an increase in reactive oxygen species (ROS) production in the mitochondria of skeletal muscle correlates to muscular atrophy. ROS are produced through cellular metabolism in the mitochondria and cause apoptosis and damage of DNA, RNA, and proteins due to oxidation - all of which contribute to the physiology of aging. One prime contributor to oxidative damage is hydrogen peroxide conceived from superoxide leaking from the mitochondria. Investigation of ROS production in skeletal muscle has often been difficult due to the complexity and energetic profile that the tissue contains. During a single fiber analysis, the heterogeneity of the fibers does not help as well. Each fiber must be analyzed individually in order to get true understanding of what the increase in ROS is doing to the specific muscle type.[4] In addition to ROS production, each single muscle fiber can be analyzed for contractile function, membrane potential, mitochondrial mass, and apoptosis. Gathering measurements for all these parameters can be vital in the understanding of why muscle contractility failure occurs and discovery of possible treatments.

One type of single muscle fiber analysis that has been in development within this lab involves microfluidics. Microfluidic devices typically consist of one or more channels and are able to precisely manipulate fluid flows within the micrometer range of length scales. Microfluidic devices rely on fundamental principles of fluid mechanics in order to function properly. The flow characteristics usually consist of a steady state, incompressible, laminar flow with a low Reynolds number.[5] An initial, simple two channel device was first developed based on preliminary rat and mouse muscle fiber length and diameter data collected by Dr. Vrata Kostal. Figure 3 shows a schematic initial drawing of the device and Figure 4 shows a CAD model made before undergoing simulations. The basis of the design was to load fibers through

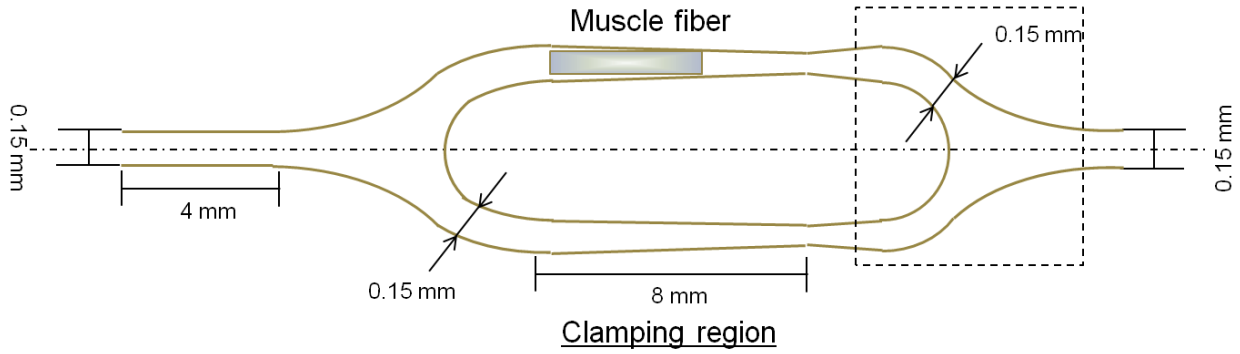


**Figure 3 - Schematic of Design of first Model**

the inlet, using a pressure difference, and then capture the fibers at a particular clamping or tapering region farther down the channels. At this region, most of the analysis of the fiber through drug treatment and image could take place. Similar technology was developed for capturing of C. elegans in the Whiteside Lab.

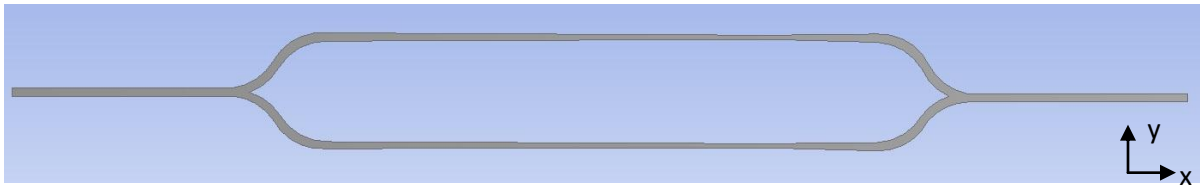


**Figure 4 - CAD Drawing of Initial 2-Channel Device**

Microfluidics can encompass a large range of systems to drive the flow. In the device we designed, flow was driven by application of a pressure gradient between the inlet and outlet. After sketching out the design, Ansys Workbench software (CFX) was used to create a 3D model, mesh, and simulation of the flow through the device with set parameters identified as steady state and laminar. Various optimizations in device design were required to ensure the flow through the device carried out properly and did not hit the sides or bifurcations of the device. Streamline and contour plots of the flow were analyzed intensively for this optimization. Figure 5a shows a full body contour plot of flow and Figure 5b shows inlet streamline of flow (indicative of proper flow splitting at bifurcation) obtained during multiple trials of simulations.

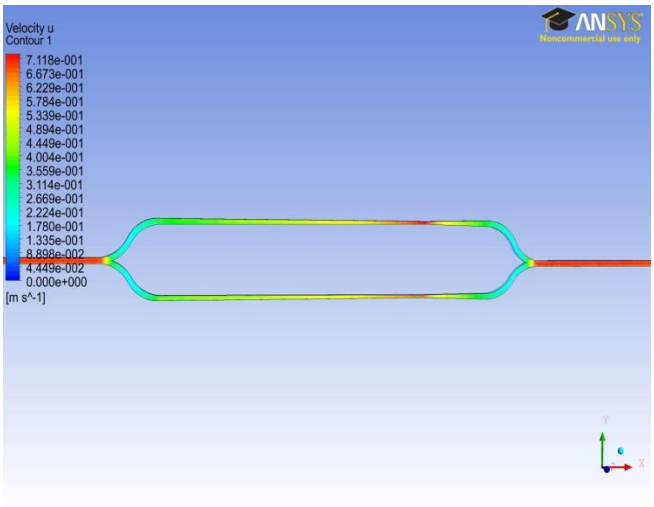


**Figure 5a. Full body Contour Plot of Flow through 2 Ch. Device**

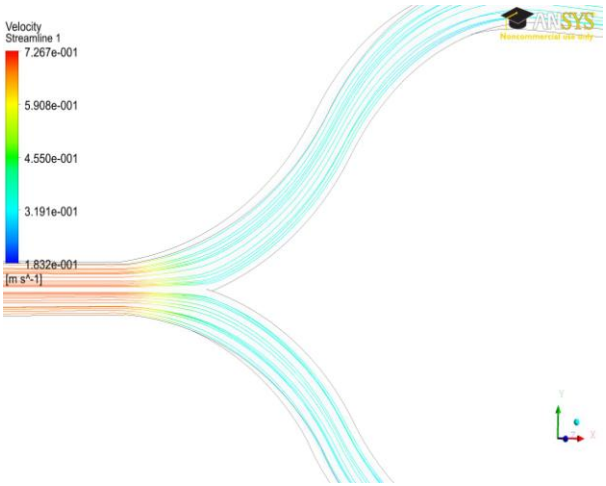


**Figure 5b. Streamline Plot of Flow through inlet bifurcation of 2 Ch. Device**

After a two channel device was successfully simulated and designed, additional simulations were conducted to model a pseudo-fiber trapped in the tapered region of one channel of the device, where the fiber would essentially be analyzed and delivered reagents to. The modeled pseudo-fiber was based upon the average length and diameter of mouse muscle fibers cultured previously in the Arriaga lab. A trapped fiber will reduce fluid flow to that

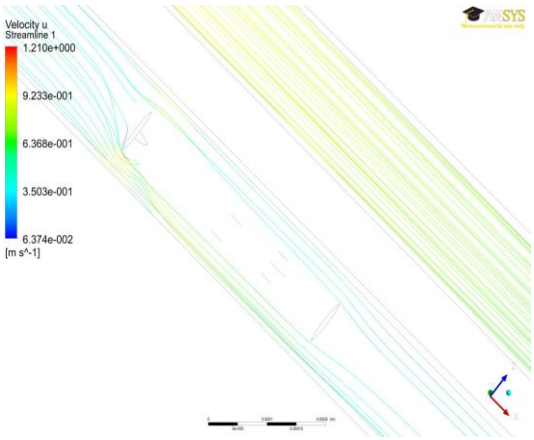


**Figure 6a. Close-up of Trapped Fiber and Flow Around**

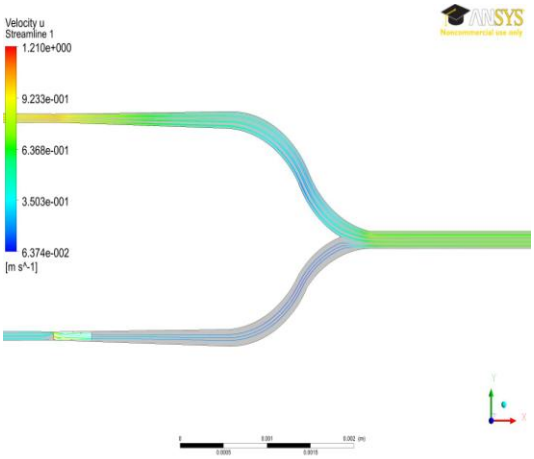


**Figure 6b. Streamline Flow Difference Between Trapped Fiber Channel and Open Channel**

channel and increase flow to the open channel – leaving both flows to converge nicely in the outlet. Figure 6a shows a close-up of the fiber trapped in the tapering region of the device and flow streamlines around the fiber and Figure 6b shows the flow difference obtained when the fiber was trapped in the bottom channel. All in all, the 2-channel device was successfully modeled and simulated. Figure 5a shows that the flow of the devices converges nicely in the outlet region and the tapering region induces max flow. Figure 5b shows the bifurcation of the device was successfully modeled in that the flow does not converge into the middle nor hit the sides of the device. Figures 6a and 6b demonstrate that if a fiber was captured, flow would occur around the fiber and more-so in the open channel. This last summer, work was done to create and simulate extended channel devices that hopefully mimic the same flow data as the 2-Channel devices. Figures 7 and 8 shows streamline flow plots of a 4-channel device and 8-channel device. CAD drawings of 54 different devices (as can be seen in Figure 9) that vary in channel number (2,4, and 8), tapering length, and tapering width were prepared for fabrication of this device for wet-lab testing. Table 1 lists the tapering

lengths and widths in the devices for rat and mouse fiber isolation. Unfortunately, some issues arose with materials and resources required for the fabrication in a timely matter.

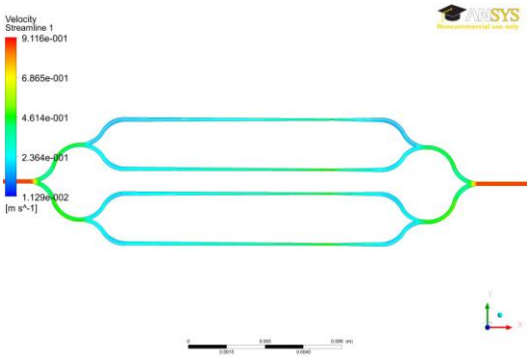


Figure 7 – 4-Channel Streamline Plot of Flow Similar to that of 2-Channel

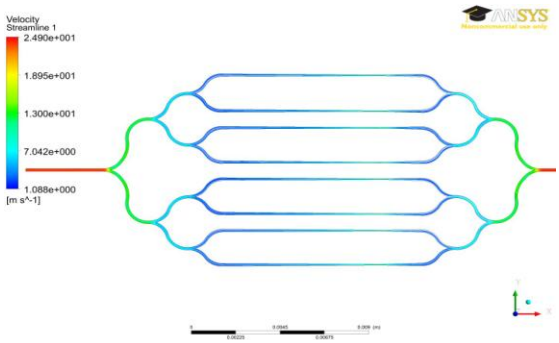


Figure 8– 8-Channel Streamline Plot of Flow Similar to that of 2-Channel

Table 1. Tapering Lengths/Widths of Devices Based Upon Preliminary Fiber Data

| | Mouse Fiber Specific Devices | | | Rat Fiber Specific Devices | | |
|---|---|---|---|---|---|---|
| Taper Width (µm) | 49 | 74 | 99 | 88 | 112 | 136 |
| Taper Length (µm) | 0 | 241 | 430 | 0 | 241 | 430 |

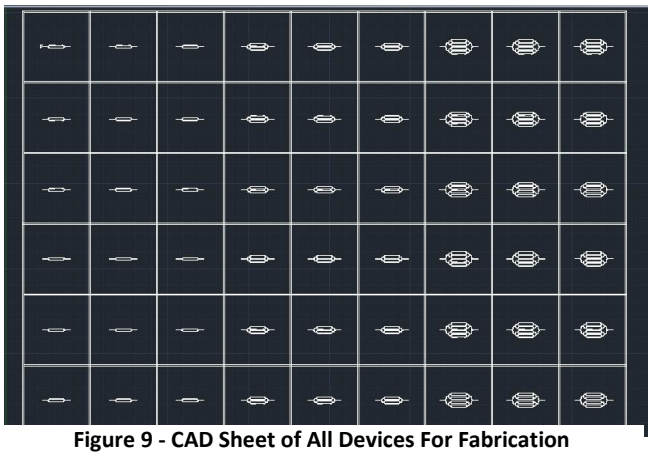

**Figure 9 - CAD Sheet of All Devices For Fabrication**

Prior to trapping single muscle fibers, details of the actual muscle fiber isolation procedure need to be worked out. Since the devices are built around the preliminary fiber data, it is important to be able to replicate the preliminary fiber dimensions. Furthermore, it is important to be able to isolate a large number of correctly sized fibers with minimal tissue debris for loading and testing of the device.

One way to obtain muscle fibers from a muscle tissue sample involves the use of collagenase. Collagen is a fibrous structural protein whose role is to provide support and structure to tissues. In fact, it makes up a large percentage of the extracellular matrix of cells/tissue. In muscle tissue, collagen fibers form fibrous connective tissue around single muscle fibers and help hold them together by providing tensile strength. Collagenase is a type of endopeptidase that attacks the structure of collagen and digests it. Typically for muscle, Type II collagenase is the enzyme used for digestion of connective tissue and isolation of muscle fibers. Due to the complicated enzymatic activity of collagenase, there is no single procedure for isolation of muscle fibers. Factors such as collagenase concentration, incubation time, and trituration method in disruption of fibers vary between experimental methods.

The main objective of this paper is to describe some of the different collagenase treatments I have performed to isolate single skeletal muscle fibers. After optimization of the concentration of collagenase acceptable for treatments, different trituration methods were assessed for tissue disruption. Each trituration method was compared by the number of fibers and average dimensions yielded, with the goal of determining which method can yield consistent fiber data that corresponds to device specifications. After knowing this, more fibers can be isolated to be used for loading into the fabricated devices and running tests.

## Materials and Methods

### Muscle Tissue Isolation

The muscle tissue used in experiments were cut and donated from Dr. LaDora Thompson's lab, in the Program of Physical Therapy at the University of Minnesota. All tissue used came from C57BL/6 strain mice with variable ages. The types of muscle tissue used include

foot adductors, thigh muscle, gracilis/SM, and hamstring – all of various size. After isolation from mice, individual types of muscle were stored on ice in Kreb solution (118 mM NaCl, 4.7 mM KCl, 1.2 mM KH2PO4, 1.2 mM MgSO4, 4.2 mM NaHCO3, 2 mM CaCl2, 10 mM glucose, 200 mM sulphinpyrazone and 10 mM Hepes, pH 7.4). All muscle tissue was used within one hour of isolation. Before collagenase treatment, the tissue was cut so that multiple treatment conditions could be used on a single muscle type. Size of tissue pieces varied, but all were cut in such a way that the incisions were made parallel to the muscle fibers. This was done with use of a scalpel, tweezers, and microscope.

**Preparation of Tissue Isolation Buffer and Collagenase Solutions**

Type II collagenase was prepared in tissue isolation buffer prepared in our lab. The buffer consisted of 185 mM Sucrose, 100 mM KCl, 5 mM $CaCl_2$, 1 mM $KH_2PO_4$, 50 mM tris-HCl, 0.2% BSA (w/v), and 500 mL sterile $H_20$. A large quantity of this buffer was prepared, titrated to a pH of 7.4, purified to 0.2 µm by filtration under a sterile hood, and kept in a 4°C refrigerator. Different concentrations of collagenase solutions were made by dissolving Type II Collagenase (Sigma Aldrich) in the tissue isolation buffer and vortexing at low speed. 0.4% (w/v), 0.6% (w/v), 1.0% (w/v), and 1.4% (w/v) solutions were made and tested.

**Determination of Optimum Collagenase Concentration and First Trituration Method – Normal Pipette**

After preparation of the collagenase solutions, the optimum concentration was evaluated on samples of mouse muscle tissue. First, the cut muscle was placed into microcentrifuge tubes. Approximately 400 µL of the specific concentration of collagenase solution was added to the tube with the cut muscle. Samples were incubated for 5 minutes at

37 °C in a Thermomixer set at a gyration of 300 rpm. After incubation, the collagenase is replaced with an equivalent volume of isolation buffer and trituration, or pulling and pushing of the mixture slowly through a simple 1000 µL pipette tip, is done five times to disrupt the bundle of fibers and release them. The fibers, in suspension of the buffer, are then washed two times in a centrifuge set at 600 g for 30 seconds to remove any residual collagenase. At this point, the suspension of fibers can be transferred to microscope slides for imaging.

**Second Trituration Method – Wide Bore Pipette**

Using the optimum collagenase concentration (0.6% (w/v)), the same treatment as above was done, but a different trituration releasing step of the fibers was induced. Instead of using the normal length of the pipette tip, we made a wider bore pipette tip by cutting it 23.8 mm from the end and creating a larger 3.5 mm diameter opening. After the treatment and washing steps, the fibers in suspension were transferred to slides for image analysis

**Third Trituration Method – Centrifugation**

The third trituration method consisted of the use of centrifugation. Contrary to placing the cut isolated muscle tissue into microcentrifuge tubes to start treatment, the muscle was placed into centrifugal filtration tubes, which contain small lipped insert/membranes stationed in deeper tubes. It was important to make sure that the muscle was gently placed all the way down to the membrane. A 400 µL aliquot of 0.6% (w/v) collagenase solution was added to the tubes of various muscle types. The centrifuge was set to run for 5 minutes at 37°C. Initially deciding on a set speed, however, was difficult. It was decided to start at 500g and work our way down. The proper speed of centrifugation was determined by observing different spinning speeds and seeing which did not let the collagenase solution simply pass all the way through

the membrane to the bottom. Eventually, 300g was the optimum speed chosen, as the solution was trapped above the membrane and engulfed the muscle tissue. Three cycles were done, at different speeds, for a final duration of 15 minutes. After centrifugation with the collagenase, the solution was washed from the muscle by spinning at 600g for 2 minutes. Then, a wash step was done with replacement of the collagenase solution with buffer and spinning down at 300g for 5 minutes. Fiber suspensions were transferred to slides for analysis.

**Differential Centrifugation – Separation of Fibers**

To further test the capabilities of the centrifugation technique, another test was done solely on one muscle type, the SM/gracilis. This test was done to verify if differential centrifugation can be utilized to separate out long muscle fibers from short fiber/debris. This separation can occur by performing the original technique of centrifugation as described above, but after washing, small samples of the fiber suspensions should be transferred with created wide bore pipette tips to new eppendorfs. In our test, a total of 4 trials were done with different speeds to test the differential centrifugation theory. At a spin time set at 5 minutes, 100g, 200g, 300g, and 400g speeds were tested with approximately 100 µL of fiber suspension sample in each tube. Supernatants (top fluid) and pellets fractions from the tubes were analyzed by microscopy after centrifugation. Numbers of large fibers and small fibers were determined in both portions with Image J. The prediction was that the pellet should have a large fiber to small fiber ratio of greater than 50% and that the supernatant should contain virtually no large fibers.

**Imaging and Analysis**

All slides with suspensions of fibers were analyzed with an Olympus IX81 microscope. The camera attachment was a Nikon KX85 and a program named MiroCCd was used to capture images. Analysis of fiber dimensions and quantity was performed by taking the captured images and importing into ImageJ software.

## Results

After compiling all of the captured images taken with the different collagenase treatments, Image J was utilized to adjust image quality/contrast and obtain measurements based off of microscope scale. Table 2 shows the average number of fibers per captured image for each collagenase concentration. This was the initial test run to see what concentration would be used for the rest of the trituration techniques. As can be seen, 0.6% (w/v) yielded the most fibers, and was thus used for the rest of the experiments. Table 3 shows a summary of the results acquired for the normal pipette tip trituration (control), the wide bore pipette tip trituration, and centrifugation. Average fiber width, length, and quantity were recorded for each set of images for different muscle types. The results for length varied quite a bit, but the widths were fairly consistent. Comparison of the images for the shortest and longest fibers is displayed in Figure 10a and 10b. Going a step farther into the centrifugation technique of the collagenase treatment, results from differential centrifugation of long and short fibers/debris can be seen in Table 4. The differential centrifugation can indeed act as one method to clean up the fiber samples. All trials, with the same muscle type, showed that long fibers dominated the bottom separation of the tubes. Figures 11a and 11b show the supernatant and bottom of the 300g trial.

Table 2. Effects of Concentration of Collagenase on Number of Fibers

| Collagenase Concentration | Average Number of Fibers |
|---|---|
| 0.4 % wt | 42 |
| 0.4 % wt | 26 |
| 0.4 % wt | 33 |
| 0.6 % wt | >50 |
| 0.6 % wt | 40 |
| 0.6 % wt | >40 |
| 1.0 % wt | 35 |
| 1.0 % wt | 40 |
| 1.0 % wt | 35 |
| 1.4 % wt | 25 |
| 1.4 % wt | 20 |
| 1.4 % wt | 14 |

Table 3. Effects of Different Triturations on Fiber Length, Width, and Number of Fibers

| Trituration Technique | Muscle Type | Ave Length (mm) | Ave Diameter (mm) | Number of Fibers in Image |
|---|---|---|---|---|
| Full Pipette | Foot | 0.257 | 0.083 | 33 |
| Full Pipette | Foot | 0.183 | 0.0786 | 15 |
| Full Pipette | Foot | 0.158 | 0.0753 | 34 |
| Full Pipette | Foot | 0.251 | 0.063 | 12 |
| Full Pipette | Foot | 0.269 | 0.083 | 13 |
| Full Pipette | Foot | 0.171 | 0.0682 | 30 |
| Full Pipette | Thigh | 0.501 | 0.0816 | 60 |
| Full Pipette | Thigh | 0.319 | 0.0862 | 26 |
| Full Pipette | Thigh | 0.319 | 0.0867 | 21 |
| Wide Bore Pipette | Thigh | 0.315 | 0.0898 | 27 |
| Wide Bore Pipette | Foot | 1.64 | 0.0685 | 6 |
| Wide Bore Pipette | Foot | 0.416 | 0.075 | 9 |
| Wide Bore Pipette | Foot | 1.498 | 0.0657 | 6 |
| Centrifugation | Foot | 0.193 | 0.0684 | 20 |
| Centrifugation | Foot | 0.174 | 0.066 | 22 |
| Centrifugation | Foot | 0.165 | 0.065 | 21 |

| | | | | |
|---|---|---|---|---|
| Centrifugation | SM/Gracilis | 0.306 | 0.0618 | 40 |
| Centrifugation | SM/Gracilis | 0.241 | 0.0683 | 47 |
| Centrifugation | SM/Gracilis | 0.289 | 0.0688 | 39 |
| Centrifugation | Thigh | 0.233 | 0.0752 | 35 |
| Centrifugation | Thigh | 0.244 | 0.0691 | 29 |
| Centrifugation | Thigh | 0.186 | 0.074 | 32 |

Table 4. Differential Centrifugation and Ratio of Long Fibers to Short Fibers in Bottom Separation

| | All SM/Gracilis | Speed in Centrifuge | | | |
|---|---|---|---|---|---|
| Location in Tube | Measurement | 100g | 200g | 300g | 400g |
| Top | # of small fibers | 0 | 0 | 0 | 0 |
| | # of large fibers | 0 | 0 | 0 | 0 |
| Bottom | Ratio of Large Fibers to Small Fibers | 0.571 | 0.538 | 0.612 | 0.512 |

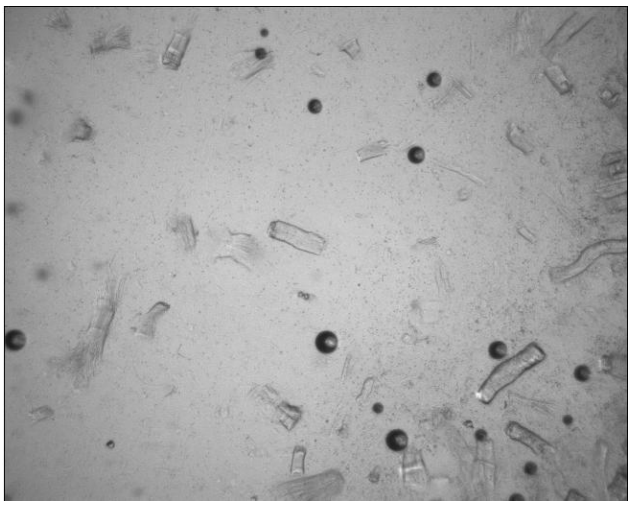

**Figure 10a - Shortest Fibers Obtained with Full Pipette Technique of Foot Muscle**

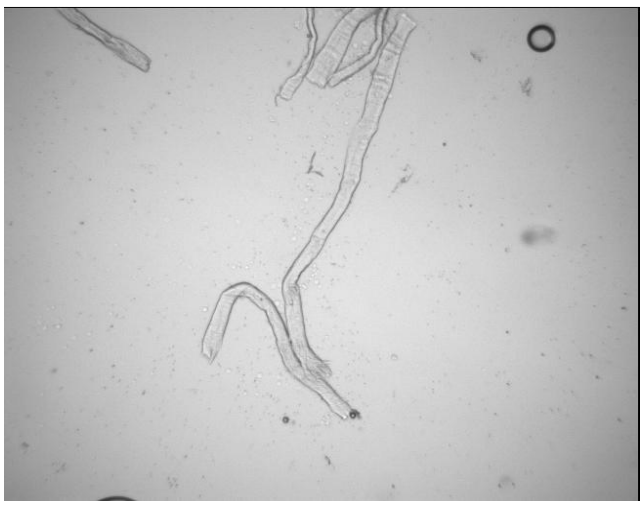

**Figure 10b - Longest Fibers Obtained with Wide Bore Pipette Technique of Foot Muscle**

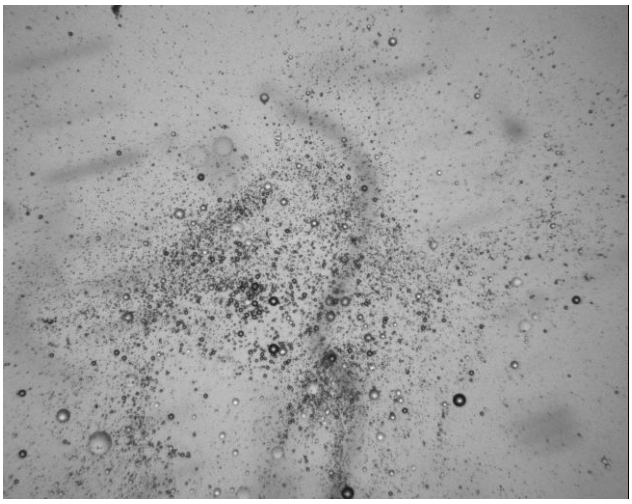

**Figure 11a – Top (Supernatant) of Centrifugation of Fibers at 300g**

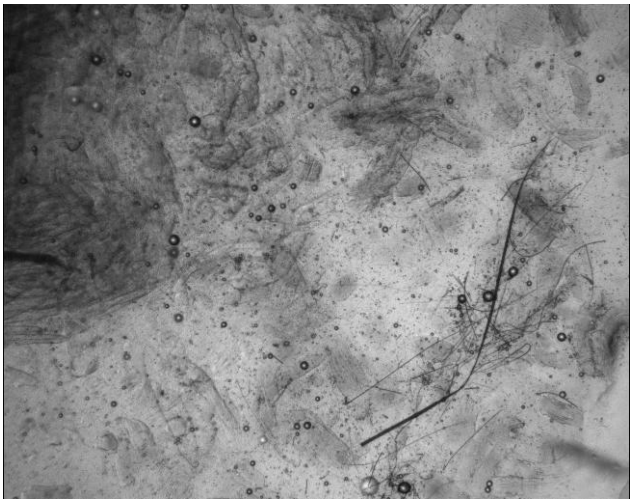

**Figure 11b – Bottom of Centrifugation of Fibers at 300g**

## Discussion

Analysis of the results of the different collagenase treatments yields some valuable information about muscle fiber isolation techniques. For the muscle tissue used, 0.6% (w/v) concentration of collagenase yielded the greatest number of fibers in treatment, but that does not necessarily mean that this is the ideal concentration for future isolations. There are a lot of factors to consider when determining fiber quantity and dimensions, including muscle tissue type, age, gender, and initial dimensions. Thus, in future experiments, a collagenase concentration assay, such as the one done in our experiment with same procedures/trituration technique, must be done in order to determine optimum concentration for each treatment and muscle tissue used.

When comparing the results of Table 3, it seems that almost all the trituration techniques meet the criteria for the tapering lengths/widths of the devices. The width is consistent across all trituration trials, but length varies quite a bit. The wide bore pipette tip

technique produced much larger fibers than the centrifugation and normal pipette trituration. In theory, the longer the fibers, the better, as we want to keep the fiber physiologically sound and preserve them to their original state within the muscle tissue. The lengths within trituration trials still vary too much to determine average lengths with confidence and really decide on what technique worked best. The full pipette and centrifugation techniques of trituration may not be getting long fibers due to the large amounts of shear force that are breaking the fibers apart. When trying to reduce the force by testing out a wide bore pipette tip, the fibers were indeed longer, but the quantity was low.

Further experimentation with differential centrifugation provided results as hoped. Centrifugation aided in the separation of long and short fibers, with over 50% of the bottom consisting of long fibers. In fact, the supernatant of all the speeds tested showed very small amounts of fibers and debris. Again, the sample size of muscle tissue quantity/type does not aid in statistical significance, but it can help in the future with isolation. After treatment with whichever trituration technique/collagenase concentration chosen, differential centrifugation can be performed to gather only the long fibers for loading into the devices. 300g seemed to work the best in our case for the centrifugation speed, but again like the collagenase concentration, this value would need to be optimized during treatments.

As mentioned previously, variation is indeed present within the length/quantity of fibers across treatments and can be solved by testing more muscle tissue/types. However, the statistical variation/insignificance can also be due to methodological errors. One of these errors lies within the muscle tissue preparation and cutting. Muscle tissue type and physiology do play roles in fiber isolation, but so does tissue length and structure. When cutting the tissue,

sometimes it was hard to make sure the cut was parallel to the muscle fibers according to microscopy. Furthermore, it was undecided on what was the proper length/width of tissues samples to be put into the tubes for treatment. Most studies that use fiber isolation state that because of the variability of muscle, fiber length should not be a parameter to analyze alone. Instead, fiber-length-to-muscle-length ratios are often compared to known literature values.[7] This means that before treatments, we should have measured the length of the muscle tissue cut and then determined these ratios for comparison. Another means of improvement for methods also includes using fresh preparations of collagenase solutions each experiment. To save time and material, large batches of both were made and preserved in fridge/freezer units. Loss of collagenase activity could occur and cause treatment to deviate from normal results.

## Future

Along with improvement of statistical and methodological errors, improvement can be made by introducing new possible collagenase treatments. One treatment that was attempted in this study, but yielded poor results, was a treatment involving magnetic fixation. After muscle tissue preparation, the tissue was attached to a small dissection pin and stabilized. The muscle on the pin was then placed into a small piece of tubing and held in place with a strong magnet. The goal was deliver the collagenase solution directly to the stationary muscle so that the muscle fibers were not gently peeled away with the delivery of the wash buffer. Issues did arise from the muscle being so degraded after treatments that it easily slipped from the pin. If we could figure out a better way to keep the muscle stationary, this treatment could relay valuable results. Another possibility in changing up the treatment involves different preparation and use of collagenase solution. Some research and studies use different dissolving buffers for the

collagenase and different incubation conditions for degradation of the muscle tissue. For example, Dr. Starkey, of the University of Connecticut, prepared muscle fibers in a study on satellite cells using DMEM as the dissolving buffer, performing a 90 minute incubation period with the muscle/collagnease solution mixture, and triturating with fire-polished Pasteur pipettes. Perhaps the change in the buffer/incubation alters the contraction of the fibers.[8]

Since the trituration of the fibers during the collagenase treatment induces fiber fragments and damage, it is also important to make sure that the fibers are still functional after treatment. The easiest way to do this would be to check membrane sealing. Like most cells, when the membrane is disrupted or damaged, the cell acts quickly in repair and reformation. Membrane sealing of the disrupted fibers can be tracked after trituration by using propidium iodide as a dye within the cells and using fluorescent microscopy to track changes in membrane formation.

Once the devices are fabricated, the next step in measuring how well fibers are isolated would be to try to trap them within devices. Methods on how to load the fibers would need to be experimented with, but after loading, the pressure gradient can be monitored/optimized to ensure proper flow of the fibers within devices. The flow and capture of the fibers will be analyzed through the use of bright field and fluorescent microscopy. After fibers are trapped, physiological properties of the fibers, such as ROS and mitochondrial mass, can be evaluated with florescent microscopy.

## Conclusion

Multiple collagenase treatments were performed to yield fibers that fit the criteria of the tapered regions of the microfluidic devices. The 0.6% (w/v) collagenase concentration

yielded the largest number of fibers, the wide bore pipette tip method of trituration had the longest fibers in length, and centrifugation can be used to separate lengths of strands. In all tests, however, results vary and statistical significance is lacking. Use of a larger sample size of muscle tissue quantity/type and adjustments to methodology ae required. Future work includes preparation of a new approach to applying collagenase more directly and not disrupting fibers, changing preparation/incubation of collagenase solution, researching more about intact satellite cells on isolated fibers, checking fiber functionality with membrane sealing, and eventually testing out isolated fiber response in the future fabricated devices.

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