Custom Fabricated Devices To Assess Rodent Muscle Health

Engineering Honors Program - Capstone Final Report

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Abstract
The Comparative Orthopaedic Rehabilitation Laboratory (CORL) is headed by Dr. Lindsey Lepley in the School of Kinesiology. The laboratory focuses on the discovery and development of rehabilitation techniques that prevent the onset of long-term sequelae that occur after anterior cruciate ligament (ACL) injury. The lab planned to implement electromyography (EMG) and mechanical-based performance assessments in a rodent model in order to characterize which treatment methods would optimally improve musculoskeletal health after ACL injury. Custom devices were needed to facilitate data collection from these assessments. Rodent EMG electrodes and a dynamometer knee orthosis were fabricated to address the CORL’s need. Both devices provided reliable data and allow the CORL to collect high-quality data for future experimentation.

Introduction
Anterior cruciate ligament (ACL) injury is a devastating injury that often results in protracted muscle weakness, reduced long-life physical activity levels, and an increased risk of early joint degeneration. These outcomes are especially devastating for young and active individuals like athletes who compete at high levels.

The Comparative Orthopaedic Laboratory (CORL), headed by Dr. Lindsey Lepley, PhD, ATC, conducts research within the School of Kinesiology at the University of Michigan. The CORL seeks to improve the recovery trajectory by uncovering the mechanisms of disease progression and identifying more effective treatment strategies. When more effective treatment strategies are identified, clinical outcomes can be improved, leading to an increased quality of life for ACL injury patients.

The lab uses rodent models to incur injury, quantify musculoskeletal health, and test different recovery strategies to better understand the consequences and treatment of common musculoskeletal injuries like ACL injury. To quantify musculoskeletal health post-injury, both electromyography (EMG) and machine-based performance tests are used.

EMG is used extensively to characterize muscle activation by measuring the electrical activity of motor units and their muscle fibers. In the case of ACL injury, the lab uses EMG to longitudinally quantify the time and progression of neuromuscular abnormalities and the responsiveness of the system to different rehabilitative regimes. The CORL plans to measure muscle activation via raw EMG data that is collected from the electrodes and use EMG and motion capture systems to analyze the data.

The CORL also uses mechanical tests to quantify musculoskeletal health after ACL injury, namely dynamometer testing. A dynamometer is a machine that is commonly used to measure...
muscle strength and health. These devices measure the force an individual’s muscle produces, send the reading to a force transducer, translate the force reading to a quantifiable metric, and then output the final result. For example, a clinical dynamometer used to measure and quantify human quadriceps strength is shown in Fig. 1 below.

![Clinical dynamometer](image)

**Figure 1.** Clinical dynamometer [1]

**Problems Addressed**
The CORL planned to use the above technologies to collect data and longitudinally quantify rodent musculoskeletal health to compare different treatment methods. However, certain needs had to be met before the relevant experiments could be completed and before this data could be collected.

**EMG Electrodes**
EMG electrodes designed to measure and collect data from rodents currently exist but are inaccessible for use in widespread research. Options are in the early stages of development, are difficult to implement, or are quite expensive, costing $305 per electrode [2]. Thus, the CORL demonstrated a need for inexpensive and biocompatible rodent electrodes that could be used to measure EMG signals during various recovery therapies. Electrodes produced in-house would decrease costs to the lab, minimize lead times between experiments, and better fit the lab’s experimental plans.

**Rodent Dynamometer**
The CORL currently has a dynamometer that is applicable to rodent experimentation. This dynamometer has been used to acquire data about lower limb strength and muscle health, specifically the quadriceps, after ACL injury. **Fig. 2** below shows the existing rodent dynamometer with a rodent being instrumented on the equipment.
The quadriceps muscle was chosen by the lab to be the focus of its experimentation because of its role in lower limb strength and function, importance to ACL and knee stability, and translatability to the human quadriceps. By collecting data about this muscle, the CORL can better identify which treatments are most optimal for recovery. The design of this dynamometer was to induce a contraction, through neuromuscular electrical stimulation (NMES), in the quadriceps muscle only and measure the force and torque produced about the knee joint. To collect this data, the rodent’s limb was positioned using the two small positioning rods extending from the elliptical leg restraining piece’s surface and strapping the leg to the elliptical piece by wrapping the leg and the elliptical piece together with velcro.

The existing dynamometer could collect and record data, but multiple issues were identified during its use. Most notably, the rodent’s leg was not completely restricted in its rotation; The only pieces holding the rodent’s leg in place were the two small positioning rods and the velcro so, as it would be later identified, additional muscles other than the quadriceps would contract, inducing non-ideal rotation and decreasing potential data quality.

To extend current the lab’s insight into ACL injury mechanics and help the lab prepare for future data collection, two custom devices were fabricated.

1) Rodent electrodes for the purpose of monitoring muscle activity via electromyography.
2) Rotation restriction device for the purpose of isolating muscle contraction to be only the quadriceps and rotation to be only about the rodent’s knee joint.
Methods

EMG Electrodes

Fabrication Procedure and Process To offset the cost of commercial implantable electrodes, low-cost biocompatible EMG electrodes that matches the rigor of the current industry standard were desired. All materials that composed the electrode needed to be biocompatible, as the electrodes would be surgically implanted into the rodent’s leg to measure muscle activity longitudinally. An image of the materials necessary to fabricate the electrodes is provided below in Fig. 3.

![Materials for electrode fabrication](image)

**Figure 3.** Materials for electrode fabrication [3].

The Implantech silicone mesh, A-M wire, foil, toluene, and Nusil-med silicone make up the actual biocompatible electrode. The cutting jig, tape, Xacto knife, and folding jig are materials used to set up and execute the electrode fabrication process.

After being taught the fabrication process by senior lab members, a small team began the work of creating the standard operating procedure for electrode fabrication. The step-by-step process for electrode fabrication is detailed below.

1) Place 0.007mm Implantech silicone mesh on cutting jig and tape down.
2) Perforate silicone mesh with X-acto knife using the cutting jig guides
3) Assemble folding jig
4) Depress folding jig to form U-shaped foil
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5) Insert U-shaped foil into perforations in silicon mesh
6) Insert second U-shaped foil to form bipolar electrode
7) Tape down base of the foils and remove silicone mesh from cutting jig
8) Fold down one upright arm of U-shaped foil
9) Denude wire and weld to the remaining upright arm of foil
10) Fold down the welded arm of foil
11) Weigh 1 gram of Nusil-Med liquid silicone
12) Draw up 75 CC’s of toluene in a syringe and mix with liquid silicone
13) Allign wires and apply silicone-toluene mixture to seal electrodes
14) Let sealant dry overnight before cutting mesh to fit and sanitizing in ultrasonic bath
15) Store bipolar electrodes as seen fit

**Electrode Quality Assurance** To ensure the custom electrodes could perform and produce high-quality results relative to the industry standard, they were tested by a fellow lab member using the ex-vivo experimental setup in **Fig. 4** below.

![Figure 4. Ex vivo Test Bed to compare custom electrodes to industry standard [2].](image)

Various signal types (saw, sine, and triangle) were generated by the sign and function generator and sent to the saline bath. The saline bath mimicked the in vivo conditions that the electrodes would experience when implanted into the rodent and conducted the signal so that it could be measured by both the custom and industry electrodes.

The signals read by the electrodes were sent to an electrode interface board, which essentially collected the electrode reading and transported it to a Neualynx data acquisition platform (DAQ). This DAQ recorded and provided the data that was used to compare the two electrode options. The results of this testing are discussed in the **Results** section of this report.
Dynamometer Knee Orthosis

Root Cause and Problem Analysis As mentioned earlier in this report, the CORL noticed that during stimulation, the rodent’s leg was not fully constrained and muscles other than the quadriceps were contracting. This led to the rotation of the hip joint and ankle, leading to non-ideal data and results. A method to constrain any accessory motion was needed.

As part of creating a solution to address the identified need, a root cause analysis was performed. The following steps were taken to best address the issue and are discussed in more detail in the ensuing report section.

1) Background Research
2) Speaking with Stakeholder
3) Visiting Active Set Up and Taking Measurements of the System
4) Making Design Recommendations
5) Design Implementation
6) User Feedback

Background Research To better understand the issue of non-ideal rotation that the lab was seeing, background research was performed. It was very important to understand the anatomy of the rodent, how the leg actually works, and how components move and rotate relative to each other. More specifically to the CORL’s dynamometer system, this stage helped inform why electrical stimulation of the quadriceps may lead to both leg extension and hip flexion. Relevant research articles and anatomy figures were used to better understand the background of the situation.

Speaking with Stakeholders Dr. Lepley, who first identified the non-desirable rotation, shared her understanding of the problem: the leg is not constrained enough to induce rotation solely about the knee joint. This knowledge was incredibly insightful, as human dynamometers, like the one shown in Fig. 1 above, fully latch or strap the human leg to the machine framework to limit any extraneous rotation. The current setup was not very stable and allowed for much more movement of the leg. After this stage, the problem was fully identified and a solution could confidently be designed.

Visiting Active Set Up and Taking Measurements of the System Before any design work was completed, the existing system was investigated. A live rodent was instrumented into the dynamometer and a contraction was induced. The step-by-step process was observed and recorded. Similar to verbal descriptions, the rotation was not solely about the knee joint, confirming the identified problem. Next, measurements of the existing system needed to be taken so dimensions of a potential solution could be determined. The existing dynamometer, initial planning, and a rodent’s anatomical dimensions were measured and are shown below in Fig. 5.
Making Design Recommendations With initial dimensioning complete, designing a solution could begin. Dr. Lepley expressed interest in a solution that could easily hold the rodent’s limb in place, easily attach to the existing setup, and not cause any harm to the rodent. These user inputs were used to guide the ideation and design processes. It was determined that a simply produced, easily removable, and uncomplicated framework that allowed access to the quadriceps muscle and restrained the rodent’s leg would be ideal. An idea to accomplish these goals provided by Dr. Lepley was designing a device similar to a rigid ankle-foot orthosis (AFO), which is shown in Fig. 6. below.

![Figure 5. Dimensioning of leg restraining piece, initial planning, and rodent anatomy data](image)

**Figure 5.** Dimensioning of leg restraining piece, initial planning, and rodent anatomy data

![Figure 6. Ankle-foot orthoses used as design inspiration [4,5.](image)

**Figure 6.** Ankle-foot orthoses used as design inspiration [4,5.]
The actual design, its implementation, and user feedback – described as the stages of Design Implementation and User Feedback in the list above – are discussed in the Results section of this report.

**Results**

**EMG Electrodes**

_Fabrication Procedure_ The steps detailed above were summarized and formatted into the detailed workflow shown below in **Fig. 7**.

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.</td>
<td>Place silicone mesh on cutting jig and perforate with X-acto knife.</td>
</tr>
<tr>
<td>B.</td>
<td>Place foil into cutting jig and use the cutting jig to fold foil into U-shape.</td>
</tr>
<tr>
<td>C.</td>
<td>Insert U-shaped foil into the perforated silicone mesh. The mesh should contain 12 foils for 6 bipolar electrodes.</td>
</tr>
<tr>
<td>D.</td>
<td>Remove the mesh from cutting jig and fold one arm of the U-shaped foil flush with the mesh.</td>
</tr>
<tr>
<td>E.</td>
<td>Denude wire and weld to the remaining upright foil arm and fold foil arm flush with the mesh.</td>
</tr>
<tr>
<td>F.</td>
<td>Align denuded wires and apply silicone (1g) - toluene (75 g) mixture to seal electrode and set for 72 hours.</td>
</tr>
<tr>
<td>G.</td>
<td>Denude wire and weld to the remaining upright foil arm and fold foil arm flush with the mesh.</td>
</tr>
<tr>
<td>H.</td>
<td>Align denuded wires and apply silicone (1g) - toluene (75 g) mixture to seal electrode and set for 72 hours.</td>
</tr>
<tr>
<td>I.</td>
<td>Divide silicone mesh sheet into 6 individual electrodes measuring “10 x 5” mm and clean in ultrasonic bath</td>
</tr>
</tbody>
</table>

**Figure 7.** Flow chart of steps for electrode fabrication [2].

Another purpose of developing this standard operating procedure was to create a knowledge base for and train future lab members and other members of the scientific community. This knowledge and fabrication process could benefit hundreds of other people and labs across the country and world if published and shared, which is an active area of work for the CORL.

**Produced Electrodes** Using this proprietary fabrication process, the team produced electrodes for the lab according to the demonstrated need. Nearly 100 electrodes were produced over the course of three weeks, as shown in **Fig. 8** below.
Figure 8. Two of the final batches of electrodes.

This portion Capstone project required great coordination, project management skills, and many hours performing hands-on fabrication work to meet the deadlines set by and needs of the CORL.

Testing Electrode Performance The performance of the custom electrodes was compared to an on-the-market standard using various waveforms. Fig. 9 shows this below.

Figure 9. Subplots A, D, and G compare the signals read by the industry standard and the custom electrodes. Subplots B, E, and H show the correlation between the two electrode options. Subplots C, F, and I display the percent difference between the custom and industry electrodes across different average voltages of the signals [2].
Visible in subplots, A, D, and G are the sensed signals from both electrodes. Because both signals are so similar, it is difficult to distinguish between custom and industry except in a few rare circumstances. The correlation between the custom electrodes and the industry electrodes is shown in subplots B, E, and H; With an R² of between 0.9317-0.9901, the CORL can confidently say that the electrodes perform similarly. This result is confirmed by subplots C, F, and I, as the percent difference between the custom and industry electrodes is minimal at physiologically relevant voltage values [2].

**Dynamometer Knee Orthosis**

*Design Implementation* After completing the dimensioning, ideation, and design work using SolidWorks, the design inputs detailed earlier were used to create the knee orthosis to meet the lab’s needs. Multiple views of the model in SolidWorks are shown in **Fig. 10.** below.

![Press Fit Clip For Attachment To Dynamometer](image1.png)  
![Knee Orthosis Body To Hold Rodent Leg](image2.png)  
![Slots For Velcro Straps](image3.png)

**Figure 10.** Labeled images of the final design of the knee orthosis.

The press fit clip allowed for simple attachment and detachment of the orthosis to the existing dynamometer device and framework. The knee orthosis body fit the unique geometry of the rodent’s leg and slots were designed to position and hold the velcro straps that would keep the rodent’s leg in place while a contraction was induced. This device was 3D printed and implemented into the existing rodent dynamometer, as shown in **Fig. 11** below.
Figure 11. Initial implementation of rodent knee orthosis.

The press-fit clip design and slots for velcro straps allowed for simple positioning and repositioning of the knee orthosis and the rodent’s limb. Once it was determined that the newly printed orthosis could be used, a rodent was instrumented and the orthosis was used to constrain the motion of its limb during stimulation, as shown in Fig. 12 below.

Figure 12. Knee orthosis in use during experimentation.

User Feedback Although qualitative in nature, the knee orthosis was a success. When the rodent’s quadriceps muscle was stimulated, only the quadriceps contracted and the leg rotated solely about the knee joint. The knee orthosis held the rodent’s leg in place relative to the elliptical support piece and allowed for the only rotation to be caused by the rodent’s quadriceps. Dr. Lepley was pleased with the orthosis and it is believed this device can be used in the future to stabilize the rodent’s leg and lead to improved data quality.


Discussion and Conclusions

EMG Electrodes
For future data collections involving EMG signaling, biocompatible electrodes were needed. Instead of purchasing industry standards for over $300 per electrode [2], the CORL determined it was possible to fabricate these electrodes in-house. After compiling the necessary resources, a standard operating procedure was developed to provide instructions on how to fabricate these electrodes.

Using these instructions, nearly 100 electrodes were produced successfully and were shown to function similarly to industry standards while costing much less, at only about $32.70/electrode. The custom electrodes had a nearly 90% cost reduction for nearly identical performance. The electrodes also could be produced much more rapidly than the commercial option, as approximately 300 electrodes could be produced in the time it would take for the commercial electrode to be delivered (6 weeks). The monetary and time savings the electrodes will return over time prove their value. The CORL is currently working to publish and spread this knowledge with other researchers to increase accessibility to the tools needed to perform groundbreaking research.

Dynamometer Knee Orthosis
After observing non-ideal muscle contraction and limb rotation during instrumentation and dynamometer use, a need for a way to stabilize the rodent’s leg was identified. After the root cause was identified, a design was created and implemented. The new knee orthosis met the design inputs and user needs defined by Dr. Lepley and was successful in constraining the motion of the leg to be the desired leg extension movement. Because of its simple design, implementation, and accessibility through CAD and 3D printing, this device can easily be used by the CORL, its future members, and other researchers in the scientific community.

The simple fabrication, repeatable use, and reliability of these devices have increased accessibility for not only the CORL but other labs as well. The creation of the EMG electrodes and the Knee Orthosis devices has laid the groundwork for the CORL to continue its impactful research on ACL injury while progressing toward the ultimate goal of better understanding ACL injury mechanisms and identifying optimal treatment therapies for patients.
Acknowledgments
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References