Developing a Non-Invasive Compressive Load Osteoarthritis Model

By

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A THESIS

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Osteoarthritis is a disease that impacts millions of people's lives worldwide. A significant amount of research has been directed toward studying osteoarthritis, specifically post-traumatic osteoarthritis. The research model that is most common to study post-traumatic osteoarthritis is by performing a medial meniscus transection (MMT) on a rat. This research looks at a less common, meaningful way of examining post-traumatic osteoarthritis in rats through a non-invasive compressive load knee model (NIKI). Over two years, a non-invasive compressive load model for rats has been built at the University of Oregon in the Guldberg lab. The concept of how the device would look was formed with input from the Sharma lab, and the device was built. Following the creation of the device, the circuitry was figured out with assistance from the Ong lab. Once the machine was running, spring testing calibration/validation was done. The device was calibrated and validated to ensure that voltage inputs would output the correct loads imparted to the knee joint. Once the device was calibrated, rat cadaver testing was done until ACL rupture was confirmed on a cadaver. The implications of the machine working are that the ACL rupture model may provide more relevance to actual knee injuries compared to surgically inducing a mechanical instability. The NIKI device could produce different structural and biochemical responses compared to surgical models.

Acknowledgments

I would like to thank my primary thesis advisors, Angela Lin and Dr. Bob Guldberg for introducing me to research during my freshman year and providing me with numerous ways to be involved in their lab. They have provided me mentorship and guidance through the world of research over the last three years. I would also like to thank the other wonderful members of my thesis committee. I want to thank Dr. Jon Runyeon, who is my second reader for the thesis. His support and input on my thesis throughout the past year, has proved very valuable. I would also like to thank my third reader Dr. Nathan Tublitz whom I am very fortunate to have received input from for my thesis. I would also like to thank my CHC representative Dr. Corinne Bayerl. I am thankful for the help in the essential part of my thesis where you helped me get the writing process started by teaching my thesis prospectus class. I also owe a thank you to Dr. Salili Karipott and Dr. Keat Ong, who helped me with all of the circuitry aspects of the NIKI device. I also would like to thank Julian McAdams, who helped in the machining process of the NIKI device, as his input was invaluable in the construction of the device. I also owe a thank you to members of the Sharma lab who helped in the initial design work of the NIKI device and whose research with their own NIKI device helped immensely in creating the NIKI device at the University of Oregon. Lastly, I would like to thank any member of the Guldberg lab who helped out, specifically Sruthi Ranganathan and Nick Pancheri, who have been essential in the creation and ongoing research of the NIKI device.

Table of Contents

Introduction

Osteoarthritis is a condition where one of the major debilitating effects is that the cartilage on the ends of bones starts to wear down throughout an individual's life. The cartilage acts as a cushion between bones, and as it degrades, it causes individuals increased discomfort as there is less barrier between bone-on-bone contact. Osteoarthritis is the most common form of arthritis and impacts millions of people each year. The prevalence of osteoarthritis worldwide after the age of 65 is around 70 percent in women and 60 percent in men (Puttini, et al., 2005). Another example of its prevalence is in the United States, where 10% of men and 13% of women 60 years or older have symptomatic knee osteoarthritis (Zhang, 2010). Osteoarthritis is going to continue and even increase in prevalence as the population continues to age and obesity becomes more common worldwide than in the past. Obesity is a factor that increases the prevalence of osteoarthritis, but some other factors that increase the risks of developing it include repetitive use of joints, bone density of the individual, muscle weakness, and prior injuries (Zhang, 2010).

Despite the large prevalence of osteoarthritis and the importance of doing research in this field, the pathophysiology has been understudied, and there are no treatments available that can provide a solution (Sacitharan, 2019). As of now, the best treatment option when all other therapies for pain management have been exhausted is joint replacement surgery (Sacitharan, 2019). Other treatment options are induced as a way to manage symptoms and provide some relief to individuals but do not address the underlying issue of cartilage degeneration. Other treatment options include medications, exercise, braces, physical therapy, and losing weight if overweight. Some more innovative treatments have been published, such as a treatment that has been trying to use the gene SIRT 1, after being activated by resveratrol, to inhibit osteoarthritis progression (Deng, et al., 2019).

It is evident that for the large impact osteoarthritis is having on the general population, there has not been enough research conducted in the field, nor any effective treatments found. The University of Oregon's Guldberg Musculoskeletal Research lab has conducted a lot of osteoarthritis research. The current method for studying osteoarthritis at the University of Oregon is by performing MMT (medial meniscal tear) surgeries on rats. This method of studying osteoarthritis in rats is not only the chief method of study at the University of Oregon but throughout the country. Although this preclinical model and other similar surgical models have been used extensively in the field of OA research, it should be pointed out that the way in which osteoarthritis was being induced in rats was not how most cases of osteoarthritis occurred naturally for humans. The vast majority of osteoarthritis cases are not something that humans develop acutely from an injury, but rather it is a progressive disease that comes from repeated compression throughout one's life from activities such as walking or running.

My thesis hinges on the paper published by Brown et al.'s 2019, "Characterization of Post-Traumatic Osteoarthritis in Rats Following Anterior Cruciate Ligament Rupture by Non-Invasive Knee Injury (NIKI)," from which we heavily based our NIKI device. This study had similar goals to my thesis, which was to characterize this new model and validate it. In their study, the Brown paper added a unique feature of characterization that had not been studied using this model before, synovial fluid biomarkers (Brown et al., 2019). The paper also discusses how despite there being multiple NIKI models developed throughout the last ten years, very few involved rats, whereas most studies involved mice and rabbits (Brown et al., 2019). The lack of studies using rats with this model, as well as the overall lack of studies in the area of non-invasive ACL rupture models, turned this into an important area of research to be developed. Another benefit of performing this study in rats, rather than the typical animal model of mice, is

that rats have great amenability to behavioral tests and are larger than mice which is preferable for certain therapeutics and imaging such as MRI (Brown et al., 2019). The Brown study, in conclusion, found that their NIKI device was consistently able to induce a femoral avulsion of the ACL. The avulsion of the ACL was induced via axial compression of the tibia (Brown et al., 2019). Another conclusion the study was able to draw was to show a change in the characterization features measured in the study, such as synovium, cartilage, synovial fluid biomarkers, and joint swelling.

A large body of pre-existing literature that helped inspire the Brown paper was non-invasive compressive load models on mice. One such paper that inspired much of the compressive load model of OA research we see today was done by Christiansen et al.'s 2012 "Musculoskeletal changes following non-invasive knee injury using a novel mouse model of post-traumatic osteoarthritis." In this paper, a compressive load model was used with a single compression on the mouse's tibia. The main data point taken in the study was micro-CT scans being performed to quantify bone changes. The study found that following the compressive load injury, there was a rapid loss of trabecular bone in injured knees compared to uninjured knees, and there was partial recovery of the bone to a new steady formation 28 days after injury (Christiansen et al., 2012). This study overall demonstrated to the scientific community that this new model of inducing osteoarthritis worked and set up a time reference to demonstrate how the bones heal after a compressive load ACL rupture.

Another preclinical animal model on which compressive load ACL rupture testing has been performed is in rabbits. One such study was published by Killian et al's, 2010 "Traumatic anterior cruciate ligament tear and its implications on meniscal degradation: a preliminary novel lapine osteoarthritis model." This paper paralleled the longer-term goal of my research

ambitions, which is comparing a surgical model and a non-surgical compression model of ACL rupture. This was a 12-week study, and the data was analyzed using histological assessment (Killian et al., 2010). The conclusion of the study was that both surgical and compressive load methods of injury both lead to meniscal damage but that the compressive load led to an advanced level of degradation of the meniscus.

My thesis research has led to the creation of the first non-invasive osteoarthritis device at the University of Oregon. The NIKI device will allow me, and others from the lab to study osteoarthritis in ways we have never been able to before. The reasons behind building the NIKI device is that it is hypothesized that there will be functional, structural, and cell biomarker differences noted between the NIKI and surgical model. The NIKI model also may provide more relevance to actual knee injuries incurred during sports activities compared to the surgical model. This research will lay the groundwork for further research studying the compressive load model.

Given the above studies showing the promising future of non-invasive ACL rupture models, my thesis proposed to answer three questions:

(1) Could we at the University of Oregon build a device as similar as possible to that of the Sharma lab?

(2) Would we be able to calibrate/validate the device using spring constants to ensure that voltage inputs would output the correct loads imparted to the knee joint?

(3) Would the device that was built through compressive force cause an ACL rupture in the rats that we could verify?

All three of these questions were of equal importance in my thesis process of building the NIKI device here at the University of Oregon. I needed the device to be similar to the Sharma lab's NIKI device. I needed the device to be calibrated to ensure we accurately applied expected loads, and I needed to demonstrate that we could use the device to consistently rupture the ACLs of the rats and that we could verify it by dissection. Once all of these questions were answered properly, this could lead to the lab using the device to launch numerous studies and help progress the field of osteoarthritis research.

Materials and Methods

Construction of the NIKI device

The most time-intensive part of my thesis was the construction of the body of the NIKI device. The process began with a Zoom call to members of the Sharma lab to learn more about the NIKI device they had built for their studies on non-invasive osteoarthritis. Through multiple Zoom calls with members of their lab, I was able to order all of the initial parts we would need for the device.

Pneumatics:

- 1 Gallon, 135 PSI Max, Trim Compressor, D55140, DeWALT
- Filter Regulator P31P Electronic Proportional, P31PA92AD2VD1A
- Tubing PVC
- Central M12 connector 4 pole
- Central Pneumatic connectors
- Industrial Quick-Disconnect Hose Coupling for Air
- Push-to-connect tube fitting for air, straight adapter, for ¼ inch tube OD x ¼ NPT male

Electronics and controls:

- NI USB-6009 14-Bit multifunction DAQ USB device
- AC/DC converter 24V 15W
- Various circuitboard components (IC REG LINEAR 10V 100 MA TO92-3, TRANS NPN 40V 1A TO92-3, Terminal block pluggable 3.81, Terminal block, pluggable 5.08)

Compression and load measurement:

- Bimba Ball bearing thruster - two-shaft 2x0.250" shaft, .56"bore, cylinder, two-stroke
- TLL series 500lb capacity load cell

Framing and structural components:

- 80/20 triple slot rail (24'')
  - Corner brackets
  - Delrin blocks
  - Aluminum base plate-

Various hardware:

- ¼-28 screws
- ¼-20 screws
- 8-32 screws

Once all of the parts were shipped to the Guldberg lab, Julian McAdams in the fabrication shop of the Knight Campus took the initial CAD drawings and modified to produce a design more consistent with our conversations about needed adjustments, and from these provided the machining work to produce all the components needed for the full system, shown in Figure 1.

Figure 1: The current NIKI device that has been built, modeled after the NIKI device from the Sharma lab (Brown et al., 2019).

Throughout the testing of the NIKI device, some slight alterations were made to the original design that we had. The two main alterations that were made are that the knee cup was changed so that the inside was not as deep. This change allowed for better ACL rupture as the knee was more firmly pressed into the cup. The second alteration that was made was that we added ways to screw the rat holder to the large plate so that during compression, the holder would not move. Although the design of our NIKI device was largely based on the Sharma lab, most of the components have been altered from their model. Some notable differences between the Guldberg lab NIKI device and the Sharma lab NIKI device are that our knee cups have a different design, and our rat holders have a different design. The knee cup design was changed in that ours is shallower which we thought would lead to better ACL rupture. The rat holder was changed in that ours has adjustable sides so that we can fit different rat weights better, and ours was made of Delrin rather than 3D printed, which we thought would make it more stable. Some of our other parts such as tubing, air compressors, and DAQ, were slightly different models than the Sharma lab's NIKI device.

Electronic controls of the NIKI device

Once the NIKI device was fully built, the next step was setting up the electronics portion, which would enable the device to run and produce data as we wanted. As no one in the Guldberg lab has an electrical engineering degree, I enlisted the help of Dr. Salil Karipott and Dr. Keat Ong. Over the course of a couple of months, parts were ordered and put together in a black box. Dr. Karipott and Dr. Ong made sure that everything was connected, and that the NIKI device should be working properly and reporting data. There was only a small number of parts that needed to be ordered for the NIKI device circuits to be made.

- Signal Amplification circuit – Custom PCB – This contains the custom circuitry for amplifying the actuation signal from the DAQ to actuate the pressure regulator. The circuit contains the following 2 ICs:

1. Voltage Regulator IC - UA78L10 – Linear 10V regulator to power the amplifier IC.
2. Linear Amplifier IC – AD820 – Amplifier IC is a non-inverting amplifier with a gain of 2.

- Power Supply – Meanwell, 24V/12V power supply – This powers the Signal amplification circuit and the pressure regulator.

To confirm that everything worked properly in the device, a day was spent confirming that all of the electronics of the NIKI device worked together with the Labview software and program. The Labview program was designed to produce an output to the pressure regulator to control pressure in response to a voltage input into the program. Visually, one could see the pneumatic thruster of the NIKI device apply a downward motion in response to the voltage that was input into Labview. These two tests proved that all of the electronic components of the NIKI device worked and that I could move on to the calibration of the device, to define what the input voltage and resulting air pressure then measured as a force (in Newtons) instead of a voltage (native measurement of the load sensor).

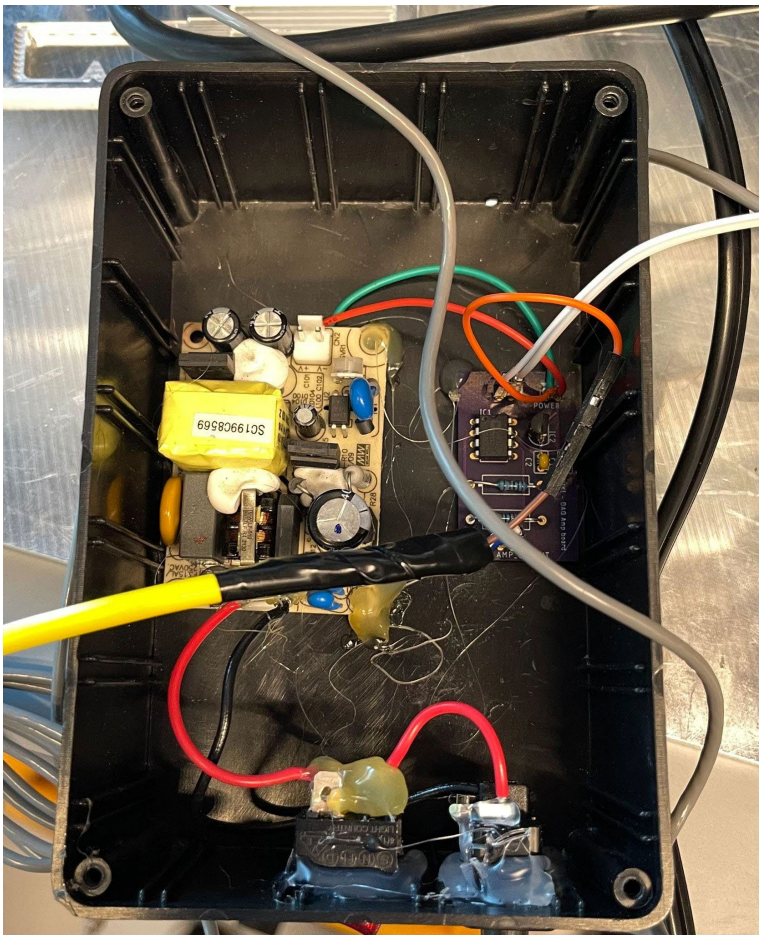

Figure 2: The black box that housed all of the electronics/circuitry for the NIKI device

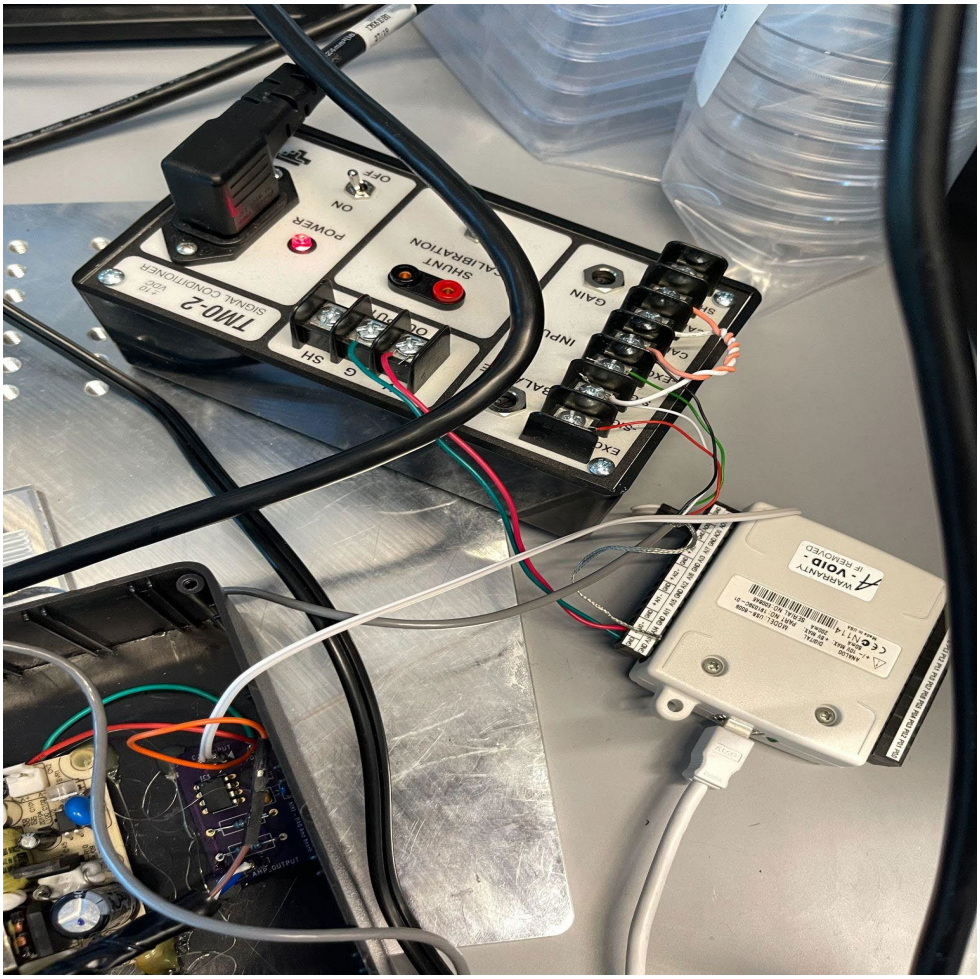

Figure 3: The black box connected to the AC/DC converter and the DAQ

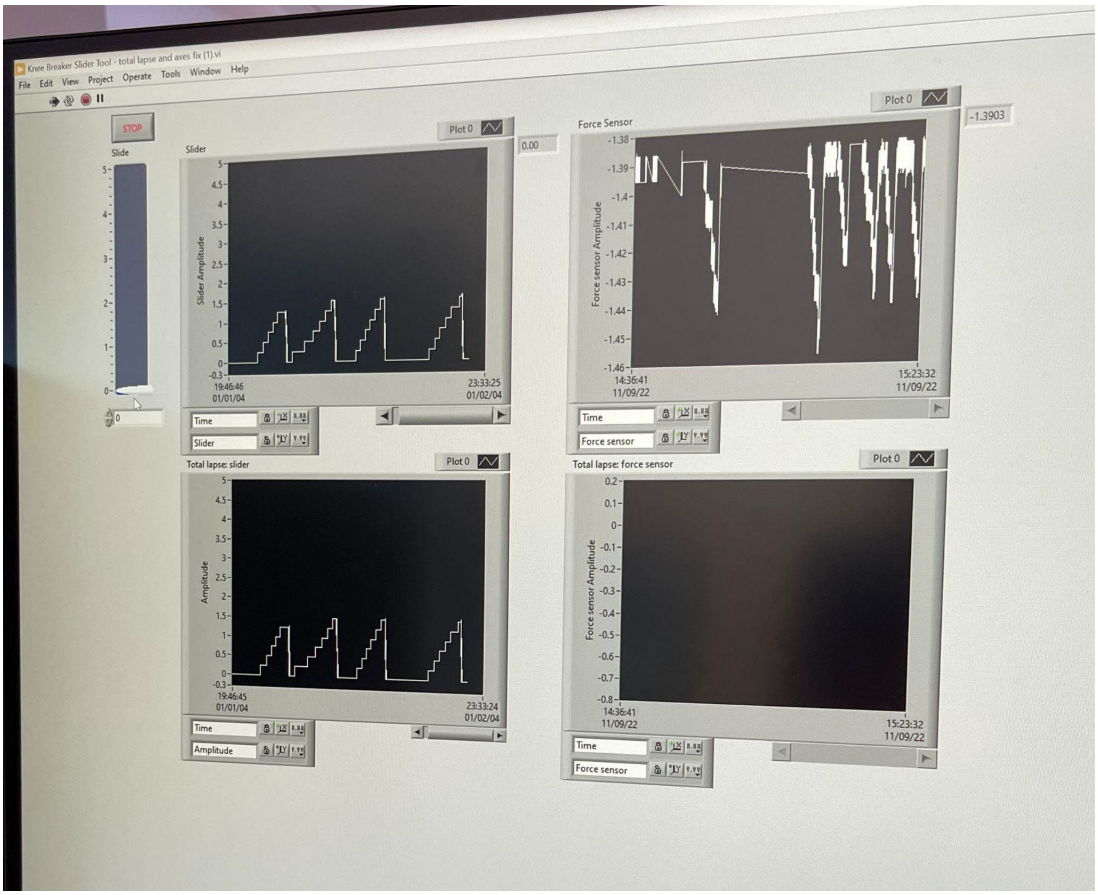

Figure 4: The Labview program. The slider on the left is where input voltage is entered that then provides pressure to the pneumatic thruster to push down onto the rat's knee. The number at the top right is the output measured voltage from the load sensor.

Calibration of the NIKI device

The calibration was done using 4 springs. The springs had varying spring constants which ranged from being compliant (1500 N/m) to the spring being stiff (9900 N/m). A series of tests were conducted on the springs where each spring was compressed at a constant voltage increment. The displacement was measured, and the readings from the load cell were plotted to generate a calibration curve for each spring. Springs were compressed at a constant input value of 0.25 (0-2.5V). When zero volts were applied, the initial length of the spring was measured using a digital caliper. Between each increment, as the spring was compressed, the new length

was measured. The differences in lengths following the increments were used to calculate the displacement at each incrementing load. Hooke's Law was then used to determine the applied loads at each increment of compression. Hooke's Law is F = -ks, where F is the applied force, k is the spring constant, and s is the measured compressive displacement. Data were then plotted for each spring to display an InputVoltage-Force calibration curve. Linear regression was used, and the linear equations were solved to create a calibration curve for each spring constant. Lastly, for each spring calibration curve, the voltage necessary to apply a 5 N force was calculated.

The predictive control calibration curve that was established was then validated by a previously calibrated high-resolution 250 N load cell on a mechanical testing frame (TA 3220). Then the voltage that was found to apply a 5 N force was measured by the 250 N load cell. The measured results were plotted against the predicted control calibration curve. Differences between the predicted control curve and load cell readings were further assessed at each 5 N increment, and percent error was plotted.

Animal Testing

The first stage of animal testing began with writing and submitting an IACUC protocol for approval of testing the NIKI device on rats. After some rounds of edits, the IACUC protocol was approved, and NIKI testing began. The first round of official testing on the NIKI device began with three full rat cadavers that had been previously frozen and then thawed. For these three rats, no ACL rupture occurred which led to a NIKI device redesign of the knee cup and addition of clamps to secure the Delrin bed to the aluminum base plate. The limbs were loaded to a maximum of 90 N/s. The cadavers underwent Faxitron X-Ray analysis to confirm that there were no bone fractures incurred.

Results and Discussion

After two years of building and working on a compressive load osteoarthritis model, the NIKI device has been completed and properly tested. The voltage necessary to apply a 5 N force was calculated to average over the range of 5-70 N for each spring. An incrementing voltage of 0.171 V was determined as the necessary input to control 5 N incrementation. The calibration/validation results are displayed in Figures 5, 6, and 7.

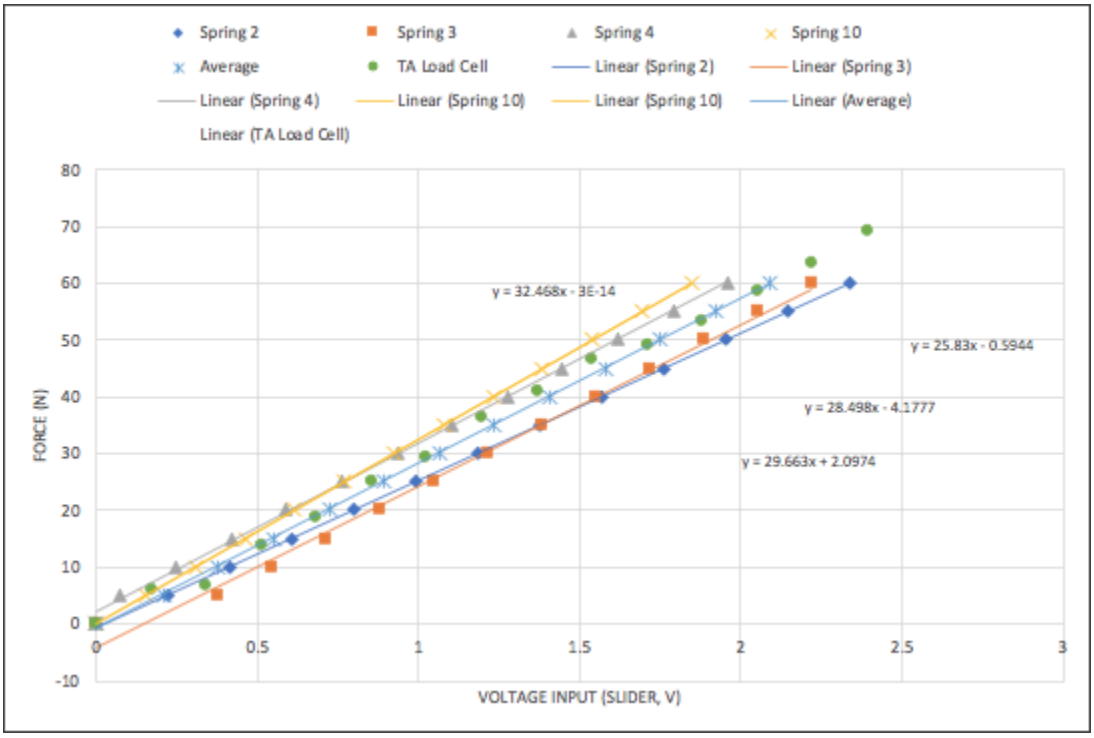

Figure 5: Calibration curve for each of the four springs comparing Force (N) with Voltage Input (Slider, V).

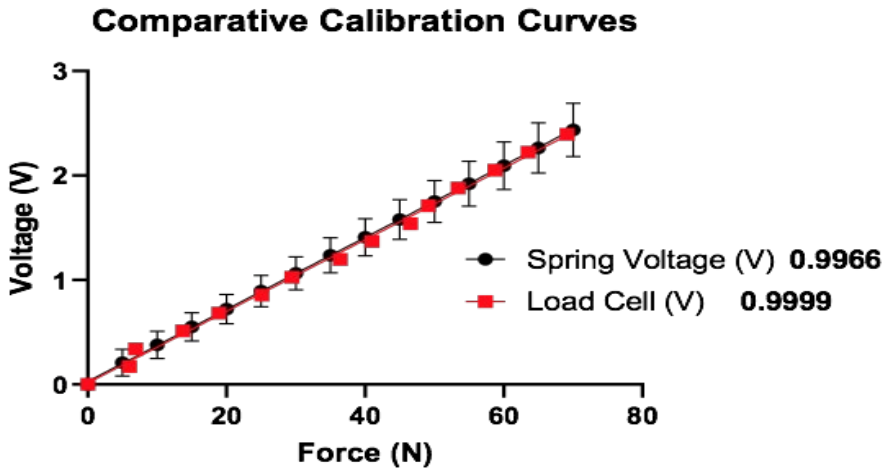

Figure 6: Input voltages of 0.171 V were applied incrementally, and forces were measured by the 205 N load cell. Measured results plotted against the predicted control calibration curve.

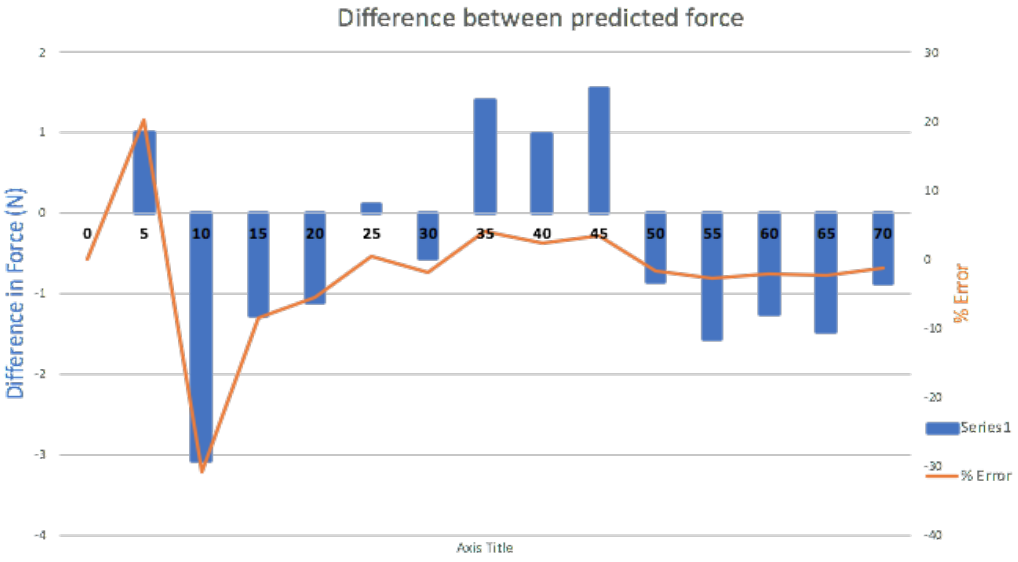

Figure 7: Difference between the predicted control calibration curve and load cell readings at 5 N increments with percent error plotted.

The greatest differences in the magnitudes between measured and predicted forces were near the bottom of the calibration curve (5-15 N). There was a maximum of 25% error

(deviation from the predicted/desired value) in the 5-15 N range. Variations in the mid-and upper range of the calibration curve were less than 1.5 N or a maximum of 2.5% error. In conclusion, the calibration/validation of the NIKI device demonstrated a working range of 45-65 N, so an applied load of +/- 1.5 N was determined to be acceptable.

The first round of testing on the animals was done on cadaveric tissue to ensure working components could deliver physiologically relevant loads. The initial cadaveric tests showed us that cadaveric tissue is too stiff to easily load into the NIKI device, but did also confirm multiple times via Faxitron X-ray that there were no apparent fractures of the tibia, patella, or femur. The terminal live rat test was able to show a successful ACL rupture by the NIKI device. ACL rupture was confirmed on the left hind limb via carefully dissecting the joint and visually inspecting the ACL. A full-thickness tear was confirmed and likely failure in the proximal third of the ACL. It appeared that an ACL substance failure occurred rather than avulsion out of the bone. There was no bone damage observed with the Faxitron X-ray analysis. The right hind limb appeared as a partial thickness tear that was slightly more mid-substance than the left hind limb ACL rupture. No bone damage was observed on the right hind limb rupture either.

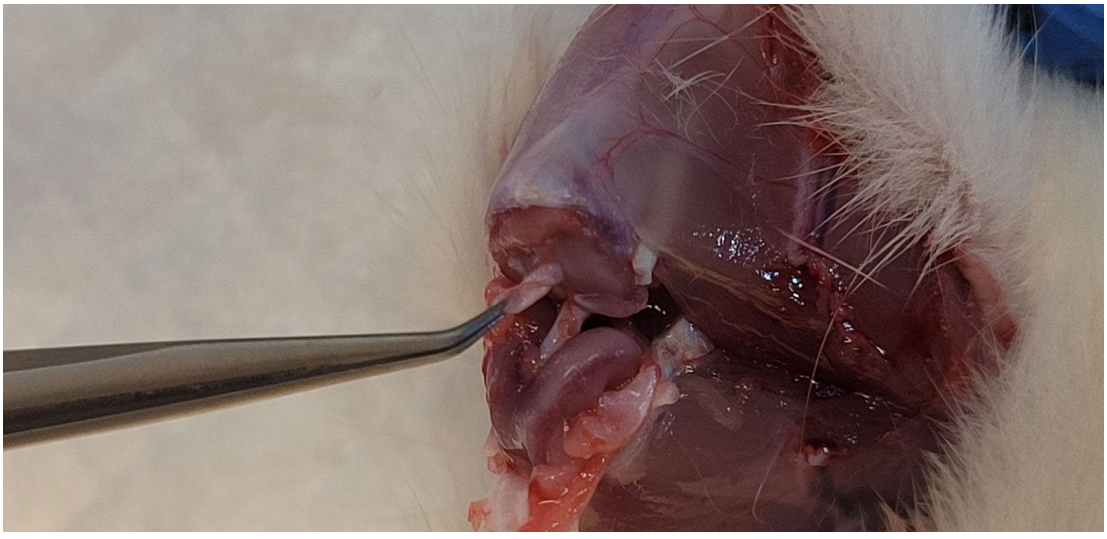

Figure 8: Image showing the left hind limb ACL rupture.

The second round of NIKI testing was completed using two cadaver rats and one terminal / non-survival live animal test. All of the rats were male Lewis rats, born in November 2022 (3-5 months of age), and had a weight between 290-350 g. The test was done bilaterally while the animal was deep under isoflurane for the duration of the device was active. Immediately after the rat underwent loading, it was euthanized, and knee dissected. Both limbs were loaded at 5 N/s until events corresponding to a large increase in displacement downward as well as a tibial translation forward. This event was noted at around 65 N for the left limb and 55 N for the right limb. The positioning of the rat is shown in Figures 9 and 10.

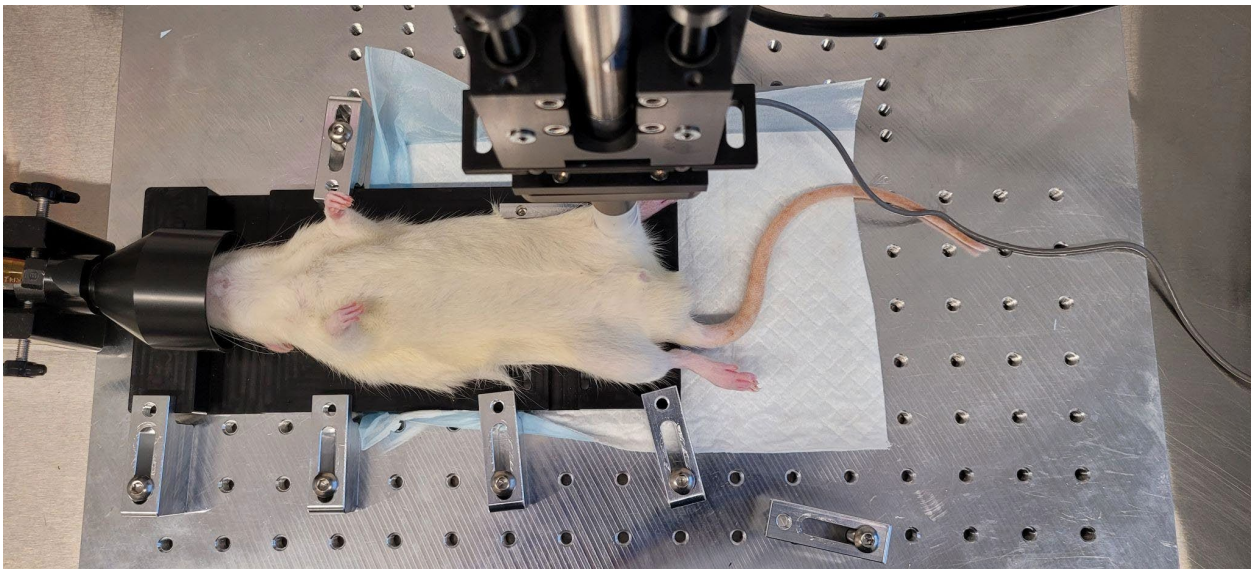

Figure 9: Aerial view of the rat positioned in the rat holder. The NIKI device knee cup is shown to be on the rat's left knee. The rat is receiving isoflurane and is properly deeply anesthetized.

Figure 10: A distal view of the rat in the rat holder of the NIKI device.

After the success of the NIKI device in creating ACL rupture, a pilot study has been planned as the next step. The pilot study will assess osteoarthritis progression at four-week and eight-week post-NIKI injury time points. The pilot study will also include a sham group (no ACL rupture, only anesthesia, and placement into the NIKI device with pre-load applied at 5N). Following euthanasia, injured limbs will be dissected and prepared for contrast-enhanced micro-CT to quantify morphometric bone and cartilage parameters and characterize osteoarthritis progression at both time points. Confirmation of ACL rupture will occur during preparation for micro-CT via dissection. The goal of this pilot study is to assess the four-week time point as a potential therapeutic window (only early-stage osteoarthritic changes) and the eight-week time point as an endpoint.

Conclusion

I proposed answering three questions by the time I completed my thesis. These three questions came in the form of goals I formulated for my research. One was to build the NIKI device similar to the Sharma lab with some possible improvements. The creation of the University of Oregon's NIKI device which I led ended up being very similar to that of the NIKI device the Sharma lab created. Some improvements were also added in the improved knee cup and the rat holder being adjustable for different rat sizes and made of a stronger material. The last improvement made was that the rat holder was able to be adjusted to different spots and screwed down on the base plate.

The second goal of the thesis was to calibrate the NIKI device using spring constants with as much accuracy as possible. The calibration and validation took place to ensure that voltage inputs would output the correct loads imparted to the knee joint. After an extensive process of calibrating the NIKI device using spring constants and validating that calibration with the TA machine, our device has proven to be calibrated to the highest degree of accuracy possible. The graphs in Figures 7,8, and 9

The third goal of the thesis was to show that the NIKI device would be successful in creating an ACL rupture in a rat. Despite the NIKI device failing to create ACL rupture in some early cadaver testing when the device was still getting optimized, ACL rupture was confirmed in the left hind limb of the non-survival animal test. Although more testing is going to have to be done to establish that we can consistently rupture the ACL, the first successful ACL rupture is promising. All three of these goals being achieved has made the NIKI device ready to have its use expanded to multiple studies that are being launched in the Guldberg and Willett labs, and I am excited to see what else we can accomplish using the device.

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