2024 Student Research Day
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Abstracts
Department of Orthopaedics
Warren Alpert Medical School of Brown University and Rhode Island Hospital

Anika Breker, B.S. in Biology
Title: Effect of Initial Graft Tension on Knee Osteoarthritis Outcomes after ACL Reconstruction: A Randomized Controlled Clinical Trial with 15-year Follow-Up
Authors: Anika N. Breker, Gary J. Badger, Ata M. Kiapour, Meggin Q. Costa, Emma N. Fleming, Stacy L. Ferrara, Cynthia A. Chrostek, Paul D. Fadale, Michael J. Hulstyn, Robert M. Shalvoy, Holly C. Gil, Braden C. Fleming
Abstract: Initial graft tension applied during ACL graft fixation could initiate PTOA.
Hypothesis: We hypothesized that the high-tension group would present improved outcomes and reduced PTOA compared to the low-tension group and show no differences from the matched control group.
Methods: Consented patients underwent ACL reconstruction with bone-patellar-bone or 4-stranded hamstring autograft. A matched control group was assembled for comparison. The low-tension group had the graft tensioned to restore normal anteroposterior (AP) laxity relative to the contralateral. The high-tension group had the graft over-constrained relative to the contralateral. Patients were followed up to 15 years post-operatively.
Results: Most outcomes presented no group differences. The low-tension group scored lower than controls in all KOOS sub-scores. The high-tension group scored lower than the controls on KOOS-Symptoms, Pain, and QOL. 42% of patients in both tension groups met the composite KOOS criteria for systematic osteoarthritis compared to controls. WORMS were higher in the surgical knee than the contralateral in both tension groups, while higher OARSI radiographic scores were observed in the surgical knee in the low-tension group.
Conclusion: Few differences were observed on 15-year outcomes between tension groups. The low-tension group was inferior to the control on more outcomes and displayed greater deficits between surgical and contralateral knees. ACL reconstruction did not prevent development of PTOA.

Wilson Chen, B.A. in Biochemistry
Title: Nonviral and Non-invasive Targeted Gene Delivery across the Blood-Brain Barrier
Authors: Xiangqin Chen, Jing Ding, Zhen Qiao, and Qian Chen
Abstract: Targeted gene therapy for treating neurodegenerative diseases is a hot and challenging research and development area. For example, current gene therapy methods for Alzheimer's Disease (AD) lack a targeted delivery system for the CNS by crossing the blood-brain-barrier (BBB) from the circulatory system and often require invasive surgical procedures such as intrathecal injections. Nanopieces™ (NPs) is a nonviral and noninvasive delivery system specialized for delivering nucleic acid (NA) cargos into cells in dense, matrix-rich tissues. Based on the prior evidence of successful NPs encapsulation and delivery of small NA cargos (siRNA, microRNA, and antisense oligonucleotides) into different regions of the brain, we hypothesized that NPs are capable of encapsulating and delivering bigger NA cargos, such as plasmid DNA, to

the CNS across the BBB. In this study, we developed the formulation of NPs encapsulating eGFP-expressing plasmid DNAs, identified a cellular receptor for NP uptake in a variety of cell lines *in vitro*, and demonstrated successful upregulation of eGFP activity in specific CNS cells *in vivo*. Our study exemplifies the promising future of targeted gene therapy for treating neurodegenerative diseases such as AD using noninvasive, nonviral NP technology.

Andrew Christenson, ScM Biomedical Engineering
Title: Design, Development, and Perception Testing of 3D Printed Insoles With Variable Longitudinal and Lateral Forefoot Stiffnesses in Athletic Footwear
Abstract: This project aimed to explore how the design of stiffening geometries in insoles have an impact on bending stiffness in both longitudinal and lateral directions and evaluate the effects of these mechanical differences on athlete perception through a unique perception-testing protocol. By leveraging the well-defined longitudinal bending axis and an estimated lateral bending axis as guidelines for design, a series of designs were developed in Rhino and Grasshopper: Null for low longitudinal and low lateral bending stiffness, Zag for low longitudinal and high lateral bending stiffness, Tori for high longitudinal and low lateral bending stiffness, and Plate for high longitudinal and high lateral bending stiffness. These insoles were 3D printed using Nylon-11 on a selective laser sintering (SLS) machine. Designs were validated through a 3-point bending test method on an Instron, informed by testing methods in the literature and recommendations from footwear industry professionals. A perception testing method was developed, employing sprinting (longitudinal bending) and cutting (lateral bending) exercises, where athletes were asked to score each insole on a scale from 1-5 in terms of comfort and stiffness. 7 male subjects were recruited for this study. The perception study revealed an inverse relationship between stiffness and comfort. Perception scores revealed that the Tori insole, designed to be flexible during cutting and stiff during sprinting, was perceived as the most stiff in both sprinting and cutting. These results suggest that more research is needed to fully understand foot biomechanics during lateral movements. By continuing to research human movement biomechanics, leveraging SLS printing as a rapid prototyping tool, and employing these methods across men, women, and youth athletes, footwear companies can work towards developing studded footwear that improves comfort and performance for athletes of all sorts.

Dylan J. Greynolds, ScM Biomedical Engineering
Title: The Rules and Science Behind Non-Wood Baseball Bat Regulations – A Scoping Review
Abstract: For the past several decades, the evolution of the game of baseball can be linked to the evolution of the baseball bat. In the 1980s, concerns regarding the integrity of America's pastime, the balance between offense and defense, and player safety began to circulate amongst parents, players, and leagues, that resulted in the regulation of non-wood baseball bat to align specifications and performance more closely to that of wood bats. Accordingly, there has been significant advancements in laboratory testing for bat performance. The objective of this study was to conduct a scoping literature review of regulations, laboratory performance testing, and field performance. Specifically, we aimed to evaluate the correlations between laboratory testing and field performance of baseball bats. A scoping review was conducted for articles reporting the physical properties of baseball bats in the lab and in the field that influence performance; after a screening process, 53 studies were extracted from full-text reviews. Through the review of studies, it was observed that metrics such as the "sweet spot" were identified in both laboratory

and field testing. However, we were unable to locate any studies that reported the rigorous validation the current laboratory bat performance measurements with field studies. To achieve this goal, it was concluded that laboratory and field studies incorporating an identical set of baseball bats will be required to rigorously determine the degree to which current laboratory bat testing procedures predict field performance.

Josephine Kalshoven, Ph.D. in Biomedical Engineering
Title: The in vitro Biomechanics of the Thumb Carpometacarpal Joint
Abstract: Osteoarthritis of the thumb carpometacarpal (CMC) joint is prevalent and debilitating, yet no surgical or therapeutic approach has proven superior, in part due to an incomplete understanding of the multidirectional biomechanics of the joint. We developed an in vitro method for multidirectional quantification of the CMC joint range-of-motion (ROM) and stiffness (K) in 6 degrees-of-freedom (DOF). First, we assessed 10 healthy specimens and determined that the CMC joint's greatest ROM lay in directions associated with activities of daily living. We also found evidence of internal/external rotation coupling with the other rotations of the joint and substantial K in the abduction-flexion region where stability is needed for grasp. Then, we assessed healthy and osteoarthritic joints to determine the correlation between osteophyte volume and ROM. We found rotational, but not translational, ROM was reduced with osteophyte growth. Finally, we transected either the anterior oblique ligament (AOL) or the dorsoradial ligament (DRL) in CMC specimens to determine their biomechanical roles. We found the AOL and DRL were complementary stabilizers and provided restraint in rotations away from and translations toward their insertions. Together, these data characterize the healthy CMC biomechanics that interventions should recoup and the impact of osteophytes and ligament degeneration.

Ashley Knebel, B.A. in PLME Program
Title: Detecting Sex Linked Differences in Osteoarthritis Development in a Genetic Murine Model using Gait Analysis
Authors: Ashley Knebel[1], Yajun Liu[1], Jing Ding[1], Qian Chen[1]
Abstract: Gait analysis has been successfully used to detect differences in a variety of conditions including joint injuries, Parkinson's, and acute limb ischemia by focusing on parameters such as stride length or stride frequency suggesting that this technique can be applied to osteoarthritis (OA). Previously, we have shown that transgenic mice with overexpression of miR-365 in Col2 lineage specific cells develop early onset of OA. To overexpress miR-365 in cartilage tissues, miR-365 mice were crossed with Col2-Cre mice to produce Col2-Cre+/miR-365 (MiR-365) mice. Beginning at two months, mice were run on a flat treadmill at 25 cm/s, and each run was recorded and analyzed using Digigait Imaging software. In female mice, there is a significant difference between MiR-365+ and Cre+ in stride length and stride duration beginning at 3 months. This difference is seen at 5 months and 7 months also. For stride length and stride duration, at no point was there a statistically significant difference between MiR-365+ or Cre+ male mice. These findings indicate that gait analysis may be sensitive to differences in the development of OA as early as 3 months and may be more sensitive to differences than other quantitative measures such as OARSI scoring with Safranin-O staining.

Layla Lynch, B.S. Honors Biomedical Engineering
Title: Proprioception Analysis of Division I Collegiate Softball Players
Abstract: Proprioception is the ability to sense one's body in space and is necessary for executing movement safely and efficiently. For athletes, proprioception may be the difference in preventing injury, maximizing rehabilitation outcomes, or ensuring optimal performance. This report aims to assess whether Division I softball players' proprioception varies between their dominant and nondominant shoulders across a variety of target positions.
Methods: Joint position sense (JPS) was assessed for dominant and nondominant shoulders of seven female softball athletes (mean ± SD, age: 20 ± 1 yr) using an Opal APDM wearable sensor to track their arm's three-dimensional position in space. The three-dimensional position was translated into an elevation angle (EA) and crossbody angle (CA) combination used to describe the shoulder joint angle. The subjects followed a joint position reproduction (JPR) protocol by replicating a series of EA and CA target positions. A two-way ANOVA test was used to identify significant differences in the error scores between dominant and nondominant shoulders, between each target position, and for both factors combined.
Results: No significant differences were found between the dominant and nondominant shoulders of the athletes across all target positions for EA (p-value: 0.766) or CA (p-value: 0.6596). Significant differences were observed only for the target position factor for EA (p-value: 0.000011) and CA (p-value: 0.0018).
Conclusion: Hand dominance does not affect shoulder JPS in female softball players, however, target positions affect proprioceptive error scores.
Clinical Relevance: Shoulder research on female athletes is an underrepresented space, despite the growing participation of women in sports and corresponding injury rates. While proprioception did not vary with hand dominance, it was heavily influenced by the target position replicated. These findings imply that certain shoulder joint angles yield different proprioceptive abilities and open the door to a better understanding of shoulder health and behavior.

Alexandria Martinez, B.S. in Biochemistry
Title: Mapping SUMOylation Sites that Regulate SOX9 Stability and Transcriptional Activity in Chondrocytes
Authors: Alexandria Martinez, Lijun Wang, Jiahui Huang, Doug Moore, and Wentian Yang
Department of Orthopaedics, Brown University Alpert Medical School
Abstract: Understanding the mechanisms that modulate SOX9 transcriptional activity and expression in cartilage is critical to understanding cartilage biology, and developing effective treatment for cartilage degenerative diseases, such as osteoarthritis (OA). This study aims to determine the impact of SOX9 SUMOylation on SOX9 protein stability and transcription activity and map SOX9 SUMOylation sites *in vitro* using mutagenesis assays and genetically modified chondrocytes.
Methods: Flag-tagged SOX9 K61R, SOX9 K253R, SOX9 K398R, SOX9 K61/253R, SOX9 K253/398R, SOX9 K61/398R, and SOX9 K61/253/398R mutants were generated using standard PCR-based mutagenesis protocols, and confirmed via DNA sequencing analysis.
Results: Western blot data shows that wildtype SOX9 and SOX9 mutants can be recognized by anti-Flag and anti-SOX9 antibodies in transfected 293T cell lysates. Importantly, our preliminary

data demonstrate that mutations of SOX9 K398R and SOX9 K253/398R decreased SOX9 abundance in murine and human chondrocytes, suggesting that these lysine residues are crucial in maintaining SOX9 protein stability.
Discussion: By analyzing the SOX9 expression in chondrocytes, we hope these mutants will allow us to determine which post-translational modification site(s) mediate(s) the regulatory effect on SOX9 in chondrocytes. We are currently repeating these experiments and analyzing the impact of other mutations on SOX9 stability and transcriptional activity.

Joseph Sebastian Stevenson, ScM Biomedical Engineering
Title: Development and Clinical Evaluation of a Device for Measuring Weight Bearing in Pediatric Standers
Abstract: Assisted standing for children requiring wheelchairs for mobility has proven to be beneficial from both psychological and physiological perspectives. However, due to the current rigid design of standers, it has been demonstrated that children may not actually be fully weight bearing. The aim of this study is to develop a weight bearing device (WBD) to measure and better understand foot loads while in standers. This development aims to assist caregivers in improving positioning, as well as provide a more precise metric in studies on the benefits of standing. With IRB approval, seven individuals ranging between 8 to 17 years old who utilize a stander in a school setting were recruited. To gather loading data, a custom WBD comprised of four full-bridge micro load cell sensors positioned across a 220 mm by 84 mm Aluminum plate was fabricated. In bench-top testing, the WBD was calibrated to acquire measurements of weight bearing on each foot, as well as distribution of body weight between left vs. right, toe vs. heal loading, and medial vs. lateral loading. The output data was used to make real-time changes to the individual's standers straps to correct and improve their weight bearing capabilities. After collecting and analyzing fourteen standing sessions, our results demonstrated that the children are not fully weight bearing, but real time adjustment in stander positioning and strapping can improve weight bearing. This information can be used by the physical therapists to correct strap tightness or brace locations in order to achieve a higher weight bearing load. Currently, physical therapists are unable to accurately tell how much the device's straps and braces are negatively affecting a patient's ability to optimally bear weight. The weight bearing device designed for this study supplies measurements that can be used for improving therapeutic techniques and ultimately an individual's health

Lara Swords, B.S. in Biology
Title: Histological Observations of Rat Anterior Cruciate Ligament after Relaxin-2 Treatment
Abstract: Female athletes are at a higher risk for anterior cruciate ligament (ACL) ruptures which may be associated with higher levels of relaxin-2 in the bloodstream. Relaxin-2 is known to upregulate matrix metalloproteinases (MMP)-1, 3 and 13, and reduce type I and III collagen expression in human ligament cells of female ACLs only, which may alter the structural integrity of the ACL over time. However, the use of relaxin-2 and its receptor antagonist as a potential therapeutic treatment option for patients with ACL reconstruction remains unknown. This pilot study aimed to explore the role of Relaxin-2 on the histology of the female rat ACL.
Methods: Treatment group rats were given relaxin-2 via oral gavage for 10 days before all rats were euthanized and underwent biomechanical testing. Histological sections of rat knees were

stained with hematoxylin and eosin to visualize any structural changes in the bone or connective tissue.
Results: Some of the relaxin-2 treatment rats showed more pronounced collagen fiber disorganization in the ACL than control rats, and some differences in femoral articular cartilage were also noted. No significant difference was found in bone composition of control vs treatment rats.
Conclusion: While relaxin-2 treatment may alter the histology of female rat ACLs, further studies should be done with intact ACLs (no biomechanical testing) and larger sample sizes.

Sam Zalk, MS.c. in Biomedical Engineering
Title: Quantifying Biomechanical Properties of the Anterior Cruciate Ligament in a Rodent Model
Authors: Samantha Zalk, Quianna Vaughan, Peter Wronski, Brett D. Owens, Jillian E. Beveridge
Abstract: Females experience ACL tears at disproportionately higher rates, potentially due to the effects of the hormone relaxin. To precisely measure ACL biomechanical properties in a rodent (rat) model in which circulating relaxin level can be manipulated, the present work sought to create a methodology to non-invasively measure the cross-sectional area (CSA) of the rat ACL and calculate its biomechanical properties. In doing so, three aims were investigated: (1) Develop camera settings to visualize the rat ACL; (2) Test intra- and inter-user agreement in CSA calculations; and (3) Test the reproducibility of the tensile testing approach from which biomechanical properties can be derived. A method to digitally measure rat ACL CSA repeatably and reproducibly was successfully developed in Aims 1 and 2 demonstrating agreement in dimensional measures to within 10%. However, large bilateral differences that ranged from 8.7 to 57.4% for ultimate failure load and stiffness between limbs suggests limitations associated with fixturing will likely overshadow potential differences attributable to relaxin administration. Modifying the fixture to test the ACL fibers more evenly and improving the fixation of the femur-ACL-tibia complex may improve reproducibility and will be the focus of future work developing the rat model to study the effects of relaxin.

Albert Wu, B.S./MS.c. in Biomedical Engineering
Title: Gait Disturbance of Alzheimer's Disease Mimics Osteoarthritis: Characterization, Treatment and Correction by Suppression of Retrotransposon LINE1
Abstract: Osteoarthritis (OA) and Alzheimer's disease (AD) are inflammatory, degenerative diseases with high prevalence in aging populations. Although these conditions affect different tissues and have distinct pathophysiology, research evidence suggests that there is an association between OA and AD at both biological and clinical levels in humans [1, 2]. Inhibition of LINE1, a highly expressed retrotransposon, with nucleoside reverse transcriptase inhibitor (NRTI) has been shown to be effective for suppressing tissue inflammaging [3]. Induction of OA is known to accelerate AD pathogenesis in the APP/PS1 transgenic mouse model of AD [4]. In this study, we characterized gait disturbance patterns in the APP/PS1 AD mouse model using DigiGait over the course of 10 months. We determined tissues and regions in which LINE1 and pro-inflammatory marker 1L-1β are over-expressed using qPCR. Gait change in AD mice is characterized by shorter swing duration, shorter stride duration, shorter stride length, and greater stride frequency.

Administration of 3TC resulted in correction of gait disturbances in AD mice and a significant reduction in LINE1 mRNA expression in the hippocampus.

[1] Weber et al. *Medicine* (2019) 98:10 [2] Ikram et al. *Osteoarthritis and Cartilage* (2019) 27:10 [3] Gorbunova et al. *Nature* (2021) 596:7870 [4] Kyrkanides et al. *Journal of Neuroinflammation* (2011) 8:112