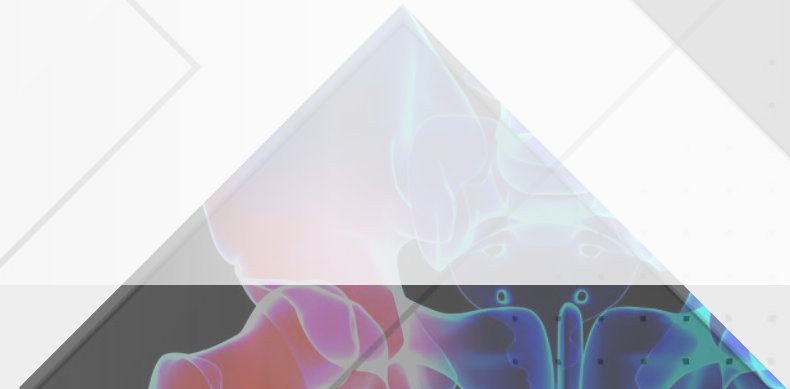
Steadman Philippon Research Institute

ORTHOPAEDIC RESEARCH JOURNAL

2022–2023



STEADMAN PHILIPPON RESEARCH INSTITUTE

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INSTITUTE MISSION

BUILDING ON OUR LEGACY OF EXCELLENCE IN ORTHOPAEDIC SPORTS MEDICINE, STEADMAN PHILIPPON RESEARCH INSTITUTE IS UNLOCKING THE SECRETS OF HEALING, FINDING CURES AND ENHANCING LIVES THROUGH GLOBAL LEADERSHIP IN REGENERATIVE MEDICINE, SCIENTIFIC RESEARCH, INNOVATION AND EDUCATION.

WELCOME

Dear Friends,

We are pleased to share the sixth edition of **Steadman Philippon Research Institute's (SPRI)** ***Orthopaedic Research Journal*** with you. This publication is a celebration of the cutting-edge science being performed at our institution, and we are thrilled to share these updates with you.

This year's edition picks up where our fifth edition left off, presenting key scientific and research updates from July 2022–June 2023. Each department at SPRI—the Linda & Mitch Hart Center for Regenerative and Personalized Medicine, Department of Biomedical Engineering, Center for Outcomes-Based Orthopaedic Research and Department of Education—has selected their top highlights from the past year to share with you.

In January 2023, SPRI's Founder, J. Richard Steadman, MD, passed away at his home in Vail. In this publication, we will share a reflection about Dr. Steadman from our own Marc J. Philippon, MD, his longtime partner and friend. It was a privilege to work with Dr. Steadman, and we are honored to continue his legacy of orthopaedic research and education at SPRI.

SPRI is home to a motivated, passionate and innovative team of scientists and researchers who conduct high-impact science in pursuit of SPRI's mission to find cures and enhance lives. Their dedication ensures that patients all over the world will benefit from SPRI's discoveries.

To all of our readers, thank you for your support of SPRI. Our research partners, community and friends are imperative in helping SPRI achieve its success in research and clinical science. We speak on behalf of the entire SPRI team when we thank you again for your support.

Respectfully yours,

Marc J. Philippon, MD
Chairman

Johnny Huard, PhD
Chief Scientific Officer

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RESEARCH AND EDUCATION DEPARTMENTS

SPRI's departments work together to enhance patient care and keep people active through leading-edge science and education. SPRI works collaboratively across its research teams and alongside physicians from The Steadman Clinic to produce high-impact, valuable clinical translation science.

LINDA & MITCH HART CENTER FOR REGENERATIVE AND PERSONALIZED MEDICINE (CRPM):

The CRPM team is focused on the basic science of regenerative medicine. CRPM's team of scientists, researchers and technicians conduct research designed to translate discoveries into practical treatments, including integral participation in each of SPRI's clinical trials.

DEPARTMENT OF BIOMEDICAL ENGINEERING (BME):

SPRI's BME team enhances patient care by focusing on injury and re-injury mechanisms and prevention, develops and validates novel surgical treatments and rehabilitation techniques and teaches advanced research protocols using state-of-the-art biomedical research techniques and technologies. The department includes a Robotics Laboratory, Biomotion Laboratory and advanced imaging, utilizing 3-Tesla magnetic resonance imaging (MRI).

CENTER FOR OUTCOMES-BASED ORTHOPAEDIC RESEARCH (COOR):

SPRI's robust outcomes database is now tracking over 48,000 surgeries. The center conducts evidence- and outcomes-based research using actual clinical data, which helps both physicians and patients in making better and more informed treatment decisions.

DEPARTMENT OF EDUCATION:

The education team administers and coordinates the clinical fellowships and international scholars programs, host conferences and international academic meetings, produces and distributes publications and educational media and organizes outreach programs with local Colorado students.

"DR. STEADMAN WAS AN INCREDIBLE SURGEON AND LEAVES A REMARKABLE LEGACY OF INNOVATION IN THE FIELD OF ORTHOPAEDICS AND SPORTS MEDICINE. HE KNEW THE VALUE OF OUTCOMES RESEARCH, AND WAS A TRUE PIONEER NOT ONLY IN TECHNIQUES, BUT ALSO IN THE IMPORTANCE OF SCIENCE AND THE PRACTICE OF EVIDENCE-BASED MEDICINE.

– DR. MARC J. PHILIPPON

J. RICHARD STEADMAN, MD (1937–2023)

A PIONEER IN SPORTS MEDICINE INNOVATION AND A CHAMPION FOR HIS PATIENTS

A Reflection by Marc J. Philippon, MD

Our sports medicine community lost one of its most influential pioneers in both surgical technique and outcomes-based research when J. Richard Steadman, MD, passed away peacefully at his Vail, Colorado, home in January.

Dr. Steadman's groundbreaking surgical innovations included the development of several advanced procedures for the knee, including "microfracture" and the "healing response," which harnesses the body's own healing potential.

It was a true honor to be asked to join Dr. Steadman here at The Steadman Clinic and to work side-by-side with him at both the clinic and Steadman Philippon Research Institute (SPRI). All of us who have had the opportunity to learn from Dr. Steadman are much better doctors and surgeons because of that experience.

Dr. Steadman was a champion for his patients, a kind and dedicated physician with a healing touch. He truly, deeply cared for every patient he met, from the world-famous Olympic and professional athletes to the "weekend warriors" who were able to stay active thanks to Dr. Steadman's talents. He prioritized their outcomes and personalized their care with the sole desired outcome being a quick return to their normal active lifestyle.

Dr. Steadman was not only a great surgeon and a great physician but also a great person. From his calming presence and bedside manner to the way that he spoke to his patients: they knew immediately that it just wasn't a typical doctor-patient relationship. "Steady" was in it for them, not only to help them, but also to make sure that their quality of life was first and foremost.

Without a question, the most important thing that I learned from him was how to always look for the best possible solution and strive for—and not settle for—anything less than the best. Dr. Steadman was the perfect example of that—"Do the right thing."

In February 2015, Dr. Steadman was honored with a dedication of a new deck on Vail Mountain. Pictured from left: Chris Jarnot, Dr. Marc Philippon, Dr. Richard Steadman, Gay Steadman, Cindy Nelson, Phil Mahre, Dan Drawbaugh, Dr. Tom Clanton, Dr. Peter Millett

His long-standing contributions to orthopaedics and sports medicine go beyond his extraordinary work in the operating room. He knew the value of outcomes research and was a true pioneer, not only in techniques, but also in the importance of science and the practice of evidence-based medicine. His love of orthopaedic surgery and his intellectual devotion to research led to his founding of two world-renowned standard bearers in the field: The Steadman Clinic and what is now Steadman Philippon Research Institute (SPRI).

Dr. Steadman was a leader in the area of physical therapy and postoperative rehabilitation. He developed rehabilitation protocols for patients with serious joint and chondral injuries—these are now used daily by orthopaedic surgeons and physical therapy professionals around the world. In the mid-1970s, he began to make a name for himself by revolutionizing postsurgical physical therapy. Instead of following the usual practice of putting a hard cast on the injured arm or leg, Dr. Steadman instituted mobilization of the limb immediately after surgery, which both expedited and improved patients' recoveries.

Dr. Steadman treated countless Olympians and professional athletes across various sports and was sought out by some of the world's best-known athletes and public figures for treatment. His ability to return high-level athletes with injuries to medal-winning victories after surgery and rehabilitation made him an iconic figure in sports medicine. Dr. Steadman's direct involvement with providing care for elite athletes got its start with his association with the U.S. Olympic ski teams in the 1970s. He served as the team physician for the U.S. Alpine Ski Team for nine consecutive Winter Olympics, beginning in Innsbruck, Austria, in 1976.

In 1982, the complexity of the knee and its critical importance to athletic performance persuaded Dr. Steadman to focus nearly exclusively on disorders and injuries to that joint. Between 1989 and 1991, he worked to develop a treatment for a specific type of injury to the anterior cruciate ligament, which came to be known as the "healing response." Dr. Steadman created his nonprofit Steadman Sports Medicine Research Foundation in 1988 at Lake Tahoe. That organization exists today as SPRI, which is known worldwide for its clinical research database and study of orthopaedic injuries. For more than three decades, SPRI has been collecting clinical data and tracking clinic patients and their treatment outcomes. It is perhaps the largest orthopaedic research database in the world that focuses on patient outcomes and the dissemination of data that promote the practice of evidence-based medicine.

In 1990, Dr. Steadman moved to Vail to open a sports medicine clinic and broaden the scope of his orthopaedic research. Thanks to Dr. Steadman's commitment to sports medicine, The Steadman Clinic has thrived in Vail and has grown to become one of the world's most respected and influential orthopaedic organizations. Combined with the research expertise of SPRI, the clinic will keep the Steadman name at the forefront of sports medicine and groundbreaking research for years to come.

Of his many passions in sports medicine, one of the most significant was his role as a mentor to sports medicine fellows. He took so much time and care in training his fellows, not only instilling in them the skills necessary to be a successful surgeon, but also imparting the tools needed to care for patients. If you ask any of his former fellows, they will tell you one of Steady's greatest lessons was how to connect and care for patients, to take time with them, and to make them feel confident in their experience.

Dr. Steadman's philosophy behind taking care of people and taking care of our profession by advancing it through research is extraordinary and has been life-changing for so many people. Many of Dr. Steadman's patients left his Vail offices with a lifelong friend. Olympic skiers and star professional athletes developed close, personal relationships with Dr. Steadman and his family. His impact went far beyond the operating room, and his patients and friends made regular social visits to Vail, even after Dr. Steadman's retirement, just to visit with him.

I will remember Steady not only for his innovations and mentorship but also for his friendship. He had a laugh like no one else and was a lover of music and spending time with the people he cared about. Dr. Steadman loved to entertain and was a true *bon vivant*—many of us treasure the great dinners we shared with him all over the world. It was, quite simply, a privilege to spend time with him.

Dr. Steadman created the unique experience of living in a small town in the mountains while conducting high-quality research and performing high-quality surgery. Since his passing in January, so many people have stopped me to point out their "Steadman knee" and share a story about what he meant to them. He was a central figure in our mountain community, and The Steadman Clinic and SPRI are honored to be a part of his continued legacy.

Dr. Marc Philippon and Dr. Steadman in Dr. Steadman's office

OREF CLINICAL RESEARCH AWARD FINDS A HOME AT SPRI

MARC J. PHILIPPON, MD RECEIVES MONUMENTAL AWARD FOR PIONEERING WORK IN HIP ARTHROSCOPY

Dr. Marc J. Philippon—Chairman of Steadman Philippon Research Institute (SPRI) and Managing Partner of The Steadman Clinic—was awarded the prestigious Orthopaedic Research and Education Foundation (OREF) Clinical Research Award from the American Academy of Orthopaedic Surgeons (AAOS). AAOS has over 39,000 members and is the world's largest medical association of musculoskeletal specialists. The OREF Clinical Research Award recognizes outstanding clinical research related to musculoskeletal disease or injury and is presented during the Academy's annual Kappa Delta Award presentations.

VALIDATING AND ADVANCING THE FIELD OF HIP ARTHROSCOPY

For over 25 years, Dr. Philippon and his team of researchers and investigators have committed to validating the hip arthroscopy procedure and advancing techniques, including labral repair and integrating orthobiologic approaches to produce the best possible outcomes for patients. Throughout his career, Dr. Philippon has helped treat painful hip injuries in countless patients, including nearly 1,000 professional athletes and Olympians—many of whom have achieved significant sports accomplishments after receiving their treatments.

Dr. Philippon's award-winning research, **"Hip Chondrolabral Dysfunction: The Road from Excision to Repair, Replacement, and Regeneration,"** explores Dr. Philippon's pioneering work in hip arthroscopy. The hip arthroscopy procedure was first introduced in 1931, but was largely considered experimental and was not adopted as a modern treatment until the 1990s. In the early days of hip arthroscopy selection, most surgeons removed much of the labrum—the cartilage lining the hip socket—but Dr. Philippon's approach preserves as much of the labrum as possible through repair or reconstruction. The labrum is a key component in an individual's hip biomechanics and tissue health, so preserving the tissue has proven to provide an improved outcome for many patients. Through this approach, Dr. Philippon has been able to successfully treat disorders like femoroacetabular impingement (FAI), a condition that is prevalent in athletes.

GROUNDED IN EVIDENCE-BASED MEDICINE

Dr. Philippon trained at McMaster University, where "evidence-based medicine" began. This approach to medical practice involves using research evidence alongside clinical expertise to optimize results for each patient. "Validating hip arthroscopy and making it a standard procedure to safely treat hip injuries, prevent hip degeneration and osteoarthritis has always been one of my goals," Dr. Philippon shared with AAOS. "In doing so, our team provided extensive evidence-based support for hip arthroscopic procedures that led to positive long-term outcomes based on four pillars: large clinical outcomes studies, biomechanics, biomotion/imaging, and therapies for regenerative and personalized medicine."

Dr. Philippon was presented the OREF Clinical Research Award at the AAOS Annual Meeting. Pictured with him at left: Grant Dornan and Karen Briggs, and at right: Dr. Johnny Huard

With these pillars in mind, Dr. Philippon and SPRI have made significant contributions in orthopaedic research including investigations into the intricacies of the hip anatomy, labral debridement, labral repair, labral reconstruction and augmentation, the use of novel orthobiologics, and identifying factors that lead to optimal patient outcomes.

ACCOMPLISHMENTS IN HIP ARTHROSCOPY

Leading up to the 2023 OREF Clinical Research Award, Dr. Philippon and his team secured many significant achievements in the field. Highlights of their work include:

- Publication of the "Most Influential Paper in Hip Arthroscopy" (Barbera et al.; *Arthroscopy* 2020), which provided evidence for the now commonly followed rule of joint space as a contraindication for hip preservation surgery.
- Development of tools to accurately measure and validate patient outcomes after labral repair and FAI, including Hip Outcome Score for Activities of Daily Living (HOS-ADL) and for sports (HOS-Sport).
- Determination that labral repair can restore significant function to the hip joint and prevent pain, instability and further wear in the cartilage. Clinical studies have shown improved outcomes in patients over the last 10 years.
- Identification of novel biologic and pharmaceutical approaches to reduce fibrosis and improve tissue healing in partnership with Johnny Huard, PhD, SPRI Chief Scientific Officer and Director of the Linda & Mitch Hart Center for Regenerative and Personalized Medicine (CRPM).
- Development of a new technique for replacing the severely damaged labrum: labral reconstruction. Positive patient outcomes have a median satisfaction score of 10 out of 10 at the 10-year follow-up.
- Since 1990, SPRI has published the most research papers in the field of arthroscopy.

DR. PHILIPPON WOULD LIKE TO THANK DR. JOHNNY HUARD, KAREN BRIGGS, MPH, GRANT DORNAN, MS AND THE ENTIRE RESEARCH TEAM AND CLINICAL STAFF FOR THEIR WORK IN SUPPORT OF DR. PHILIPPON'S CLINICAL RESEARCH.

SPRI CLINICAL TRIALS ARE HALLMARK OF CLINICAL TRANSLATION

SPRI is committed to bench-to-bedside research, translating discoveries made in the lab into treatments for patients. This commitment is exemplified by the organization's clinical trials, which are funded federally by the National Institutes of Health (NIH) and Department of Defense (DoD), as well as philanthropically and through foundation grants. Below, please find more information about SPRI's ongoing clinical trials.

CLINICAL TRIALS: CURRENTLY ENROLLING

PROSPECTIVE EVALUATION OF PLATELET-RICH PLASMA AND BONE MARROW CONCENTRATE TREATMENT TO ACCELERATE HEALING AFTER ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION

Principal Investigators: Johnny Huard, PhD and Peter J. Millett, MD, MSc

This is a prospective, randomized, placebo-controlled trial to evaluate potential beneficial effects of leukocyte-poor platelet-rich plasma (LP-PRP) and bone marrow concentrate (BMC) on the healing and health of critical joint tissues (grafts/ligaments, meniscus and cartilage) in the knee following anterior cruciate ligament (ACL) reconstruction surgery. This trial will compare the two injection procedures against a control group (no injection). BMC subjects will have bone marrow aspirated from the subject's iliac crests and the cellular rich portion will be concentrated and subsequently injected into the subjects' symptomatic knee. PRP subjects will have a venous blood draw with subsequent platelet concentration. The resulting PRP will be injected into the symptomatic knee. Follow-up visits involving imaging and biomotion testing will take place at 2 weeks, 6 months and 12 months after the ACL reconstruction surgery.

The study will be conducted at The Steadman Clinic, Steadman Philippon Research Institute, and Vail Valley Surgery Center. This study is supported by The United States Department of Defense: Office of Naval Research.

For additional information, please visit www.clinicaltrials.gov and search for: NCT04205656.

Primary Objectives

- Collect clinical outcomes data, imaging data, strength and physical function data and patient-reported outcomes to compare amongst investigational and control groups.
- Determine the signature protein and cellular profiles of PRP and BMC to compare to optimal clinical, biomechanical and MRI results after ACL reconstruction surgery.

THE USE OF SENOLYTIC AGENT TO IMPROVE THE BENEFIT OF PLATELET-RICH PLASMA AND LOSARTAN FOR TREATMENT OF FEMOROACETABULAR IMPINGEMENT AND LABRAL TEAR: A PILOT STUDY

Principal Investigators: Marc J. Philippon, MD and Johnny Huard, PhD

This study is being conducted at The Steadman Clinic and Steadman Philippon Research Institute and is supported by The United States Department of Defense. The purpose of the study is to study the effects of a pre- and post-operative senolytic medication (Fisetin) for femoroacetabular impingement and labral repair.

For additional information, please visit www.clinicaltrials.gov and search for: NCT05025956.

Primary Objectives

- To determine whether pre- and post-operative Fisetin will improve the benefits of PRP when used in conjunction with hip arthroscopy for the treatment of femoroacetabular impingement and labral tears.
- To determine whether pre- and post-operative Fisetin for hip arthroscopy decreases serum biomarkers of SASP and inflammation.
- To determine whether Fisetin prior to undergoing hip arthroscopy is safe.

FISETIN DOSING EQUIVALENCE STUDY

Principal Investigators: Johnny Huard, PhD and Dustin Anderson, MD

This is a prospective randomized controlled trial evaluating if the supplement Fisetin safely reduces senescent cells, and if so, to compare this reduction when taking a daily dose (once a day) versus a bolus dose (single dose given over a short period of time: 2 days). This clinical trial will be conducted at The Steadman Clinic and Steadman Philippon Research Institute.

Primary Objectives:

- Determine if daily and bolus doses of Fisetin reduce serum biomarkers of Senescence-Associated Secretory Phenotype (SASP).
- To define the safety and tolerability of Fisetin administered as a bolus and daily regimen.
- To evaluate changes in senescence biomarker values within bolus dosing windows when compared to a daily dosing regimen.

CORRELATION OF BONE, AGING, AND INFLAMMATION BIOMARKERS ITH BONE HEALING

Principal Investigator: Chelsea S. Bahney, PhD

It is well established that bone healing is delayed with aging, yet the fundamental mechanisms that drive this impaired healing are not understood. This is a prospective observational trial employing a novel quantitative marker of bone regeneration alongside key markers of aging and in inflammation. We hypothesize that aging causes dysregulation of the immune system that leads to protracted bone healing. This study is funded by the Orthopaedic Research and Education Foundation (OREF) Aircast Research Grant (TAF-21-059).

Primary Objectives:

- To understand the normal temporal pattern of our novel bone regeneration biomarker in patients following bone fracture, osteotomy, spine and ankle fusions.
- To determine if markers of aging and inflammation are correlated with delayed bone regeneration.
- To develop personalized healing trajectories for patients following surgical procedures to promote bone repair.

CLINICAL TRIALS: COMPLETED ENROLLING

SENOLYTIC DRUGS ATTENUATE OSTEOARTHRITIS-RELATED ARTICULAR CARTILAGE DEGENERATION: A CLINICAL TRIAL

Principal Investigators: Johnny Huard, PhD and Thos A. Evans, MD

This is a prospective randomized controlled trial evaluating the therapeutic efficacy of Fisetin (a dietary supplement) for the treatment of knee osteoarthritis symptoms. This clinical trial will be conducted at The Steadman Clinic and Steadman Philippon Research Institute. This study is supported by The United States Department of Defense: Office of Naval Research.

For additional information, please visit www.clinicaltrials.gov and search for: NCT04210986.

Primary Objectives:

- Determine if oral Fisetin treatment reduces pro-inflammatory and cartilage degenerating systemic senescence-associate secretory phenotype markers in patients with moderate to severe knee osteoarthritis.
- Determine if Fisetin treatment results in greater improvements in functional performance and physical function compared to the placebo group.
- Evaluate, quantify and validate a non-invasive magnetic resonance imaging (MRI) methodology to measure cartilage and subchondral bone health compared to the placebo group.
- Determine if Fisetin treatment groups result in greater improvements in self-reported outcomes compared to the placebo group.

THE USE OF SENOLYTIC AND ANTI-FIBROTIC AGENTS TO IMPROVE THE BENEFICIAL EFFECT OF BONE MARROW STEM CELLS FOR OSTEOARTHRITIS

Principal Investigators: Johnny Huard, PhD, Marc J. Philippon, MD, Scott Tashman, PhD, Leslie Vidal, MD

This is a prospective, randomized, double-blind, active control clinical trial is proposed to evaluate the safety and efficacy of a senolytic agent (Fisetin) and an anti-fibrotic agent (Losartan), used independently and in combination, to improve beneficial effect demonstrated by the active control which is to be injection of autologous bone marrow aspirate concentrate (BMAC) into an osteoarthritic knee. This proposed clinical trial will add supportive data to the scientific evidence of two currently active clinical trials being conducted by The Steadman Clinic and Steadman Philippon Research Institute. The study is funded by the National Institutes of Health (NIH). For additional information, please visit www.clinicaltrials.gov and search for: NCT04815902.

Primary Objectives:

- To define in combination with injection of BMA concentrate into the osteoarthritic knee the safety and tolerability of Fisetin and Losartan, alone and both given in sequence, as compared to injection of BMA concentrate alone.
- To define in combination with injection of BMA concentrate into the osteoarthritic knee the effectiveness of Fisetin and Losartan, alone and both given in sequence, as compared with injection of BMA concentrate alone into the osteoarthritic knee, specifically evaluating:

1. Structural improvement of the injected joint
2. Effect on pain, function, and quality of life
3. Characterization of synovial fluid content
4. Characterization of CONCENTRATED BMA cell content prior to injection
5. Effect on local and systemic SASP and OA-associated biomarkers as compared to placebo
6. Functional performance, strength and movement as compared to placebo
7. Time to rescue treatment

THE STEADMAN CLINIC
SURGEONS AND PHYSICIANS

MARC J. PHILIPPON, MD
Managing Partner
Sports Medicine, Hip Disorders, Hip Arthroscopy
Chairman, Steadman Philippon Research Institute

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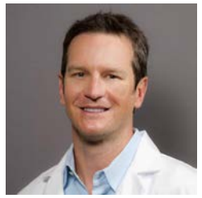
JOEL M. MATTA, MD
Hip Disorders: Preservation,
Replacement & Fractures



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JARED T. LEE, MD
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STUART D. KINSELLA, MD, MSTR
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Navigation-Assisted Surgery

WAQQAR KHAN-FAROOQI, MD
Foot, Ankle & Trauma Surgery

THE STEADMAN CLINIC **SPINE PROGRAM** WELCOMES TWO NEW SURGEONS

THE STEADMAN CLINIC EXPANDED ITS SPINE PROGRAM THIS YEAR, WELCOMING DR. SONNY GILL TO THE PRACTICE IN NOVEMBER 2022 AND DR. STUART KINSELLA IN MAY 2023. DR. GILL TREATS PATIENTS IN VAIL AND BASALT, AND DR. KINSELLA SERVES THE CLINIC'S ROARING FORK VALLEY PATIENTS IN BOTH ASPEN AND BASALT.

Now with three neck and back specialists—Dr. Michael Gallizzi joined The Steadman Clinic in March 2022—The Steadman Clinic has more spine surgeons than it has had in its history, serving more patients and offering broader treatment options for orthopaedic conditions of the spine. Both Drs. Gallizzi and Kinsella offer robotic treatments, utilizing the Globus ExcelsiusGPS—one located in Vail, and one in Aspen.

A former SPRI Fellow, **Dr. Gill** joined The Steadman Clinic from the Medical Group of the Carolinas, where he built a spine center of excellence. He specializes in complex and minimally invasive spine surgery, including athletic injuries of the spine through motion-preserving techniques. Dr. Gill was a fellow at SPRI from 2004–2005 and worked as a physician at The Steadman Clinic from 2008–2010, before moving his practice to South Carolina. Dr. Gill earned his MD at Boston University and completed his residency at the University of Virginia School of Medicine. He completed his spine fellowship at Emory University before joining SPRI for his sports medicine fellowship.

In April, Dr. Gill was named the Head Team Physician for the U.S. Freestyle Ski Team, joining Drs. Randy Viola, Tom Hackett and David Kuppersmith who serve as lead physicians for the U.S. Ski and Snowboard Team.

Dr. Kinsella joined The Steadman Clinic on May 1, 2023 from Vero Beach, Florida, where he worked in practice for 2.5 years. He has performed over 100 robotic surgeries, and is the first full-time spine specialist located in The Steadman Clinic, Aspen and Basalt. Dr. Kinsella obtained his medical degree from the University of Pennsylvania where he also earned a Master of Science in Translational Research (MSTR). He completed his internship and residency in the Harvard Combined Orthopaedic Residency Program and later completed his spine fellowship at the Harvard Medical School Combined Spine Fellowship.

BOTH DRS. GILL AND KINSELLA ARE COMMITTED TO THE PRACTICE OF EVIDENCE-BASED TREATMENT OF THE SPINE AND ARE LOOKING FORWARD TO EXPANDING SPRI'S RESEARCH INTO SPINE-SPECIFIC PROJECTS.

LINDA & MITCH HART CENTER FOR REGENERATIVE AND PERSONALIZED MEDICINE

FACULTY AND STAFF

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SUDHEER RAVURI, PHD
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PING GUO, PHD
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Program Director of Genetic and Cellular Engineering

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Post-Doctorate Scholar

ANNA LAURA NELSON, MS
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LUCAS MINAS
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DYLAN BOYES
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Vice President, Operations

LUZ THEDE, MD
Clinical Trials Clinician

SARA ROBINSON
Clinical Trials Data Coordinator

CHLOE BARTON
Clinical Trials Coordinator

THE LINDA & MITCH CENTER FOR REGENERATIVE AND PERSONALIZED MEDICINE (CRPM) was launched in 2015 as the Center for Regenerative Sports Medicine. In 2021, the team's name was broadened, acknowledging the array of science being conducted in the lab.

Scientists and researchers at CRPM are underway on several research projects, including SPRI's federally funded clinical trials, preclinical projects and research programs sponsored by philanthropy. CRPM is conducting research supported by the National Institutes of Health (NIH), Department of Defense (DoD), industry and foundation grants and awards. The team employs six full-time PhDs and three full-time MDs, five of whom are principal investigators and program directors for CRPM's research categories. The team is led by Johnny Huard, PhD, who also serves as SPRI's Chief Scientific Officer.

CRPM's scientific programs investigate cellular science and regenerative medicine techniques and therapies. Each program is led by a Principal Investigator, who is a subject matter expert in their program. These programs are:

- Fracture Repair and Regenerative Therapeutics
- Muscle Repair and Stem Cell Biology
- Bone and Cartilage Biology and Tissue Engineering
- Genetic and Cellular Engineering
- Stem Cell Engineering and Cartilage Regeneration
- Health Aging Diagnostics

The CRPM team is committed to conducting high-impact science and earns major funding awards each year. In addition to its federal funding and industry grants, CRPM receives significant philanthropic funding each year, which is used to launch vital pilot studies and research programs, many of which include major university collaborators. The findings made from CRPM's pilot studies are often used to develop major grant applications, including numerous trials awarded by federal agencies.

FEDERAL AND STATE FUNDING AWARDS

NATIONAL INSTITUTES OF HEALTH (NIH)

- **4** Primary Grants
- **3** Subaward Grants
- **1** Regenerative Medicine Innovation Project (RMIP) Clinical Trial

DEPARTMENT OF DEFENSE (DOD)

- **1** Contract: **3** Clinical Trials, **1** Clinical Study
- **1** Grant: **1** Clinical Trial

STATE OF COLORADO

- **1** Grant

SPRI's Clinical Trials Team Advances Clinical Translation

In 2019, SPRI received its first primary National Institutes of Health (NIH) Grant, and since that first award, the research team has steadily increased its number of awards, including clinical trials and studies supported by federal agencies. SPRI's Clinical Trials Team works collaboratively with SPRI and The Steadman Clinic to facilitate and support the ongoing clinical science efforts.

Co-located with CRPM, the team is led by SPRI's VP of Operations Suzanne Liv Page and includes Dr. Luz Thede, Clinical Trials Clinician; Sara Robinson, Clinical Trials Data Coordinator and Chloe Barton, Clinical Trials Coordinator.

CRPM WELCOMES TWO NEW RESEARCH SCIENTISTS

CRPM WELCOMED DRS. KIMI KATAOKA AND DUSTIN SNAPPER TO THE DEPARTMENT IN 2023. DR. KATAOKA IS A POST-DOCTORATE SCHOLAR, AND DR. SNAPPER IS A RESEARCH SCIENTIST.

KIMINARI KATAOKA, MD

Kiminari Kataoka, MD has almost 10 years of experience as an orthopaedic surgeon. Originally from Himeji, Japan, Dr. Kataoka earned his medical degree from Saga University School of Medicine in Japan. He is currently enrolled at Kobe University Graduate School of Medicine, studying the effects of micro-fragmented adipose tissue (MFAT) on articular cartilage regeneration. He also had experience working in sports medicine in Japan, working as a game physician for a professional soccer team. Dr. Kataoka joined SPRI in April of 2023 as a post-doctorate scholar and is working on cartilage regeneration using pluripotent stem cells.

Dr. Kataoka is currently working alongside CRPM's Dr. Naoki Nakayama on an NIH-funded R01 grant focusing on articular cartilage tissue engineering with human pluripotent stem cells.

DUSTIN SNAPPER, MD, MS

Dr. Dustin Snapper is a Research Scientist at CRPM. Dustin completed his BS at the University of Maryland, Baltimore County in the biological sciences with a minor in chemistry. He subsequently received his MD from West Virginia University School of Medicine and completed a general surgical internship at Charleston Area Medical Center. He went on to obtain a Master of Science degree in biomedical engineering at Case Western Reserve University.

Dr. Snapper has also completed two post-doctoral fellowships, one at the FDA in the Vascular Biology Laboratory and the second at the Uniformed Services University in the Department of Pharmacology. At the FDA, he studied the functional effects of nickel chloride and nickel-titanium (nitinol) nanoparticles, commonly used as a component of vascular stents and metal-on-metal hip implants, on cultured human endothelial cells and macrophages with relevance to biocompatibility. He also determined the in vivo effects of nickel chloride, nickel-titanium, and cobalt-alloy on angiogenesis within silicone bioreactors surgically implanted into mice.

At the Uniformed Services University, he conducted research on the effects of blast-induced traumatic brain injury. He utilized a novel bioengineered 3D brain-like tissue for identifying biomarkers of primary blast-induced traumatic brain injury. He also studied the role of angiotensin-(1-7) in mediating the radio-protective effects of captopril, and performed transcriptomic analysis of mouse brains after traumatic brain injury.

His research has been published in several peer-reviewed journals and has been presented in a number of scientific conferences.

HONORS AND AWARDS:

- 1st place Post-Doctoral Fellow Poster Award, National Capital Area TBI Research Symposium, 2023
- Post-Doctoral Outstanding Poster Award, USU Research Days, 2021
- FDA Group Recognition Award, Essure Stent Post-Market Safety Review Team, 2017
- Case Western Reserve University Scholarship, 2015–2017
- Global Health Travel Award, West Virginia University School of Medicine, 2012–2013
- Mountaineer Medical Scholarship, West Virginia University, 2012–2013

DR. JOHNNY HUARD, CHIEF SCIENTIFIC OFFICER

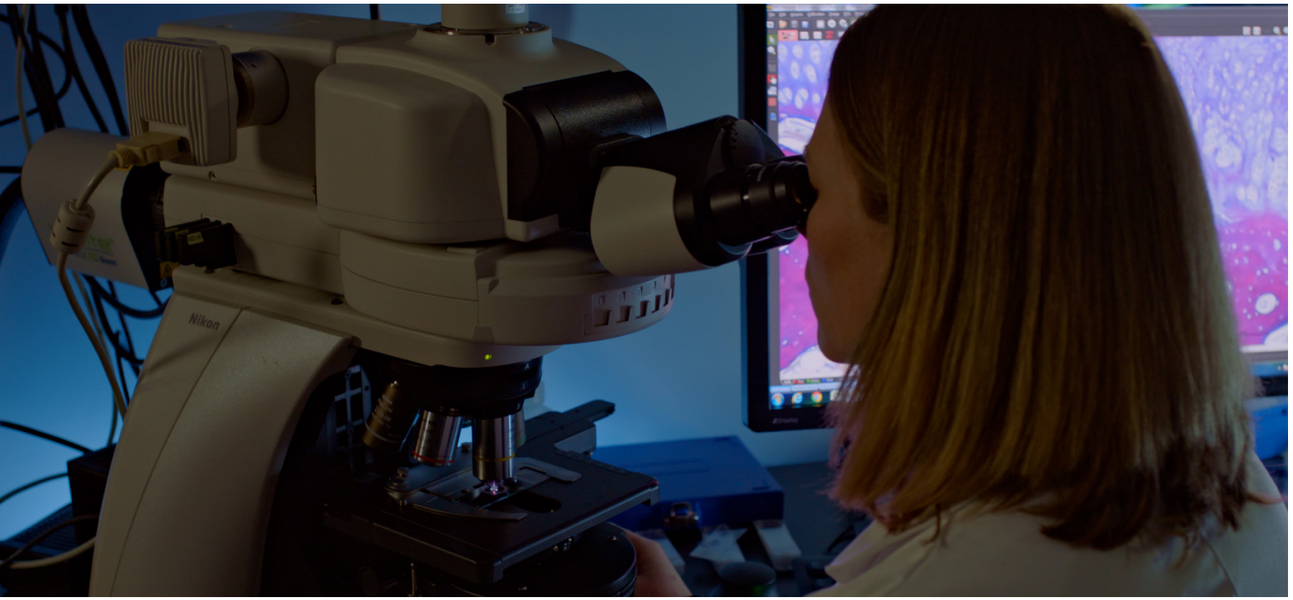
Dr. Johnny Huard is the Director of CRPM and SPRI's Chief Scientific Officer. He is a Principal Investigator on five National Institutes of Health (NIH) grants and four Department of Defense (DoD) grants at SPRI and has been successful in bringing in philanthropic funding to support SPRI's regenerative medicine program. Philanthropic gifts support research projects that fill the pipeline of federally funded grants and awards.

Dr. Huard has extensive knowledge in the areas of gene therapy, tissue engineering and regenerative medicine applications based on the use of muscle-derived stem/progenitor cells (MDSCs). Dr. Huard and his team have published over 430 peer-reviewed papers, over 90 reviews/book chapters, and have had over 900 abstracts accepted for presentation at national and international conferences (Citations 46990, h-index: 113, i10-index: 379). Dr. Huard and his team's current major research interests include:

- Muscle stem cells isolation and characterization
- Bone and articular cartilage regeneration and repair
- Senotherapeutics in musculoskeletal repair, cardiac and skeletal muscle injury repair, regeneration and fibrosis prevention
- Peripheral nerve regeneration
- Healthy aging and the use of adult stem cells as a source for paracrine factors to alleviate the phenotypic changes associated with natural and accelerated aging

The focus of Dr. Huard's laboratory is to develop biological medicine approaches to improve musculoskeletal tissue repair after injury, disease and aging. His team of scientists uses a variety of technologies that fall into four different categories including:

- **BIOLOGICS**
  - Adult stem cells, including muscle-derived stem cells, adipose-derived stem cells
  - Bone Marrow Cell Concentrate
  - Platelet-Rich Plasma
- **REGENERATIVE MEDICINE APPROACHES**
  - Gene therapy approaches
  - CRISPR-Cas9
  - Protein delivery like coacervate, microspheres, PA-nanofibers and magnetic nanoparticles
- **THERAPEUTICS**
  - FDA-approved drugs such as anti-fibrotic agents and pro-angiogenic agents
  - Telomerase activity (hTERT)
  - Senotherapeutics like senolytic and senomorphic drugs
- **ANIMAL MODELING**
  - Dystrophic and progeria mice models
  - Super healer mice (MRL/MpJ)
  - Osteoarthritis model/microfracture
  - Tibia fracture
  - Calvarial defect
  - Ovariectomy



CRPM SCIENTISTS SHARE PROGRAM UPDATES

CRPM's scientists work collaboratively across their programs to support the efforts of SPRI's clinical trials, the department's grants and philanthropically funded studies. Each scientist reviewed their past year of research (July 2022–June 2023) for inclusion in this edition of the *Orthopaedic Research Journal*.

CHELSEA S. BAHNEY, PHD – PRINCIPAL INVESTIGATOR

Dr. Chelsea Bahney is the Program Director of Fracture Repair and Regenerative Therapeutics in CRPM. Dr. Bahney is an NIH-funded scientist and serves in leadership roles on several scientific societies including the International Society of Fracture Repair, Orthopaedic Research Society, the Tissue Engineering and Regenerative Medicine International Society, the Orthopaedic Trauma Association, and the AO R&D Commission.

HIGHLIGHTED NEW PUBLICATIONS

Dr. Bahney has over 50 peer-reviewed publications, with 14 new articles in the last year. Google Scholar reports almost 2400 citations, an h-index of 23 and an i10-index of 31. Featured articles from the last year include:

1. Rivera KO, Cuylear DL, Duke V, O'Hara KM, Kharbikar BN, Kryger AN, Miclau T, **Bahney CS**, Desai TA. *Localized delivery of β-NGF via injectable microrods accelerates endochondral fracture repair*. Frontiers in Bioengineering & Biotechnology. 22 May 2023.
2. Fok SW; Gresham RCH; Ryan W; Osipov B; **Bahney C**, Leach JK. *Macromolecular crowding and decellularization techniques increase the growth factor binding potential of cell-secreted extracellular matrices.* Frontiers in Biomaterials and Biotechnology. Volume 11, 06 February 2023.
3. Miclau K; Hambright WS; Huard J; Stoddart, M; **Bahney CS**. *Cellular Expansion of MSCs: Shifting the Regenerative Potential.* Aging Cell. 19 Dec 2022.
4. Marcucio RS, Miclau T 3rd, **Bahney CS**. *A Shifting Paradigm: Transformation of Cartilage to Bone during Bone Repair.* J Dent Res. 27 Oct 2022.
5. Nelson AL; Fontana G; Miclau E; Rongstand M; Murphy W; Huard J; Ehrhart NE; **Bahney CS.** *Therapeutic approaches to activate the canonical Wnt pathway for bone regeneration.* Journal of Tissue Engineering and Regenerative Medicine. 16 Sept 2022.

PRESENTATIONS

In the last year, Dr. Bahney had 15 abstracts accepted for podium or presentation at national or international conferences, was an invited speaker at multiple national venues and served as a conference program organizer/ moderator. She would like to highlight the following invited seminars:

- **ORS Musculoskeletal Biology Workshop.** Park City, UT. August 2022. "Towards Personalized Orthobiologics: What's New and What's Still Unknown?"
- **Extremity War Injuries XVI.** September 2022, Washington DC. "Orthobiologics: From the Bench to Clinical Practice."
- **ORS Program Organizer** Feb 2023, Dallas TX. "Unmet Clinical Needs in Sports Medicine."
- **ORS Moderator Feb 2023,** Dallas TX. "Business Plan Competition"
- **International San Francisco Orthopaedic Trauma Course.** April 2023. San Francisco, CA. *Bone Healing: Are there any growth Factors on the horizon? (Invited Speaker)*
- **International San Francisco Orthopaedic Trauma Course.** April 2023. San Francisco, CA. *Cell-based therapies - how are they being optimized? (Invited Speaker)*

AWARDS

- President International Society of Fracture Repair
- NIH Study Section

GRANT FUNDING

Dr. Bahney's research is funded by two independent NIH R01s, one NIH supplement, three foundation grants, and philanthropy. She also manages the CRPM efforts on the DOD- and NIH-funded clinical trials.

YEAR IN REVIEW

In 2022–2023 Dr. Bahney was honored to serve as President of the International Society of Fracture Repair and Chair the 17th Biennial Conference in Edinburgh Scotland. The meeting was part of the World Orthopaedic Research Congress 30-Year Anniversary and the theme of this meeting was *Transdisciplinary Science to Solve Unmet Needs in Fracture Repair*. As President, Dr. Bahney organized and chaired the two-day meeting and obtained NIH funding to increase student participation in the event. In addition to featuring Dr. Bahney as President, Drs. Philippon and Huard were invited keynote speakers and Dr. Bahney's Program of Fracture Repair and Regenerative Therapeutics supported four student abstracts/presentations. In addition to this meeting, our team had strong national representation at the Orthopaedic Research Society, the Extremity War Injuries, and Orthopaedic Trauma conferences with three students winning awards for their presentations.

LOOKING AHEAD

In the upcoming year, the Program of Fracture Repair and Regenerative Therapeutics looks forward to advancing our foundational research program that aims to test and develop novel strategies to accelerate fracture repair. This basic science research program is complemented by an active clinical trial investigating biomarkers of bone repair and immunosenescence with over 250 enrolled patients at our site and another 100 patients from a secondary site at Oregon Health and Science University (OHSU). This year we have also begun a new research initiative to both understand pain associated with fracture repair and develop novel non-opioid strategies to reduce pain. This research focus is complementary to our current funding and relates to the critical NIH initiative "Helping to End Addiction Long-term® Initiative" (NIH HEAL).

XUEQIN GAO, MD, PHD – PRINCIPAL INVESTIGATOR

Dr. Xueqin Gao is the Program Director of Bone and Cartilage Biology and Tissue engineering at SPRI's CRPM. Over the past year, Dr. Gao continued to work on her basic research projects and made significant contributions to three DOD- and one NIH-funded clinical trials.

PUBLICATIONS

Below is a selection of five of Dr. Gao's publications from this year. *# Co-first authors, * Corresponding authors*

1. **Gao X,** Hwang MP, Wright N, Lu A, Ruzbarsky JJ, Huard M, Cheng H, Mullen M, Ravuri S, Wang B, Wang Y, Huard J. The use of heparin/polycation coacervate sustain release system to compare the bone regenerative potentials of 5 BMPs using a critical sized calvarial bone defect model. **Biomaterials.** 2022 Sep;288:121708. PMID: 36031459, **Impact factor 15.304**

2. **Gao X*,** Sun X, Cheng H, Ruzbarsky JJ, Mullen M, Huard M, Huard J*. MRL/MpJ Mice Resist to Age-Related and Long-Term Ovariectomy-Induced Bone Loss: Implications for Bone Regeneration and Repair. **Int J Mol Sci**. 2023 Jan 25;24(3):2396. PMID: 36768718, **Impact factor 6.208**

3. Cheng H, **Gao X***, Huard M, Lu A, Ruzbarsky JJ, Amra S, Wang B, Huard J*. Bone morphogenetic protein 4 rescues the bone regenerative potential of old muscle-derived stem cells via regulation of cell cycle inhibitors**. Stem Cell Res Ther.** 2022 Jul 30;13(1):385. PMID: 35907860, **Impact factor 8.08**

4. Zhang L, Hajebrahimi S, Tong S, **Gao X**, Cheng H, Zhang Q, Hinojosa DT, Jiang K, Hong L, Huard J, Bao G. Force-Mediated Endocytosis of Iron Oxide Nanoparticles for Magnetic Targeting of Stem Cells. **ACS Appl Mater Interfaces**. 2023 May 5. Online ahead of print. PMID: 37145890, **Impact factor, 10.383**

5. Lacheta L, **Gao X**, Miles JW, Murata Y, Fukase N, Utsunomiya H, Dornan G, Tashman S, Kashyap R, Altintas B, Ravuri S, Philippon M, Huard J, Millett PJ. Losartan in Combination with Bone Marrow Stimulation Demonstrated Synergistic Effects on Load to Failure and Tendon Matrix Organization in a Rabbit Model. **Arthroscopy**. 2023 Jun 1: S0749-8063(23)00423-1. Online ahead of print. PMID: 37270113, **Impact factor 5.973**

ABSTRACTS AND CONFERENCES PRESENTATIONS:

Dr. Gao had a total of ten presentations in the past year, including six first author presentations, and two co-first author presentations. The following are five of her first author presentations. *# Co-first authors, * Corresponding authors*

1. **Xueqin Gao**, Mintai Hwang, Aiping Lu, Matthieu Huard, Joseph J Ruzbarsky, Yadong Wang*, Johnny Huard,* Senescent cells accumulation during human muscle-derived stem cells expansion negatively affects BMP2 mediated bone regeneration. ASGCT 2023 Annual Meeting, May 16-21, 2023, Los Angeles, CA, **Poster Presentation.**

2. **Xueqin Gao***; Joseph J. Ruzbarsky; Charles Huard; Aiping Lu; Sarah White; Jessica Ayers; Bing Wang; Johnny Huard*. Targeting Muscle Cell Senescence In Dystrophin-/-/utrophin-/-Double Knockout Mice Improves Muscle Histopathology And Enhances Bone Mass And Lifespan. ORS 2023, Feb 10-14, 2023, Dallas, TX, **Podium Presentation**.

3. **Xueqin Gao***; Joseph J. Ruzbarsky; Matthieu Huard; Sudheer Ravuri; Ping Guo; Laura Chubb; Nicole Ehrhart; Bing Wang; Johnny Huard*. The Role Of Sclerostin In Bone Osteopenia Of Muscular Dystrophy. ORS 2023, Feb 10-14, 2023, Dallas, TX, **Poster Presentation**.

4. **Xueqin Gao,** Mintai Hwang, Matthieu Huard, Aiping Lu, Joseph J Ruzbarsky, Sudheer Ravuri, Yadong Wang*, Johnny Huard*. The Use of Coacervate Sustained Release System to Identify the Most Potent BMP for Bone Regeneration. AOSSM, 2022, July 13-17, 2022, Colorado Springs, CO, **Poster Presentation.**

5. Ingrid K. Stake#; **Xueqin Gao**#*; Matthieu Huard; Naomasa Fukase; Sudheer Ravuri; Marc Philippon; Thomas O. Clanton; Johnny Huard*. Effects Of Losartan And Fisetin On Microfracture Mediated Cartilage Repair Of Ankle Cartilage In A Rabbit Model. ORS 2023, Feb 10-14, 2023, Dallas, TX, **Poster Presentation**.

KEY ACHIEVEMENTS

Dr. Gao's research program had a productive year with many publications in high-impact journals and conference presentations. Dr. Gao has made significant contributions to SPRI clinical trials by running all markers for ELISAs. Dr. Gao contributed to the research that was awarded the ON Foundation/AOSSM Orthoregeneration Research Award, presented in July 2022.

Dr. Gao provided significant support to SPRI's federally funded grants, including the NIH Regenerative Medicine Innovation Project (RMIP), three DoD clinical trials, and NIH R21 and R01 grants. In addition to the federal grants, Dr. Gao provided significant support of three philanthropically funded projects.

Dr. Gao applied for four NIH awards as co-principal investigator, one as co-investigator, and a foundation award as co-investigator.

LOOKING AHEAD

Dr. Gao will continue to apply for federal grants by submitting new grants or revised grants from the NIH or DOD. She will continue leading philanthropically funded projects under Dr. Huard's direction and supporting SPRI's federally funded clinical trials and projects.

PING GUO, PHD – PRINCIPAL INVESTIGATOR

Dr. Ping Guo is the Program Director of Genetic and Cellular Engineering at SPRI's CRPM. For the past year, Dr. Guo has focused his research on osteoarthritis and diabetes-related musculoskeletal diseases.

PUBLICATIONS

1. Guo P, Zhang T, Lu A, Shiota C, Huard M, Whitney KE, Huard J. "Specific reprogramming of alpha cells to insulin-producing cells by short glucagon promoter-driven Pdx1 and MafA" *Mol Ther Methods Clin Dev*, 2023; Feb 11;28:355-365.

2. Hambright WS, Mu X, Gao X, **Guo P**, Kawakami Y, Mitchell J, Mullen M, Nelson AL, Bahney C, Nishimura H, Hellwinkel J, Eck A, Huard J. "The Senolytic Drug Fisetin Attenuates Bone Degeneration in the Zmpste24 -/- Progeria Mouse Model" *J Osteoporos,* 2023; Feb 22;2023:5572754.

3. Lu A, Tseng C, **Guo P**, Gao Z, Whitney KE, Kolonin MG, Huard J." The role of the aging microenvironment on the fate of PDGFRβ lineage cells in skeletal muscle repair" Stem Cell Res Ther, 2023; Aug 5;13(1):405.

PRESENTATIONS

- Slowly Release TIPE2 Is Capable to Attenuate OA by Reducing Cellular Senescence in A Progeria Mouse Model, **Poster presentation: ORS 2023**

AWARDS

- NIH R01 AR077045-01A1(PI: Dr. Nakayama), 04/1/2021-3/31/2026, **Articular Cartilage Tissue Engineering with Human Pluripotent Stem Cells**
- NIH 1R21AR079075-01 (PI: Dr. Huard)**,** 03/12/2022-01/31/2024, **SMART stem cells that autonomously down-modulate TFG-β signaling for Articular Cartilage Repair**

KEY RESEARCH UPDATES

1. Primary focus on TIPE2 gene therapy for OA using Z24 mouse model. There is a new finding in the Z24 mice knee that senescent cells were found in the five-month-old cartilage and TIPE2 gene-therapy-reduced senescent cells in the knee of Z24 mice. Collected data, wrote a grant application and a manuscript has been written.
2. Dr. Guo submitted three NIH grants as a co-investigator.
3. Worked on NIH- and DoD-funded clinical trial projects. Mainly performed multiplex assay for patient samples.
4. Involved in other PI's projects, performed *in vitro* and *in vivo* experiments.

LOOKING AHEAD

Dr. Guo plans to work on osteoarthritis and therapy, performing excellent research in stem cell therapies for skeletomuscular diseases. Dr. Guo will continue work on the NIH-funded project SMART stem cells that autonomously down-modulate TFG-β signaling for Articular Cartilage Repair (1R21AR079075) and Articular Cartilage Tissue Engineering with Human Pluripotent Stem Cells (NIH 5R01AR077045). Dr. Guo is revising and will resubmit the R01 grant submitted 10-16-2023.

AIPING LU, MD – PRINCIPAL INVESTIGATOR

Dr. Aiping Lu is the Program Director of Muscle Repair and Stem Cell Biology at SPRI's CRPM.

PUBLICATIONS

1. Ping Guo, Ting Zhang, **Aiping Lu**, Chiyo Shiota, Matthieu Huard, Kaitlyn E Whitney, Johnny Huard. Specific reprogramming of alpha cells to insulin-producing cells by short glucagon promoter-driven Pdx1 and MafA. Mol Ther Methods Clin Dev . 2023 Feb 11;28:355-365
2. Chathurika Henpita, Rajesh Vyas, Chastity L Healy, Tra L Kieu, Aditi U Gurkar, Matthew J Yousefzadeh, Yuxiang Cui, **Aiping Lu**, Luise A Angelini, Ryan D O'Kelly, Sara J McGowan, Sanjay Chandrasekhar, Rebecca R Vanderpool, Danielle Hennessy-Wack, Mark A Ross, Timothy N Bachman, Charles McTiernan, Smitha P S Pillai, Warren Ladiges, Mitra Lavasani, Johnny Huard, Donna Beer-Stolz, Claudette M St Croix, Simon C Watkins, Paul D Robbins, Ana L Mora, Eric E Kelley, Yinsheng Wang, Timothy D O'Connell, Laura J Niedernhofer. Loss of DNA repair mechanisms in cardiac myocytes induce dilated cardiomyopathy. Aging Cell . 2023 Apr;22(4):e13782.
3. Xueqin Gao, Mintai P Hwang, Nathaniel Wright, **Aiping Lu**, Joseph J Ruzbarsky, Matthieu Huard, Haizi Cheng, Michael Mullen, Sudheer Ravuri, Bing Wang, Yadong Wang, Johnny Huard. The use of heparin/polycation coacervate sustain release system to compare the bone regenerative potentials of 5 BMPs using a critical sized calvarial bone defect model. Biomaterials. 2022 Sep;288:121708.
4. **Aiping Lu**, Chieh Tseng, Ping Guo, Zhanguo Gao, Kaitlyn E Whitney, Mikhail G Kolonin, Johnny Huard. The role of the aging microenvironment on the fate of PDGFRβ lineage cells in skeletal muscle repair. Stem Cell Res Ther. 2022 Aug 5;13(1):405.
5. Haizi Cheng, Xueqin Gao, Matthieu Huard, **Aiping Lu**, Joseph J Ruzbarsky, Sara Amra, Bing Wang, Johnny Huard. Bone morphogenetic protein 4 rescues the bone regenerative potential of old muscle-derived stem cells via regulation of cell cycle inhibitors. Stem Cell Res Ther. 2022 Jul 30;13(1):385.

ABSTRACTS

1. **Aiping Lu**, Katie Sikes, Ping Guo, Matthieu Huard, Kelly Santangelo, Scott Tashman, Vihang A. Narkar, Johnny Huard. Skeletal Muscle Atrophy After ACL Rupture Can Be Mitigated By ERRγ Overexpression In Skeletal Muscle. ORS Annual Meeting 2023. Accepted for poster presentation**.**
2. Xueqin Gao, Joseph J Ruzbarsky, Charles Huard, **Aiping Lu**, Sarah White, Jessica Ayers, Bing Wang, Johnny Huard. Targeting Muscle Cell Senescence in Dystrophin-/-/Utrophin-/-Double Knockout Mice Improves Muscle Histopathology andEnhances Bone Mass and Lifespan. ORS Annual Meeting 2023. Accepted for podium presentation.
3. Chelsea S. Bahney, Adam D. Goff, Victoria R. Duke, Kelsey O'Hara, Alex W. Goff, Charles Huard, Xueqin Gao, Ping Guo, **Aiping Lu**, Suzanne Page, Scott Tashman, William S. Hambright, Thos Evans, Marc J. Philippon, Johnny Huard. Clinical Assessment of Senescence Cell Burden and Correlation to Severity of Osteoarthritis. ORS Annual Meeting 2023. Accepted for poster presentation.
4. Ping Guo, Xueqin Gao, Matthieu Huard, **Aiping Lu**, and Yadong Wang, Johnny Huard. Slowly Release TIPE2 Is Capable to Attenuate OA by Reducing Cellular Senescence in A Progeria Mouse Model. ORS Annual Meeting 2023. Accepted for poster presentation.
5. Xueqin Gao, Mintai Hwang, **Aiping Lu**, Matthieu Huard, Joseph J Ruzbarsky, Yadong Wang, Johnny Huard. Senescent cells accumulation during human muscle-derived stem cells expansion negatively affects BMP2 mediated bone regeneration. ASGCT 2023 Annual Meeting. Accepted for poster presentation.

GRANT SUBMISSIONS:

Dr. Lu submitted four grants to the NIH this year as co-investigator.

CURRENT RESEARCH PROJECTS

Project #1: Development of biological approaches to enhance skeletal muscle rehabilitation after anterior cruciate ligament injury (NIH R21AR075997-01). This grant will potentially reveal the cellular and molecular mechanism linking muscle weakness, MPCs depletion/FAPs activation with muscle vascularity after ACL injury and could consequently lead to the development of more effective novel therapeutic approaches and rehabilitation strategies for delaying/reversing muscle weakness after ACL injury.

Project #2: Reducing Fatty Infiltration and Fibrosis after Rotator Cuff Injury to Preserve Musculoskeletal Function (3030 Salah Study of RC Disease). Rotator cuff (RC) pathology is a common, age-related degenerative musculoskeletal disorder, leading to pain and dysfunction that is exacerbated by degenerative changes in the RC muscles, including atrophy, fatty infiltration, and fibrosis, that typically cannot be reversed by exercise alone. We propose to investigate a new therapeutic pathway for preserving or improving muscle function by increasing blood vessels (angiogenesis), using a unique mouse model. The results of this study could lead to the development of drugs that can preserve muscle and improve outcomes after rotator cuff injury as well as facilitating more effective surgical repairs.

Project #3: RMIP clinical trial. A clinical trial is proposed to evaluate the safety and efficacy of a senolytic agent (Fisetin) and an anti-fibrotic agent (Losartan), used independently or in combination, to improve the beneficial effect of one marrow derived stem cells (derived from bone marrow aspirate concentrate) for improving outcomes in patients with knee osteoarthritis. These proposed

clinical trials will build upon a currently active clinical trial on orthobiologics at TSC/SPRI for OA treatment at SPRI utilizing the same patient population and outcomes assessments to effectively provide a 6-arm, comprehensive assessment of biological therapies for improving treatment of osteoarthritis.

Project #4: Development of approaches to optimize stem cell banking. The goal of this study is to develop a BMAC harvesting, banking and delivery service that facilitates multiple injections from a single bone marrow harvest without expansion or significant manipulation of the cells.

Project #5: Can Aged FAPs be Utilized to Promote Muscle Regeneration Through Improved Mitochondrial Biogenesis? This study is a mechanistic evaluation of how ß-adrenergic stimulation of intramuscular fibroadipoprogenitors (FAPs) can amplify the endogenous process of mitochondrial biogenesis and activity to improve myoblast differentiation and muscle function in the setting of aging.

Project #6: Development of Biological Approaches to Alleviate Aging-Related muscle sarcopenia via Skeletal Muscle Specific Over-Expression of Estrogen-Related Receptor Gamma. Muscle health declines during aging. Strategies to delay muscle aging could improve health span with aging. We are using a unique transgenic mouse model, with enhanced muscle vascularity and function, to demonstrate that preserving muscle vascularity and mass during natural and accelerated aging, will significantly delay age-related musculoskeletal diseases, preserving musculoskeletal tissue regeneration and repair during the aging process.

NAOKI NAKAYAMA, PHD – PRINCIPAL INVESTIGATOR

Dr. Naoki Nakayama is the Program Director of Stem Cell Engineering and Cartilage Regeneration in SPRI's CRPM.

PUBLICATIONS

- Pothiawala, A., Sahbazoglu, B.E., Ang, B.K., Matthias, N., Pei, G., Yang, Q., Davis, B.R., Huard, J., Zhao, Z., and **Nakayama, N.** (2022) "GDF5^{+} chondroprogenitors derived from human pluripotent stem cells preferentially form permanent chondrocytes" *Development 149 (11).*

ABSTRACTS

- Minas, L., Easton, S.M., Huard, J., and **Nakayama, N.** (2023) "Signaling Mechanisms for Generating Permanent Cartilage Forming Activity from Human Pluripotent Stem Cell-derived Mesodermal Cells: Implication of Activin A and Interleukin-6 family cytokines" **The 75th ORS Annual Meeting Abstract** 0126.

ORAL PRESENTATIONS

1. **Stem Cell Interest Group, the 75th Orthopaedic Research Society (ORS) Annual Meeting**, Hilton Anatole, Dallas, TX "In vitro embryology using pluripotent stem cells for cartilage tissue engineering" (February 2023).
2. **The 7th Vail Scientific Summit**, Steadman Philippon Research Institute, Vail, CO "Cellular rejuvenation using the iPS technology for cartilage repair" (August 2022)

RESEARCH SUPPORT:

1. **R21AR079075**, Nakayama, N. (mPI), (Huard, J. cPI, Guilak, F. mPI) 3/01/22-2/29/24. NIAMS, National Institutes of Health (PA20-195) "SMART Stem Cells that Autonomously Down-modulate TGF-β signaling for Articular Cartilage Repair"

This project aims to test the novel technical concept of autonomous inhibition of extracellular (i.e., TGF-β) signaling for cartilage repair.

2. **R01AR077045**, Nakayama, N. (PI) 04/01/21-03/31/26. NIAMS, National Institutes of Health (PA19-056) "Articular Cartilage Tissue Engineering with Human Pluripotent Stem Cells"

This study seeks to develop and characterize the GDF5^{+} permanent cartilage-forming cells from human pluripotent stem cells.

ACHIEVEMENTS

1. Dr. Nakayama's major research activity was funded by NIH (R01) as PI (Third period, approved 2021, applied earlier in 2020).
2. Dr. Nakayama's second NIH grant (R21) was also funded by NIH as a co-PI (Second period, approved 2022 applied June 2021).
3. Dr. Nakayama was invited to give a talk at the 2022 VSS and 2023 ORS Annual Meeting.
4. Dr. Nakayama has initiated a Stem Cell Interest Group in ORS, as co-organizer.
5. Dr. Nakayama became an Associate Editor of the journal *Frontiers in Bioengineering and Biotechnology.*

KEY RESEARCH/LAB UPDATES

The key outcomes of this year's efforts are to start Dr. Nakayama's own group in a new place (we moved from Fort Collins to Vail in July 2022) and then to make progresses in both R01 and R21 projects. With the R01, Dr. Nakayama discovered key signaling mechanisms leading to GDF5^{+} "articular cartilage forming cells" from mesodermal progeny of human pluripotent stem cells (PSCs). He also acquired a postdoctoral fellow in April 2023 from Kobe University, Japan, and has been training him for pursuing and expanding the R01 project. With the R21, Dr. Nakayama has introduced the fibrosis-preventing Smart Cell construct into mouse PSCs and is currently characterizing mesenchymal stromal cells (MSCs) derived from them. He also had a new research intern in May 2023 from U. Wisconsin and has been training him for pursuing the R21 project. SPRI also acquired a Cell Sorter in March 2023. Dr. Nakayama trained Mr. Lucas Minas to become a specialist in FACS (Cytek Northern Light Spectral Flow cytometer) and Cell sorting (Sony MA900).

LOOKING AHEAD:

This coming year, Dr. Nakayama's group will focus on making progress on his R01 and R21 grant projects. Dr. Nakayama is also interested in initiating a tissue (especially lymphoid tissue) rejuvenation project using epigenetic drugs with Mr. Minas. Dr. Nakayama plans to submit another research grant with Dr. Huard on this project. Time has also come for Dr. Nakayama to plan and strategize renewal of his R01 grant. For this purpose, he will focus mainly on pushing the discovery of signaling mechanisms for generating GDF5^{+} articular-like cartilage forming cells from mesodermal progeny of human PSCs to publication.

SUDHEER RAVURI, PHD – DEPUTY DIRECTOR, CRPM

Dr. Sudheer Ravuri works closely with Director and Chief Scientific Officer Dr. Johnny Huard and leads and manages multi-disciplinary research projects at CRPM, involving basic science studies and clinical translation.

Dr. Ravuri's main areas of research focus each year are the study of adipose-derived stem cell biology and applications, biomaterials, characterization of orthobiologics, senotherapeutics and technology development with implications for healthy aging and active living.

Dr. Ravuri is an investigator on NIH, DoD, state and philanthropy-funded research projects and is actively involved in translational studies under Dr. Huard's supervision. He is also serving as reviewer and editorial board member of several research journals, member of research societies, member of leadership committees, and co-chair of research conferences.

Dr. Ravuri has played a significant role in expanding the CRPM laboratory to different locations in Colorado, including leading the team's move from Fort Collins to Vail and into the new regenerative medicine laboratory in Basalt, Colorado.

CRPM 2023 ORTHOPAEDIC RESEARCH SOCIETY (ORS) ABSTRACTS

Signaling Mechanisms for Generating Permanent Cartilage-Forming Activity from Human Pluripotent Stem Cell-derived Mesodermal Cells: Implication of Activin A and Interleukin-6 family cytokines

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Disclosures: Johnny Huard (Leadership for ORS; Cook Myosite).

INTRODUCTION: Chondrogenesis from human pluripotent stem cell (hPSC)-derived mesodermal cells is considered to be a human model of cartilage development. The mesodermal cells are expandable in the presence of inhibitor of the receptors for fibroblast growth factor (FGF) and transforming growth factor-beta (TGF-β), SB431542. The types of cartilage the cells preferentially generate have been characterized *in vitro* and *in vivo.* Resistance to endochondral ossification is one of the most important characteristics of tissue engineered cartilage for use in cartilage regenerative therapy. Although directed derivation of endochondral ossification-resistant chondrocytes from hPSCs has been reported, when the precursor chondrogenic mesenchymal cells expressing SOX9 protein and mRNA ($SOX9^+$) are expanded to large numbers in culture, the resulting cartilage pellets tend to express hypertrophic chondrocyte markers *in vitro* and become fully mineralized (i.e., proceed to the endochondral ossification process) after transplantation, yielding bony tissue. We have recently reported successful derivation of novel *GDF5*-gene expressing ($GDF5^+$), but $SOX9^{lo}$, chondrogenic mesenchymal cells from hPSC-derived $SOX9^+$ chondrogenic mesodermal cells, which seemed committed to the generation of *in vivo* endochondral ossification-resistant (permanent) cartilage [1]. Interestingly, transcriptome analyses revealed that the same cells seemed to display expression of tendon/ligament progenitor genes, such as *SCX, MKX,* and *TNMD.* Therefore, we studied whether heterogeneity in the $GDF5^+$ cell population (e.g., a mixture of SCX^+ and SCX^- cells that do not necessarily express *GDF5*) or generation of homogeneous $GDF5^+SCX^+$ cells is required for the formation of permanent cartilage. Our approach was to establish methods for the preferential specification of SCX^- cells or SCX^+ cells during differentiation of $SOX9^+$ mesodermal cells to $GDF5^+$ cells, which would enable comparison of the chondrogenic activities of the resulting cells.

METHODS: Human PSC lines were differentiated toward paraxial mesodermal progeny in a chemically-defined medium (CDM) as previously described [1-3], and the mesodermal fraction was isolated by cell sorting [1, 4]. The isolated mesodermal cells were then cultured in CDM containing FGF2, SB431542 and CHIR99021 (canonical WNT signal activator) [1] to generate $SOX9^+$ mesenchymal cells with fibroblastic morphology. To generate $GDF5^+$ mesenchymal cells (with round morphology), the cells were shifted to CDM supplemented with platelet-derived growth factor (PDGF), and Noggin (an inhibitor of bone morphogenetic protein [BMP]) (**PN** medium) [1]. Based on our previous RNA-seq data [1], we tested the effects of secreted factors whose genes are differentially expressed between $GDF5^+$ cells and $SOX9^+$ cells on the differentiation capacity and growth properties of the two cell types. Endochondral ossification-resistant (i.e., $COL2A1^+COL10A^{lo}$) cartilage formation from $GDF5^+$ cells was demonstrated by 3-dimensional chondrogenesis culture using PDGF, TGF-β3 and BMPs as described previously [1-3]. The resulting cartilage pellets were subjected to RT-PCR analyses.

RESULTS: As reported previously [1], $GDF5^+$ cells developed in PN medium expressed *SCX* (somewhat more weakly than previously reported). Accordingly, they are referred to herein as $GDF5^+SCX^{lo}$). The cells gave rise to cartilage pellets that expressed *COL10A1* at 1-10% of the level, and *PRG4* at a higher level (data not shown) than that derived from expanded $SOX9^+$ cells *in vitro* (Fig. 2). Among the factors tested during genesis of the $GDF5^+$ cells from $SOX9^+$ cells in PN medium, Activin A strongly induced *SCX* expression and round morphology, but slightly suppressed *GDF5* expression (+A, Fig. 1), leading to $GDF5^{+/lo}SCX^{hi}$ cells. In contrast, the addition of Interleukin (IL)-11, Leukemia inhibitory factor and/or Oncostatin M (OSM) (IL-6 family cytokines) strongly induced *GDF5* expression, but had little effect on *SCX* levels (+OSM, Fig. 1) and resulted in $GDF5^{hi}SCX^{lo}$ cells. Cartilage pellet analyses revealed that Activin A- or OSM-treated $GDF5^+$ cells generate chondrocytes that express increased levels of *COL10A1* (Fig. 2). Co-treatment with Activin A and OSM resulted in $GDF5^{hi}SCX^+$ cells (+A+OSM, Fig. 1) that gave rise to chondrocytes expressing even higher levels of *COL10A1* (Fig. 2). Interestingly, however, treatment with OSM followed by Activin A (+OSM→A, Fig. 1) generated $GDF5^+SCX^{lo}$ cells that produced $COL2A1^+COL10A^{lo}$ permanent-like cartilage resembling that of the original $GDF5^+$ cells (GDF5+ cell genesis, +OSM→A, Fig. 2).

DISCUSSION: Our results suggest that Activin A and IL-6 family cytokines, whose genes are highly expressed in $GDF5^+$ cells, are functional, and sequential action of the two is important for developed $GDF5^+$ cells to gain/maintain the capacity to preferentially generate permanent-like cartilage *in vitro*. To obtain evidence for the suggestion, we are currently determining the time-dependent expression of the Activin A (*INHBA*) and IL-6 family cytokine genes and their receptor genes during the genesis of $GDF5^+$ cells in standard PN. However, at present, it remains unclear whether the generation of a mixture of $GDF5^{+/lo}SCX^{hi}$ cells and $GDF5^{hi}SCX^{lo}$ cells, or the presence of homogeneous $GDF5^+SCX^{lo/+}$ cells, is key to eliciting permanent cartilage-forming activity. Accordingly, further functional studies (e.g., co-culture experiments) are under way. We also plan to perform single cell RNA-seq analyses to determine which cell-type/population within the $GDF5^+$ cells are critical to the appearance of permanent cartilage-forming activity.

SIGNIFICANCE/CLINICAL RELEVANCE: These observations support our hypothesis that the post-transplantational fate of chondrocytes developed from mesenchymal cells can be determined at the mesenchymal cell stage. That fate seems to depend on how the mesenchymal cells are generated and maintained in culture. Further biological studies will not only enable hPSC-derived mesenchymal chondroprogenitors to be used for cell-based cartilage regenerative therapy, but may also lead to delineation of a critical mechanism that allows adult chondrogenic cells, such as mesenchymal stromal cells, to reproducibly regenerate permanent hyaline cartilage necessary for cell-based cartilage therapy in adults.

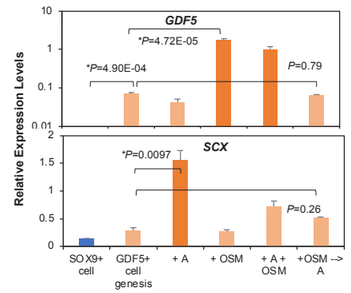

Fig. 1. *GDF5* and *SCX* levels during the $GDF5^+$ cell genesis culture in PN medium. A: activin A. N=2-6.

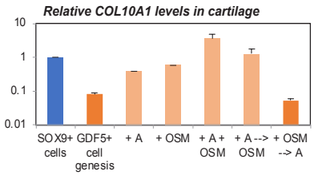

Fig. 2. *COL10A1* levels in cartilage pellets formed from $GDF5^{hi/+}$ cells generated under different conditions in the presence of TGFβ3+BMP4. N=2.

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Novel therapy for cartilage regeneration using nanofibers with localized sustained release of losartan

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Disclosures: CSB (1 Iota Biosciences; 8 ORS ISFR, TERMIS, OTA, AO Foundation; 9 Associate Editor Journal of Tissue Engineering & Regenerative Medicine), MJP (1 Smith & Nephew, Arothsurface, Conmed; 9 AJSM Editorial Board); JH (1 Cooke Myocyte)

INTRODUCTION: Osteoarthritis (OA) reached 52.2 million in the United States alone in 2012 and is projected to increase by 49% to 78.4 million by 2040. The central pathologic feature of OA is the progressive loss of articular cartilage, which currently shows no regenerative capacity. The TGF-β1 inhibitor, losartan, has recently attracted attention for its beneficial effects on promoting articular cartilage regeneration by reducing fibrocartilage formation following microfracture. However, there are concerns about systemic side effects associated with oral administration of losartan or the short drug half-life early if delivered by intra-articular injections. To address this concern, we aimed to develop an injectable biomaterial for the local and sustained release of losartan within the affected joint space. We accomplish this by modifying the charge structure of our self-assembling peptide amphiphiles nanofibers to effectively bind losartan. Given that losartan has a net negative charge, we hypothesized that a positively charged peptide amphiphile could prolong the release kinetics of losartan and promote chondrogenic regeneration *in vitro* in a dose-dependent manner.

METHODS: Positively (K-type) or negatively (E-type) charged peptide amphiphile nanofibers (**Fig. 1A**) were created using our established technology and loaded with 0, 0.05, 5, or 50 mg/dl of losartan. The microstructure of losartan sustained release nanofibers was evaluated by transmission electron microscope (TEM) images and the release kinetics of losartan were quantified over the time course of 2 weeks. The various losartan nanofibers were co-cultured in 0.4-μm semipermeable transwell over the ATDC5 chondrocyte cell line in a 24-well plate. Cell proliferation was evaluated longitudinally at 24 hours, 48 hours, 5 days, and 14 days after losartan nanofiber treatment using the non-destructive PrestoBlue assay. To access the regenerative potential of these nanofibers in an OA pathology, ATDC5 chondrocytes were primed with IL-1β (10ng/ml) for 24 hours. The glycosaminoglycans (GAG) assay was used to evaluate proteoglycan production following treatment with the nanowires. Gene expressions of chondrocyte-related markers (*Il6, Mmp13, collagen X, collagen II, aggrecan (acan), and Tgf-β1*) were evaluated 14 days after treatment using quantitative real-time PCR. All graphs were plotted using GraphPad Prism 9.0. ANOVA followed by Tukeys HSD post-hoc testing was used to test for significant difference between groups in Prism.

RESULTS: There were no obvious morphological differences in the structure of the positive or negatively charged peptide atmophile nanofibers or following the binding of losartan when visualized under TEM. Positively charged K nanofibers demonstrated a slower release of the negatively charged losartan (**Fig. 1B**). After 14 days, both nanofibers with/without losartan led to cell proliferation, with no significant differences in metabolic cell activity noted between the K and E nanofibers (**Fig. 2**). No cellular toxicity was noted at any losartan dose (data not shown). Increased GAG production was noted in the K nanofibers compared to the negatively charged E nanofibers (**Fig. 3A**). Among the K nanofibers, 0.5 and 5 mg/dl losartan doses demonstrate increased chondrogenic activity compared to 50 mg/dl losartan based on the increased *collagen II* and *aggrecan* gene expression (**Fig. 3 B**) with all nanofibers promoting a reduction in the pro-inflammatory response measured *Il6* (**Fig. 3B**). Furthermore, mRNA expression of Tgf-β1, which was elevated by IL-1β loading, was significantly suppressed by 5 mg/dl losartan nanofibers (**Fig.3 B**).

DISCUSSION: This study showed that both positively and negatively charged peptide amphiphiles can effectively load losartan in a non-cytotoxic manner for controlled drug release, but that prolonged drug release was observed with the positively charged K nanofibers due to electrostatic interactions between the drug and nanofiber. Furthermore, these results suggest that the nanofibers with or without losartan can promote chondrocyte proliferation and that the K nanofibers enhance GAG matrix production from chondrocytes. Interesting the low to moderate dose of losartan were found to maximize chondrogenic activity and minimized inflammatory and fibrotic responses at a gene level. Taken together these data suggest that the K nanofibers bound with low (0.5 mg/dl) to moderate (5 mg/dl) losartan can have a pro-regenerative phenotype in normal or OA-activated chondrocytes.

SIGNIFICANCE: Positively charged losartan sustained-release nanofibers may be a novel and clinically useful treatment for promoting cartilage regeneration and reducing fibrosis in conditions of OA by blocking TGF-β1.

ACKNOWLEDGMENTS: We gratefully thank the Shannon Foundation for their generous support of this collaborative research.

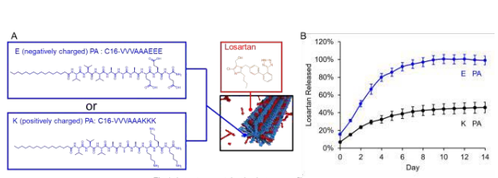

Fig.1: Losartan sustained release nanofibers
A: Structure of losartan sustained release nanofibers
B: Losartan release properties from nanofiber

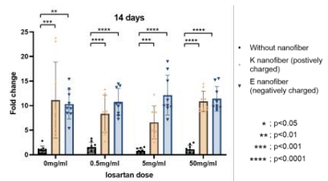

Fig. 2: Comparison of cell proliferation for each losartan dose 14 days after treatment

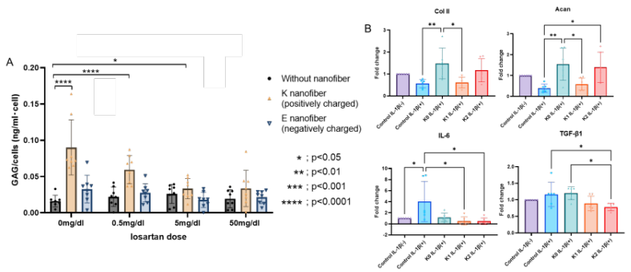

Fig.3
A: Comparison of GAG production per unit cell
B: Comparison of mRNA expression of Col II, Acan, IL-6, and TGF-β1

Quantification of stem cell number, their secretome, and cellular senescence in fractionated clinical bone marrow

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Disclosures: CSB (1, 3A, 4, 8, 9, detailed electronically); MJP (2, 3B, 5, 9, detailed electronically); JH (1, detailed electronically)

Introduction: Bone marrow aspirate can be harvested from the iliac crest to harness the therapeutic effect of mesenchymal stem cells (MSCs), other progenitor cells, cytokines, and growth factors. Before injecting this treatment into the site of injury, the aspirate is typically processed into bone marrow concentrate (BMC), which is intended to improve the therapeutic effect of BMA. However, MSCs only account for 0.001% to 0.01% of mononuclear cells present within BMA and there is a paucity of literature that illustrates the benefits of laboratory manipulation during bone marrow concentration. Most importantly, the number of MSCs, and thereby the amount of BMA, required to be therapeutically useful is not known. In our clinic, 60 -120 mL of BMA is typically harvested for ~10-fold concentration (BMC) prior to clinical application. Protocols vary across clinic/clinician/procedure, emphasizing the lack of standardization in the preparation of orthobiologics such as BMC. Thus, the primary purpose of this study is to evaluate whether MSC concentration or aspirate secretome (cytokines and growth factors) in BMA exhibit any difference from the beginning to end of a 60mL draw. We further investigated whether patient demographics and senescence varied significantly across our study cohort. We hypothesize the first 5 mL of BMA harvested will exhibit the highest concentration of MSCs and regenerative analytes, while subsequent fractions will be diluted due to peripheral blood infiltration.

Methods: This clinical observation study was approved by our Institutional Review Board and strict protection of human data was followed. A total of 12 patients undergoing an elective bone marrow harvest and clinical injection procedure between the ages of 18 and 77 were enrolled. Patients donated whole blood (WB) (5-30mL), BMA that was fractionated across the 60 mL harvest volume (BMA-1=1mL from the first 5mL, BMA-2= 1mL from the subsequent 30mL, and BMA-3=1mL from the 31-60 mL), and 1 mL of the BMAC (product). Samples underwent red blood cell (RBC) depletion and were processed into a cellular component or plasma. Senescence (CD87, C_{12}FDG) and MSC content were quantified on a Cytek® Northern Lights (16v, 14b) flow cytometer. MSC-negative markers: CD11b, CD19, CD34, CD45; MSC-positive markers: CD44, CD73, CD90, CD105. Inflammatory and regenerative cytokines and growth factors were measured via multiplex immunoassays on the Luminex® 100/200™ System according to the manufacturer's protocols. ANOVA, Pearson Correlation, and linear regression analyses were completed in GraphPad Prism V 9. Each patient is represented as an individual dot on the graphs.

Results: No statistically significant differences were found in the percentage of MSCs across the three different BMA fractions or within the BMC, but BMC did reduce the amount of patient-to-patient variation (**FIG 1A**). %MSCs did positively correlate with the pro-inflammatory cytokines IP-10/CXCL10 and TNFα (**FIG 1B,** $r > 0.5$). Additionally, the analytes IL-1RA and OC were significantly elevated in the first 5mL of BMA (BMA-1) compared to the subsequent fractions and the final BMAC product (**FIG 2A-B,** $p = <0.001$*)*. The analytes TGFβ1 (**FIG 2C**) and MMP-1 all trended lower in BMA-1 compared to subsequent fractions. Within this small cohort, %MSCs in fractionated BMA did not correlate with patient demographics such as sex or BMI (data not shown). However, our large cohort study has shown that age significantly correlates with an increase in the number of senescent PBMCs cells (**FIG 3A**) and age-related growth factors (GDF15 & FGF23), proinflammatory cytokines, and the bone inhibitor sclerostin (SOST) (**FIG 3B**, blue bars $r>0.05$). As such, we next tested whether the number of senescent cells influences the %MSCs in the BMC product. Our current data set shows a positively trending correlation that is non-significant at this sample size (**FIG 3D**), but %MSCs in BMA-1 and BMA-3 were significantly and positively associated with the respective percentage of $C_{12}FDG^{++}$ cells in each fraction ($p = 0.004$, $r=0.8553$; $p = 0.0283$, $r = 0.6564$; data not shown). Similarly, while no significant correlations were found between age and analyte in the BMC product, similar trends were noted in the correlation tree (**FIG 3C**).

Discussion: These preliminary results suggest that MSC concentration does not vary across BMA fractions; however, the secretome does change across with BMA harvest volume. Interestingly, anti-inflammatory and osteogenic factors were increased in BMA-1, while fibrotic and SASP-related analytes were decreased. Thus, we cannot propose a specific volume of BMA needed to achieve a therapeutic effect based on this MSC analysis; However, this secretome analysis highlights the dynamic protein levels in BMA. It might suggest that specific BMA fractions are more clinically valuable than others due to heightened concentrations of a myriad of therapeutic factors. The positive correlation between %MSCs and senescence may indicate that a senescent milieu in bone marrow recruits MSCs to a site of injury and/or inflammation to promote healing, which is potentially exacerbated by increasing age. However, the relationship between senescence and MSC content in bone marrow is a novel focus in the field and requires further study to delineate the actual mechanism. Finally, %MSCs in BMC was negatively correlated to time to injection and time of BMA harvest during the day; therefore, this could inform clinicians on when to schedule these treatments and how quickly to inject their patients with the BMAC product. Based on these results, clinicians may recommend scheduling these treatments earlier in the day and injecting the BMAC product as quickly as possible post-BMA harvest to achieve the most therapeutic effect.

Significance: A more thorough characterization is imperative to determine which beneficial components of bone marrow can be utilized in the clinical setting. This complete analysis will permit novel laboratory manipulation and clinical optimization to specialize the therapeutic effect.

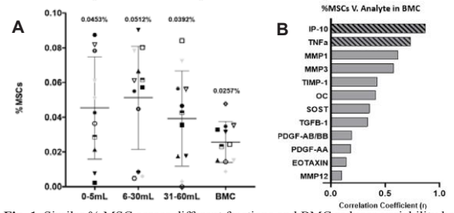

Fig. 1. Similar % MSC across different fractions and BMC reduces variability between patients (**1A**). 26 Analytes were analyzed in BMC and found the following correlation with % MSC. Interestingly, pro-inflammatory cytokines were significantly and positively associated with % MSC (**1B**).

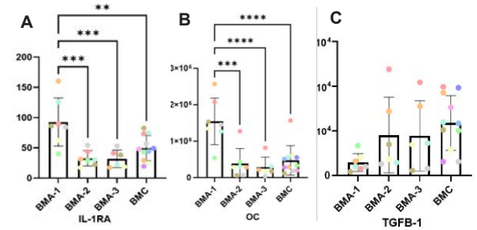

Fig. 2. The anti-inflammatory analyte, IL-1RA, was significantly lower in BMA-1 compared to subsequent fractions and BMC (**2A**). TGFB-1, which induces fibrosis and cellular senescence, is non-significantly lower in BMA-1 (**2B**). Additionally, the level of osteocalcin (OA), a biomarker for bone formation, is significantly higher in BMA-1 compared to subsequent

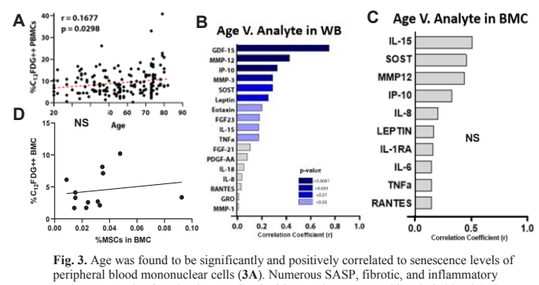

Fig. 3. Age was found to be significantly and positively correlated to senescence levels of peripheral blood mononuclear cells (**3A**). Numerous SASP, fibrotic, and inflammatory analytes were also found to be correlated with age when measured in whole blood (**3B**). Similar trends were found when analyzing BMC, but there were no significant findings (**3C**). A positive but nonsignificant correlation was observed between %MSCs and senescence in BMC.

Clinical Assessment of Senescence Cell Burden and Correlation to Severity of Osteoarthritis

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Disclosures: CSB (1 Iota Biosciences; 8 ORS ISFR, TERMIS, OTA, AO Foundation; 9 Associate Editor Journal of Tissue Engineering & Regenerative Medicine); ST (5 Canon, Siemen, Arthrex, SubioMed, Icaru; 9 Editorial Board for JOR and J Biomechanics); MJP (1-9 as electronically detailed); JH (1 Cooke Myocyte); others (none)

Introduction: Osteoarthritis (OA) is a progressive degenerative disease of the articular cartilage and is associated with subchondral bone pathology and synovial joint damage. This disease permeates all demographics causing it to affect over 300 million people worldwide. The severity of OA is traditionally graded using the Kellgren-Lawrence radiography scoring system of classification (KL grade), which diagnoses OA based on marginal osteophytes, joint space narrowing, subchondral bone sclerosis, and altered bone shape. Despite the assessment of numerous OA indicators, limitations to the repeatability and reliability of KL grading for diagnostic purposes remains. In response, various putative biomarkers have been characterized as potential indicators of structural, biochemical, or physiological severity of OA pathogenesis. However, there are currently no reliable quantitative biomarkers that are diagnostic or prognostic of OA. Senescence is a cell state defined by loss of proliferative capacity and the release of pro-inflammatory cytokines/chemokines, proteases, and other factors, otherwise known as the senescence-associated secretory phenotype (SASP) that contribute to systemic aging and drive age-related conditions, especially OA. Peripheral senescent T-cells specifically have been linked to conditions such as frailty, rheumatoid arthritis, and bone loss. Thus, the ability to accurately detect and/or track peripheral senescent cells, may be useful to define OA severity and potentially assist in deciding interventional strategies. In our lab, we have recently developed a flow cytometry-based assay to detect senescent peripheral blood cells (PBMCs), including subsets such as CD3+ T-cells, using the fluorescent compound $C_{12}FDG$. A key interest of our group is investigating the contribution of senescence to age-associated musculoskeletal decline and understanding peripheral dynamics of senescent PBMC subsets associated with orthopaedic conditions including OA.

Methods: Patients between the ages of 40-80, with radiographic evidence of KL grade II-IV osteoarthritis in one or both knees, were enrolled (N=64) in an IRB and FDA approved randomized, prospective double-blinded clinical trial. Age and gender matched healthy controls were enrolled into a separate, IRB approved clinical observation study. A standard venipuncture procedure collected 30mL of peripheral blood from OA and healthy. Peripheral blood mononuclear cells (PBMC), T cells, serum, and plasma were isolated and used for subsequent quantification of senescent cell burden, inflammatory cytokines, and growth factors. Senescence in the cellular component was quantified using the $C_{12}FDG$ marker of cellular β-galactosidase activity by flow cytometery and gated to define a distinct population of $C_{12}FDG^{++}$ (high) cells. Thirty-four serum/plasma analytes were measured via multiplex immunoassays (Luminex®) or ELISA according to manufacturer's protocols. Senescence-associated gene expression was measured using qRT-PCR for *p16* and *p21*. A comprehensive set of patient-reported outcomes were collected, with only the SF-12 physical component score (PCS-12) reported here. A higher PCS-12 score indicates better physical health. Mann-Whitney testing for comparison of two groups, ANOVA, Pearson correlation, and/or Linear Regression were used for statistical analysis. Analysis was done using Prism V9 and each individual patient is represented as a unique point on the plots.

Results: The number of $C_{12}FDG^{++}$ senescent cells in PBMCs and T-cells were significantly higher in the OA population compared to the healthy population (**FIG 1A**). The senescence-associated genes (*p16* and *p21*) were also higher in T-cells of OA patients, but only *p21* displayed a significant difference (**FIG 1B**). Of importance, the number of senescent cells negatively correlated with the PCS-12 score (**FIG 2A**, r=-0.274, p=0.027), indicating that higher senescent cell burden resulted in poorer physical health. However, we found no difference in senescence cell number based on KL grade (**FIG 2B,** p=0.31 PBMC or 0.35 T-Cell). In the T-cell compartment 8-analytes were found to be significantly positively correlated with the percentage of $C_{12}FDG^{++}$ senescent cells, including growth factors associated with angiogenesis, inflammation, and aging (**FIG 3A,** r>0.5, red color). TGFβ1 was significantly negatively correlated (data not shown for space). No significant correlations were noted between the biomarkers and senescent PBMCs (**FIG 3B**) despite the previously noted increase in OA and association with physical health **(FIG 1-2**).

Discussion: Here, we report significant increases in circulating senescent total PBMCs and T-cells in OA patients when compared to healthy volunteers. We also found positive correlations between senescent PBMCs/T-cells and serum OA biomarkers and patient reported pain/health scores in individuals with OA, but not with KL grade. Importantly, this study includes patients only at baseline of enrollment in a double blinded prospective clinical trial testing FDA approved therapeutic strategies to reduce senescence.

Significance: This is the first clinical evidence that senescent cell burden is correlated to the severity of OA. Our comprehensive assessment of the senescence phenotype within OA is critical to our understanding of its role disease pathology. Overall, senescent detection may serve as a companion diagnostic tool that permits a greater comprehension of OA pathology and serve as a biomarker to grade efficacy of therapeutic interventions.

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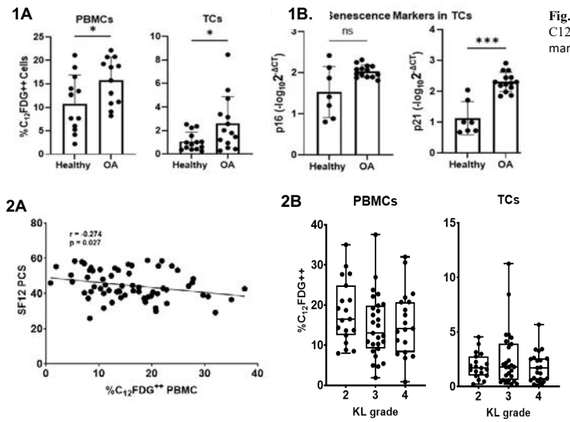

Fig. 2. SF12 PCS was observed to be negatively and significantly correlated with percent C12FDG++ (bright) PBMCs (**3A**). Percent C12FDG++ (bright) PBMCs and T cells were categorized based on the corresponding subject KL grade. In both cell populations, there were no significant differences between KL grade (**3B**).

Fig. 1. Percent C12FDG++ (bright) PBMCs and T-cells in healthy versus OA patients. C12FDG++ T-cells were significantly elevated in OA patients (**1A**). qPCR results for senescence markers p16 and p21 indicate increased senescence expression in enriched CD3+ T-cells (**1B**).

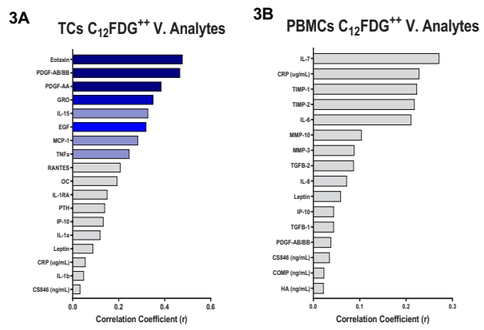

Fig. 3. Percent C12FDG^{++} (bright) T cells was found to be significantly correlated with numerous analytes associated with angiogenesis, inflammation, and aging (**2A,** blue color). There were no significant correlations observed between percent C12FDG^{++} (bright) PBMCs and the analytes measured (**2B**).

Effects of Fisetin Treatment On Cellular Senescence In Multiple Organs Of Aged Sheep

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Introduction: Fisetin is a natural flavonoid that has been shown to delay aging via multiple mechanism and extended life and health span in a mice model[1]. Fisetin has also been shown to reduce age related decline in brain function using different injury models [2-7]. Fisetin exerts an anti-inflammatory effect on the microglial cells, the immune cells of the brain as well [8],while reducing apoptosis in cardiomyocytes. Majority of the previous studies used mice. The goal of this study is to take advantage of large animal model's translational value and investigate whether systemic fisetin treatment of aged sheep would have beneficial to multiple organs via reduction of senescent burden and senescent associate phenotype (SASP).

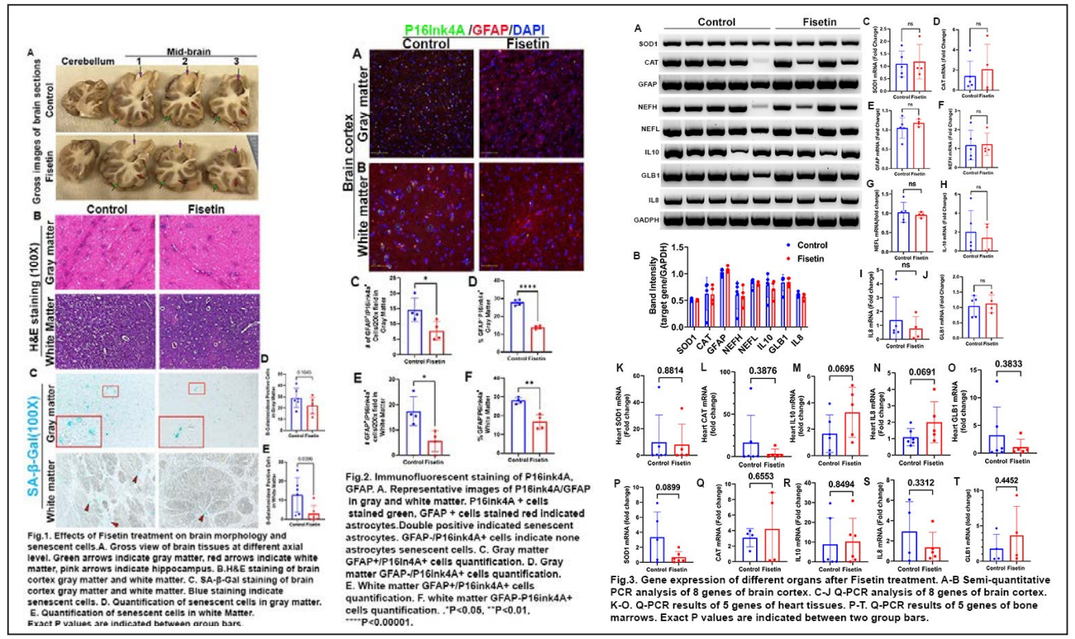
Materials and Methods: This study was approved by Institutional animal care and use committee (IACUC) of Colorado State University. 8 years old Nordic sheep was divided into two groups(N=6/group), one group receive fisetin at 20mg/kg by intravenous infusion two days every week for 8 weeks, the other group received vehicle intravenous infusion. Brain tissues were harvested after 8 weeks, left hemisphere were dissected and fixed in neutral buffered formalin for 9 days and then midbrain was sliced into three different level and processed using 15% sucrose 30% sucrose in PBS and embedded in NEG freezing medium. Mid-brain sections were cut into 10µm thickness. H&E staining, senescent associated β-galactosidase (SA-β-Gal) staining were performed. Immunofluorescent staining of P16ink4A and GFAP, an astrocytes marker was also performed. The right hemisphere was used to collect brain cortex for isolation RNA and real time quantitative polymerase chain reaction (Q-PCR). We also collected left ventricle muscle of the heart for isolation of RNA and Q-PCR analysis. Furthermore, bone marrow was aspirated from sheep iliac crest and nucleated cells were isolated and RNA was extracted for Q-PCR.

Results: 1. Senescent cells in the aged sheep's brain. Gross images showed no obvious differences on the midbrain between fisetin treated sheep and vehicle treated sheep (Fig.1A). H&E staining did not reveal significant difference between fisetin treated and vehicle treated sheep brain (Fig.1B). However, many SA-β-Gal positive cells were detected in the gray matter of the sheep's brain tissues, insets highlighted positive cells (Fig.1C). Fisetin treatment showed trend of decreased SA-β-Gal positive cells (P=0.1645) (Fig.1D). White matter also has many SA-β-Gal cells, but relative fewer than gray matter. Fisetin treatment significantly decreased the number of SA-β-Gal cells in white matter (Fig.1 C, E). **2. Identification of senescent astrocytes.** Immunofluorescent staining indicated $GFAP^+/P16Ink4A^+$(astrocytes) and $GFAP^-/P16ink4A^+$cells (non-astrocytes) were significantly decreased by Fisetin treatment in the gray matter (Fig.2 A,C,D). $GFAP^+/P16Ink4A^+$ and $GFAP^-/P16ink4A^+$ cells were also significantly decreased by Fisetin treatment in the white matter (Fig.2 B,E-F). **3. Q-PCR results of brain cortex**. Semi-quantitative-PCR indicated fisetin treatment did not change gene expression of SOD1, CAT, GFAP, NEFH, NEFL, IL10, IL8 and GLB1(Fig.3A-B). These results were further validated by Q-PCR (Fig.3C-J). **4.Q-PCR results of heart tissues.** We found fisetin treatment did not significantly change gene expression of SOD1, CAT and GLB1 in the sheep's heart, but demonstrated a trend of increase of IL10 (P=0.0695) and IL8 (P=0.0691(Fig.3K-O**). 5.Q-PCR results of bone marrow**. No statistical significance was found for bone marrow gene expression (Fig.3P-T).

Discussion and conclusion: This study revealed that Fisetin treatment does not affect general morphology of the brain. Both gray matter and white matter demonstrated presence of senescent cells in old sheep brain. Fisetin treatment significantly decreased senescent cells in both gray and white matter of the brain. Fisetin treatment significantly reduced senescent astrocytes ($GFAP^+/P16Ink4A^+$) and non-astrocytes ($GFAP^-/P16ink4A^+$) in both brain gray and white matter. Fisetin treatment increase IL10 and IL8 at marginal statistical level in the heart tissues and did not significantly change bone marrow gene expression. More organ analyses are ongoing. In conclusion, fisetin treatment decreased brain cortex senescent cells in both gray and white matter and increased IL10 and IL8 level in the heart without inducing significant changes to other gene expression.

Significance: Senescent cells are widely present in the brain cortex of old sheep and decreased by fisetin treatemnt. Fisetin may improve brain, heart health.

Acknowledgement: This project was funded by the Philanthropic gift from Borgen Family and the Hart Family.



Effects of Losartan and Fisetin on Microfracture Mediated Cartilage Repair of Ankle Cartilage in a Rabbit Model

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Disclosures: Authors' disclosures are available on ORS website.

Introduction: Microfracture is the standard treatment of osteochondral lesions of the talus (OLTs); however, the repair tissue is predominantly fibrocartilage which has led to concerns regarding the quality of the repair and long-term outcome. Biological regulation of microfracture has shown to improve the quality of the repair tissue. Losartan is an anti-hypertension drug that can inhibit the activation of transforming growth factor beta 1 (TGFβ1), and has shown to improve knee cartilage repair after both oral administration and intra-articular injection [1, 2]. Fisetin is a flavonoid that has demonstrated senolytic properties by elimination of senescent cells and extend life and health span of aged mice [3]. It has been shown that Fisetin can attenuate destabilized medial meniscus (DMM) induced osteoarthritis progression via anti-inflammatory effects [4]. The aim of this study was to determine if the administration of Losartan, Fisetin, or Losartan and Fisetin after microfracture of OLTs result in better cartilage quality compared to microfracture alone in a rabbit model.

Materials and Methods: This study was approved by the Institutional Animal Care and Use Committee (IACUC) of Colorado State University. An osteochondral defect was made in the talar dome of 32 female New Zealand White rabbits. Thereafter, the rabbits were divided into 4 groups (N=8/group): 1) microfracture only (Microfracture), 2) microfracture + Losartan (Losartan), 3) microfracture + Fisetin (Fisetin), and 4) microfracture + Losartan and Fisetin (Losartan+Fisetin). After surgery, the rabbits were fed daily with Losartan 10mg/kg/d and/or Fisetin 20mg/kg/d that were mixed with shredded carrots and apples. All rabbits were euthanized at 12 weeks after surgery, and the healing of the cartilage defect was evaluated macroscopically and with microcomputed tomography (micro-CT), histology, and immunohistochemistry. The capsule tissue was evaluated with quantitative polymerase chain reaction (Q-PCR). The serum was evaluated with enzyme-linked immunosorbent assays (ELISA) for detection of Interleukin 1-β (IL1-β), IL6, matrix metallopeptidase 9 (MMP9), and superoxide dismutase 1(SOD1). All continuous data were analyzed using Analysis of Variance (ANOVA) followed by Tukey's multiple comparisons test.

Results: Both Losartan and Fisetin groups showed significantly better macroscopic healing than the Microfracture group with increased ICSR score (P=0.0036, P=0.0146, respectively) .Losartan+Fisetin showed a trend towards increased ICRS score compared to the Microfracture group (P=0.0675) (Fig.1A-D). MicroCT showed that the subchondral bone had healed almost completely in the Losartan and Fisetin groups, while incomplete healing was seen in the Microfracture and Losartan+Fisetin groups (Fig.1E-F). With Safranin O staining, the regenerated cartilage was stained in orange red and showed best healing and natural cartilage curvature in the Losartan and Fisetin groups. The worst repair showed that the subchondral bone was not healed, with fissure and lack of integration with host cartilage (Fig.2A-C). Losartan and Fisetin treatment improved the cartilage repair by increasing O'Driscoll histology score, but the difference did not reach statistical significance when compared to the Microfracture group (P=0.065, P=0.149, respectively) (Fig.1D). Surprisingly, the modified O'Driscoll histology score of Losartan+Fisetin group was significantly lower than Losartan group (P=0.0124) and Fisetin group (P=0.0178). There was no significant difference between Losartan+Fisetin and Microfracture group (P=0.2181) (Fig.2D). H&E and Alcian blue staining showed similar results as Safranin O staining. Immunohistochemistry showed that collagen 2 (Col2) was expressed in all regenerated cartilage. The best cartilage repair in the Losartan and Fisetin groups showed organized hyaline like cartilage with intense Col2 staining (Fig.3A-C). Immunohistochemistry of P16 showed strong nuclear staining in all groups, but there were no differences between groups (Fig.3D). Q-PCR revealed that the Fisetin group had significantly decreased catalase compared to the Microfracture, Losartan, and Losartan+Fisetin group. The Fisetin group also had increased MMP9 expression when compared to the Losartan group. The Fisetin group showed a trend of decreasing TAK1 compared to the Microfracture group (P=0.057) and Losartan group (P=0.054). The Losartan+Fisetin group also showed decreased TAK1 compared to the Microfracture group (P=0.05). No differences were found between any groups regarding expression of SOD1, GPX1, MMP2, SMAD4, SMAD2, or TGFβ1 in capsule tissues. ELISA showed that IL1β and IL6 was not detectable in serum and SOD1 and MMP9 were not significantly different between any groups.

Discussion: We found that both Losartan and Fisetin improved the cartilage healing after microfracture of OLTs, both grossly and histologically. Compared to microfracture only, both Losartan and Fisetin resulted in increased hyaline cartilage regeneration. This is the first study to show that Fisetin has beneficial effect on microfracture mediated repair of OLTs. Unexpectedly, Losartan and Fisetin in combination did not improve the cartilage healing compared to microfracture only. The mechanism why there is no beneficial effect when Losartan and Fisetin are combined is unknown. Fisetin as a senolytic agent for cartilage repair may depend on the dose and regimen of drug administration. Since we mixed Losartan and Fisetin with food, the two drugs may affect each other metabolically, which needs to be further studied.

Significance/clinical relevance: This study suggests that biological regulation of microfracture using either Losartan or Fisetin alone may improve the cartilage healing of OLTs. Clinical studies are needed to evaluate the potential benefits in humans.

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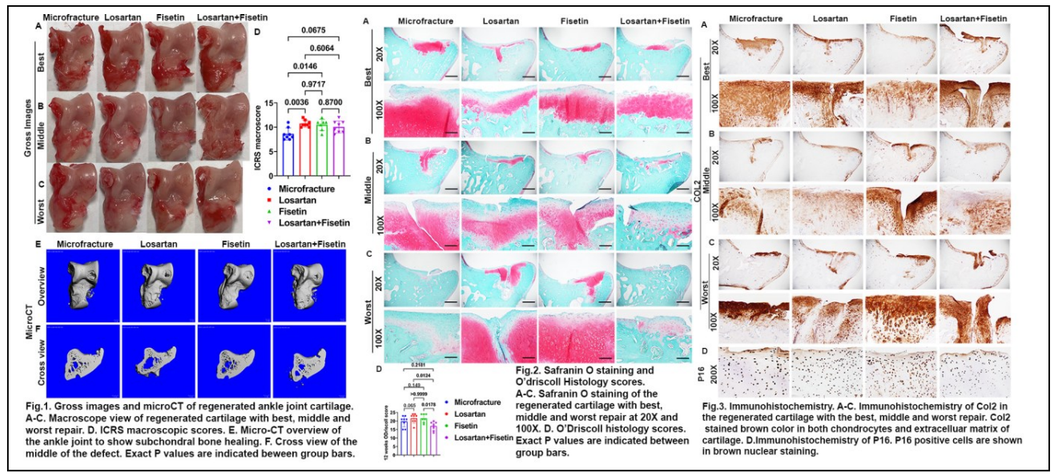

Fig.1. Gross images and microCT of regenerated ankle joint cartilage. A-C. Macroscope view of regenerated cartilage with best, middle and worst repair. D. ICRS macroscopic scores. E. Micro-CT overview of the ankle joint to show subchondral bone healing. F. Cross view of the middle of the defect. Exact P values are indicated beween group bars.

Fig.2. Safranin O staining and O'driscoll Histology scores. A-C. Safranin O staining of the regenerated cartilage with best, middle and worst repair at 20X and 100X. D. O'Driscoll histology scores. Exact P values are indicated between group bars.

Fig.3. Immunohistochemistry. A-C. Immunohistochemistry of Col2 in the regenerated cartilage with the best, middle and worst repair. Col2 stained brown color in both chondrocytes and extracelluar matrix of cartilage. D.Immunohistochemistry of P16. P16 positive cells are shown in brown nuclear staining.

Targeting Muscle Cell Senescence in Dystrophin$^{-/-}$/Utrophin$^{-/-}$Double Knockout Mice Improves Muscle Histopathology and Enhances Bone Mass and Lifespan

Xueqin Gao[1], Joseph J Ruzabarsky[1,2], Charles Huard[1], Aiping Lu[1], Sarah White[3], Jessica Ayers[3], Bing Wang[4], Johnny Huard[1*]
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Introduction: Duchenne's Muscular Dystrophy (DMD) is a severe genetic muscle disease due to the mutation of the dystrophin gene. In addition to the muscular manifestations of the disease, DMD patients also have a high risk of bone fracture. Dystrophin$^{-/-}$/Utrophin$^{-/-}$ (DKO-Hom) is a mouse model that recapitulates the clinical manifestations of DMD better than *Mdx* mice with severe muscle histopathology including muscle necrosis, fibrosis, fat infiltration, heterotopic bone formation (HO), kyphosis and short lifespan[1]. Previous investigations have shown that DKO-Hom mice exhibit bone osteopenia as early as 4 weeks secondary to muscle pathology as well as delayed fracture healing [2, 3]. A previous study also demonstrated that both DKO-Hom mice and Mdx showed cell senescence and senescence-associated phenotype in the muscle tissues but not bone tissues [4] (Gao X, ORS 2020). The aim of this study is to investigate if targeting cell senescence with Ruxolitinib can improve the muscle and bone phenotypes as well as the general health in DKO-Hom mice.

Methods: 1. Animal breeding. DKO-Hom mice were generated using dystrophin$^{-/-}$/Utrophin$^{+/-}$ mice bred at the Animal Facility of Colorado State University. All experiments were approved by IACUC animal protocol of CSU (#1234). **2. Mice treatment:** DKO-Hom mice at 4 weeks-old were then divided into two groups (n=8/group including both males and females) and treated with Ruxolitinib (60mg/kg/d), a JAK1/2 inhibitor, and vehicle (5%DMSO in PBS) by oral gavage daily. Mice were then sacrificed at 12 days after treatment. Gastrocnemius muscle tissues were collected for histology and immunofluorescent staining. Thigh muscle tissues were collected for RNA isolation and Q-PCR. Lumbar spine, right tibia, femur bones were collected for Micro-CT, histology. **3.Lifespan**: To detect whether targeting cell senescence could extend lifespan, DKO-Hom mice were treated with either Ruxolitinib or vehicle (control) for 12 days (n=8-10 both males and females) and allow the mice to live to natural death. The lifespan was compared between treated and untreated mice. All data were analyzed using Graphpad Prism 9.

Results: 1. Muscle histology results. Muscle H&E staining showed decreased inflammatory cell infiltration and improved muscle regeneration after ruxolitinib treatment compared to the control dKO-Hom mice (Fig.1A). Immunofluorescent staining demonstrated that CD68$^+$ macrophages, CD68$^+$/P21$^+$ cells and CD68$^-$/P21$^+$ cells were all significantly decreased by Ruxolitinib treatment compared to the control dKO-Hom mice (Fig.1B-E). Furthermore, Q-PCR results indicated that Ruxolitinib treatment decreased senescent β-Galactosidase mRNA (GLB1) (Fig.1F), migration inhibitor factor (MIF)(Fig.1G), tartrate resistant acid phosphatase (TRAP) (Fig 1H) and bone morphogenetic protein 4 (BMP4) (Fig.1I). **2. Bone Micro-CT and histology results**. Treatment of DKO-Hom mice with Ruxolitinib for 12 days significantly increased bone volume /total volume (BV/TV), trabecular thickness (Tb.Th) of spine trabecular bone (Fig.2A-C) and showed a trend of increased BV/TV, Tb.Th of proximal tibia trabecular bone (Fig.2D-F), but no changes in femur cortical bone thickness (Ct.Th) or bone volume density(Fig.2G-I). Furthermore, Ruxolitinib treatment sightly decreased muscle heterotopic bone formation (data not shown) and did not change body weight of DKO-Hom mice when compared to vehicle-treated mice. Herovicis' staining showed thicker trabecular bone in Ruxolinitib-treated mice when compared to control mice (Fig.3A). H&E staining also showed thicker bone trabeculae in the Ruxolitinib group compared to the vehicle treated DKO-Hom mice (Fig.3B). Osterix (OSX)$^+$osteoprogenitor cells in spine L5 trabecular bone were not significantly affected by Ruxolitinib treatment (Fig.3C,E). However, Ruxolitinib treatment significantly decreased TRAP$^+$ osteoclasts in comparison to vehicle-treated mice (Fig.3D, F). **3. General health**. Ruxolitinib-treated DKO-Hom mice demonstrated significantly increased average lifespans when compared to the vehicle-treated mice (Fig.3G). Further analysis on diaphragm and heart tissues are ongoing.

Discussion and conclusion: This study reveals that targeting cellular senescence with Ruxolitinib improves muscle histopathology in DKO-Hom mice by decreasing inflammation especially M1 macrophages (CD68+ cells) as well as other cells (CD68$^-$P21$^+$). P21 positive cells are also present in the newly regenerated multiple nuclear fibers. Interestingly, Ruxolitinib also decreased GLB1 and MIF expression in the dystrophic muscle tissue. TRAP and BMP4 expression were found in the DKO muscle which relates to muscle heterotopic bone formation and was decreased in Ruxolitinib-treated mice. More importantly, Ruxolitinib treatment was found to improve trabecular bone microarchitecture of the spine as well as proximal tibia without significantly affecting cortical bone. Ruxolitinib treatment also extends lifespan of dKO-Hom mice indicating general improvement of health. **In conclusion, targeting cell senescent in the muscle tissue of DKO-Hom mice improved both muscle histopathology and bone microstructure while extending mice lifespan.**

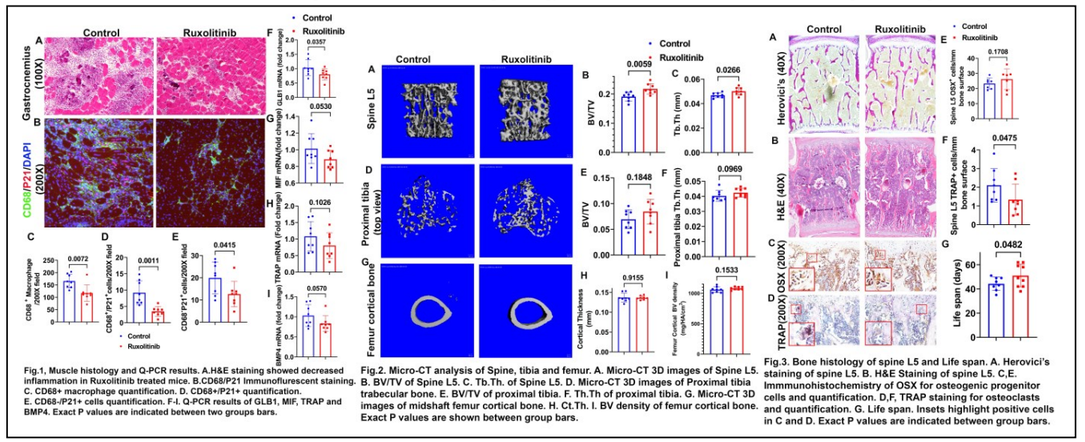

Fig.1. Muscle histology and Q-PCR results. A.H&E staining showed decreased inflammation in Ruxolitinib treated mice. B.CD68/P21 Immunoflurescent staining. C. CD68+ macrophage quantification. D. CD68+/P21+ quantification. E. CD68-/P21+ cells quantification. F-I. Q-PCR results of GLB1, MIF, TRAP and BMP4. Exact P values are indicated between two groups bars.

Fig.2. Micro-CT analysis of Spine, tibia and femur. A. Micro-CT 3D images of Spine L5. B. BV/TV of Spine L5. C. Tb.Th. of Spine L5. D. Micro-CT 3D images of Proximal tibia trabecular bone. E. BV/TV of proximal tibia. F. Tb.Th of proximal tibia. G. Micro-CT 3D images of midshaft femur cortical bone. H. Ct.Th. I. BV density of femur cortical bone. Exact P values are shown between group bars.

Fig.3. Bone histology of spine L5 and Life span. A. Herovici's staining of spine L5. B. H&E Staining of spine L5. C,E. Immmunohistochemistry of OSX for osteogenic progenitor cells and quantification. D,F, TRAP staining for osteoclasts and quantification. G. Life span. Insets highlight positive cells in C and D. Exact P values are indicated between group bars.

Significance/clinical relevance: Targeting cell senescence with Ruxolitinib, FDA-approved drug can be an adjunct therapy for muscular dystrophy patients in combination with steroids or gene therapy.

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The Role of Sclerostin in Bone Osteopenia of Muscular Dystrophy

Xueqin Gao[1], Joseph J Ruzbarsky[1,2], Matthieu Huard[1], Sudheer Ravuri[1], Ping Guo[1], Laura Chubb[3], Nicole Ehrhart[3], Bing Wang[4], Johnny Huard[1*]
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Disclosures. Authors' disclosures are shown in ORS website.

Duchenne's muscular dystrophy (DMD) is a severe genetic muscle disease due to a mutation of the dystrophin gene. DMD patients are at an increased risk of bone fracture. Dystrophin$^{-/-}$/Utrophin$^{-/-}$ (DKO-Hom) is a mouse model that recapitulates the clinical manifestations of DMD better than *Mdx* mice, with the manifestations of severe muscle histopathology including muscle necrosis, fibrosis, fatty infiltration, heterotopic bone formation (HO), kyphosis and shortened lifespan[1]. Previously, it has been shown that DKO-Hom mice exhibit bone osteopenia as early as 4 weeks secondary to muscle pathology and demonstrated delayed fracture healing [2, 3]. It has also been shown that sclerostin (SOST) was increased in the serum of 4- and 6-week-old DKO-Hom mice. A recent human study demonstrated that SOST increased in human DMD patients and was correlated with lower bone mineral densities [4]. However, the source and role of SOST in both osteopenia and increased fracture risk in DMD is unknown, thus prompting the current study.

Methods: 1. Animal breeding. DKO-Hom mice were generated using dystrophin$^{-/-}$/Utrophin$^{+/-}$ mice and bred in the Animal Facility of Colorado State University. All experiments were approved by IACUC animal protocol of CSU (#1234). **2. Identify the source of SOST expressions in DKO-Hom mice**. 4-week-old DKO-Hom mice were sacrificed, and their bone, muscle and serum were collected (N=12 for WT mice, N=18 for DKO-Hom mice). Bone tissue immunohistochemistry of SOST, serum SOST ELISA and muscle Q-PCR of SOST were performed. **3. SOST antibody (SOST ab) treatment.** 4-week old DKO-Hom mice were divided into 2 groups (N=8-10, both male and females) and treated with FDA approved SOST ab (Evenity at the clinical dose, 3.5mg/Kg/week) or PBS once a week for 2 weeks. Body weights were recorded weekly to adjust drug dosages. Mice were sacrificed and bone and muscle tissues were collected for Micro-CT analysis and histology using established protocols. All data were analyzed via T-tests using GraphPad Prism 9 software.

Results: 1. SOST expression in muscle and bone tissues. We found SOST expression in osteocytes of tibia cortical bone was significantly decreased in DKO-Hom mice compared to WT mice (Fig.1A-B). SOST expression in the trabecular bone of the proximal tibia also trending decrease in the DKO-Hom group (Fig1.C-D). However, the serum SOST was significantly increased in the 4-week-old DKO-Hom mice (Fig.1E). Furthermore, SOST mRNA is significantly increased in the thigh muscle tissue in DKO-Hom mice compared to WT mice (Fig.1F). These results indicated high SOST in the serum is likely derived from dystrophic muscle. **2. Treatment of SOST ab increased bone microarchitecture of both spine and long bones.** SOST ab treatment did not significantly change body weight. Micro-CT results demonstrated that two weeks of treatment with SOST ab significantly increased both bone volume/total volume (BV/TV) (30% increase) and trabecular thickness (Tb.Th) in spine L5 trabecular bone compared to control mice (Fig.2A-C). The BV density of spine also was significantly increased in the SOST ab treated mice (P=0.0005) compared to the control mice (data not shown). The trabecular separation (Tb.Sp) showed trend of decrease (P=0.26). No differences were found for trabecular number (Tb.N) of spine L5. For the proximal tibia, BV/TV (increased by 45%) and Tb.Th were significantly increased in SOST ab-treated mice compared to the control mice (Fig.2D-F). Tb.N also showed an increasing trend (P=0.11) while the Tb.Sp showed a decreasing trend in the SOST ab treated mice (data not shown). Cortical thickness (Ct.Th) of the midshaft femur was significantly increased in SOST ab treated mice than PBS treated dKO-Hom mice (Fig.3G-H). No statistically difference was found for BV density (data not shown). There was no difference of muscle heterotopic bone formation in micro-CT analysis (data not shown). **3. Histology.** Herovici's staining showed collagen 1 bone matrix in red-pink color. The thickness of col1agen 1 positive trabecular bone was higher in the L5 vertebrae of SOST ab treated mice than control mice (Fig.3A). H&E staining also demonstrated a greater trabecular bone thickness in the SOST ab treated mice than the control mice (Fig.3B). Furthermore, the H&E staining of muscle tissues surrounding the spine showed no differences between SOST ab treated mice compared to control mice (Fig.3C).

Discussion and conclusion: Patients with muscular dystrophy have bone osteopenia and are at a high risk of fracture which often causes them to become wheelchair bound. Most scholars thought the bone loss in muscular dystrophy was caused by chronic corticosteroid treatment, however, this theory has recently been challenged. A recent study showed DKO-Hom mice without corticosteroid treatment also demonstrated osteopenia as early as 4 weeks, however, the cause of osteopenia is still not clear. FGF 21 released by diseased skeletal muscle have been shown to contribute to the bone loss in DKO-Hom mice [5]. Our previous study showed SOST was increased while RANKL was significantly decreased in DKO-Hom mice [2]. In current clinical practice, biphosphates, an anti-resorption drug, and RANKL antibody was used for preventing bone loss in DMD patients, but these treatments only maintained bone mass without improvement of bone health [6]. Therefore, new treatment for bone loss in DMD is necessary. A recent study showed serum SOST is increased in DMD patients which is consistent with earlier findings in DKO-Hom mice[2]. This study identified that increased SOST in the serum is mainly derived from diseased muscle tissues in DKO-Hom mice. Treatment with SOST ab dramatically increases spine and long bone microarchitecture without significantly affect muscle pathology and general health. SOST ab has been approved in clinical for treatment of osteoporotic patients with dual effect of both increase bone formation and decreased bone absorption. Therefore, SOST ab may be a potential new therapeutic agent for DMD patients to improve their bone health. **In conclusion, this study demonstrates that increased SOST in muscle of DKO-Hom mice may contribute to bone loss, and SOST ab dramatically improve bone health in DKO-Hom mice and can be a potential therapy for DMD patients.**

Significance: This study identified SOST as a potential factor that contributed to the bone loss in DKO-Hom mice and SOST ab used alone or in conjugation with steroids may achieve beneficial effects for both bone and muscle for DMD patients.

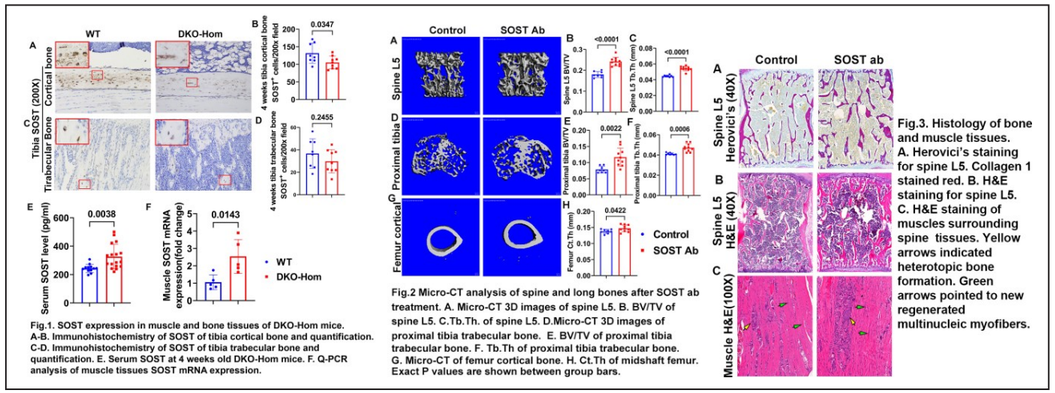

Fig.1. SOST expression in muscle and bone tissues of DKO-Hom mice. A-B. Immunohistochemistry of SOST of tibia cortical bone and quantification. C-D. Immunohistochemistry of SOST of tibia trabecular bone and quantification. E. Serum SOST at 4 weeks old DKO-Hom mice. F. Q-PCR analysis of muscle tissues SOST mRNA expression.

Fig.2 Micro-CT analysis of spine and long bones after SOST ab treatment. A. Micro-CT 3D images of spine L5. B. BV/TV of spine L5. C.Tb.Th. of spine L5. D.Micro-CT 3D images of proximal tibia trabecular bone. E. BV/TV of proximal tibia trabecular bone. F. Tb.Th of proximal tibia trabecular bone. G. Micro-CT of femur cortical bone. H. Ct.Th of midshaft femur. Exact P values are shown between group bars.

Fig.3. Histology of bone and muscle tissues. A. Herovici's staining for spine L5. Collagen 1 stained red. B. H&E staining for spine L5. C. H&E staining of muscles surrounding spine tissues. Yellow arrows indicated heterotopic bone formation. Green arrows pointed to new regenerated multinucleic myofibers.

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Fisetin Treated Human Bone Marrow Aspirate Concentrate Rapidly Reduces Senescence Signatures

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INTRODUCTION: Human bone marrow aspirate concentrate (hBMAC) is a readily available source of mesenchymal stem cells (MSCs) that is a common regenerative medicine treatment for various orthopedic applications. Senescence is a cell fate characterized by the loss of metabolic function, proliferative capability, and increased resistance to apoptosis which leads to tissue decline. Fisetin is a naturally occurring flavonoid with demonstrated senolytic activity through the targeted elimination of senescent cells. The purpose of this study was to investigate the therapeutic effect of fisetin on senescence in bone marrow derived MSCs (BM-MSCs) and hBMAC. We hypothesized that fisetin would decrease senescence in both hBMAC and cultured BM-MSCs, which we posit may improve the quality of the hBMAC orthobiologic product. The utilization of fisetin to improve patient derived products requires a therapeutic index. Here, we demonstrate that 50µM of fisetin was able to reduce senescence signatures in BM-MSCs and hBMAC in as little as 2 hours.

METHODS: BM-MSCs were obtained from ATCC used for *in vitro* experiments to determine dose and time course. BM-MSCs were cultured in normal growth media (Low Glucose DMEM, 10% MSC FBS, 1% Penicillin/Streptomycin, and 10 ng/mL FGF). Cells were treated with 50 µM of the senescence inducing agent etoposide for 24 hrs then treated with 50 µM of fisetin for 24hrs. Senescence was determined using the *β*-gal staining kit (Cell Signaling Technology) and further verified using the senescence stain C_{12}FDG staining via flow cytometry (Sigma-Aldrich). Cells were pretreated with Bafilomycin for 1hr prior to C_{12}FDG staining for 1hr then washed, collected, and analyzed with a Guava EasyCyte flow cytometer. Gene expression was determined for senescence transcripts *p21*, and inflammatory transcripts *IL-1β* and *IL-6* via qPCR. For hBMAC, samples were obtained from our clinic (**IRB#2020-50).** To confirm the efficacy of fisetin treatment for reduction of senescence; the combination of four different time-courses (0, 2, 6, 24 hrs) and four dose concentrations of fisetin (0, 20, 50, 100 µM) were investigated. All the data points are presented as the mean ± standard deviation (SD). Statistical significances were calculated based on one-way analysis of variance (ANOVA) and p-value ≤ 0.05 was considered significant.

RESULTS: *β*-gal staining confirmed senescence induction with 24 hr etoposide treatment (50 µM) that could be rescued by 24 hr etoposide treatment (50 µM) (**Fig. 1A**). Senescence induction by etoposide and reduction with fisetin in BM-MSCs was further confirmed by flow cytometry analysis using C_{12}FDG staining highlighting the C_{12}FDG assay as a valid detection method of senescence (**Fig. 2A**). qPCR analysis demonstrated elevated gene expression of the senescence marker *p21* as well as proinflammatory markers *IL-1B* and *IL-6* (**Fig. 2B**) indicating the efficacy of fisetin on reducing these specific markers (**Fig. 2B**). We next tested senolytic efficacy in hBMAC which contains MSCs to see if senescence signatures could be reduced with senolytic drugs in a dose and time dependent manner. Interestingly, it was found that 50µM fisetin treatment of hBMAC could be an effective therapeutic dose capable of reducing senescent cell number within 2 hrs (**Fig. 3**). It was also interesting that 50µM did not signifyingly alter senescence reduction capability or increase cell death between 20µM and 100µM after 2 hrs (**Fig. 3A)** suggesting that higher doses of fisetin could be safe and tolerable. The time course also revealed a decrease in C_{12}FDG cells even though there was no significant difference between 2, 6, or 24 hr time points (**Fig. 3B**).

DISCUSSION: Aging is the progressive physiological change that leads to the decline of fundamental biological functions and the onset of numerous age-related pathologies. Orthobiologics, specifically hBMAC has garnered significant attention for its regenerative properties and ability to help delay the onset of osteoarthritis. Here, we used BM-MSCs as a proxy for cell specific effects of senolytic treatments that target a fundamental property of aging, senescence. Our overarching goal was to link the therapeutic effect of fisetin on BM-MSCs and compare the outcomes through an *ex vivo* experiment and apply it to an upcoming *in vivo* experiment in a preclinical rat model of OA. FDA regulations regarding supplementation of specific drugs like fisetin applied to hBMAC presence a burden on an exciting, easily accessible source of a depleted senescent hBMAC product. Through our upcoming *in vivo* rat study, we hope to understand the translational aspects of our in vitro toxicity dose experiments to reveal why there is a wide range of efficacy of fisetin (0, 20, 50, 100 µM) with no significant difference in the cell death. This could imply a large therapeutic index of fisetin in a biological setting. Understanding the metabolic process of fisetin will help bring treatments of this senolytic drug to life as we understand the optimal therapeutic effect in different biological specimens and cell types.

SIGNIFICANCE/CLINICAL RELEVANCE: The ability to enhance hBMAC therapy in a timely and safe manner is important for the future therapies of orthobiologics. As we look to personalize medicine, fisetin treatment of hBMAC over a 2hr treatment course can improve the product given to patients. An *in vivo* rat study will be conducted via an osteoarthritis model to confirm these preliminary results and reveal the efficacy of this therapy in a biological setting.

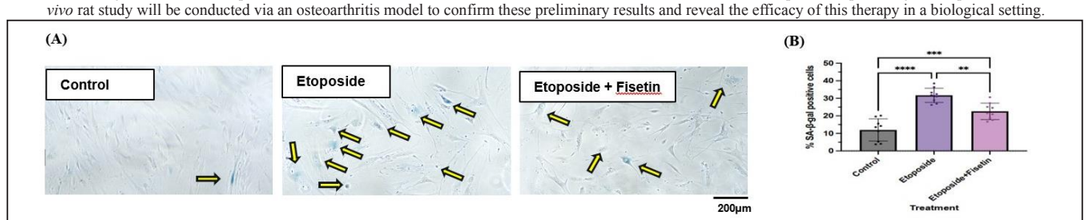

Figure 1. Senescence induction and Fisetin treatment using human bone marrow derived mesenchymal stem cells
(A) Microscopic images of β-gal staining, (B) % SA- β-gal positive cells

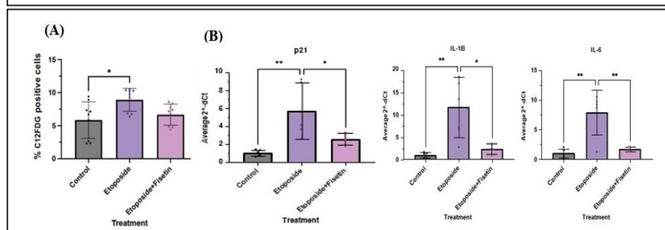

Figure 2. Senescence induction and Fisetin treatment using BM-MSCs
(A) Delta % C12FDG positive cells using flow cytometry, (B) qPCR after after senescence induction (50 µM of Etoposide, 24hr) with or without Fisetin treatment (50µM, 24hr). Mean±SD. Significant different: p< * 0.001, p< ** 0.0001, p< *** 0.001, p< **** 0.0001

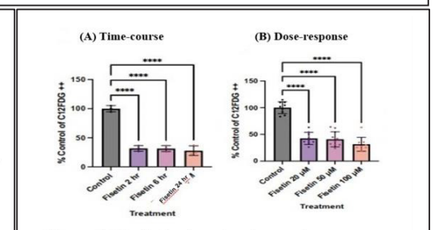

Figure 3. Fisetin treatment on human bone marrow aspirate concentrate
(A) Time-course experiment with 50 µM Fisetin.
(B) Dose-control experiment with 2 hr Fisetin treatment.
Mean's. Significant different: p< **** 0.0001

Skeletal Muscle Atrophy After ACL Rupture Can Be Mitigated By ERRγ Overexpression In Skeletal Muscle

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INTRODUCTION

Anterior cruciate ligament (ACL) reconstruction is the 6th most common orthopedic procedure performed in the United States (1,2). ACL injury leads to altered knee joint function, significantly increasing the risk of developing knee osteoarthritis. There is substantial evidence to suggest that muscle weakness contributes to adverse outcomes after ACL injury/reconstruction (3). Despite efforts to improve rehabilitation methods, current strategies often fail to restore pre-injury muscle strength in ACL-injured limbs. Our team has identified that estrogen-related receptor gamma (ERRγ) is a crucial regulator of paracrine angiogenesis in skeletal muscle (4). Selective over-expression of ERRγ in skeletal muscle (Err-gamma transgenic mice, TG) activates a robust paracrine angiogenic gene program involving myofibrillar induction and secretion of a battery of angiogenic factors resulting in promotion of muscle vascularization (4). The goal of this study was to determine if ERRγ-driven muscle angiogenesis can mitigate muscle atrophy after ACL injury by comparing TG mice to age-matched wild-type (WT) littermate control mice using a mechanically induced ACL injury model.

METHODS

Animals: 12 week old male and female TG and WT mice obtained from Dr. Narkar's laboratory were used for this study. The mechanically induced ACL rupture was conducted by our collaborators, Dr's Santangelo and Sikes at Colorado State University, who have specialized equipment for and prior experience with this injury model. The ACL rupture was performed on the right leg, and the left leg was used as non-injured control. The mice were euthanized four weeks after injury. The muscle tissues were harvested and the gastrocnemius muscle (GM) mass was flash-frozen in liquid nitrogen-cooled 2-methylbutane, and cryo-sectioned. The myofiber cross-sectional area (CSA) was measured based on the H&E staining on the GM muscle of TG and WT mice. **Cell Isolation and Myogenic Differentiation Assay**: Muscle progenitor cells (MPCs) from TG and WT mice were isolated using a modified preplate technique (5) and myogenic differentiation was performed by replacing the proliferation medium with fusion medium (DMEM with 2% FBS). The MPCs were isolated from TG ad WT control mice and the diameters of myotubes were measured based on desmin staining after myogenic differentiation. **H&E** staining was performed on 10 µm cryosections from GM according to the manufacturer's instructions. **Immunohistochemical staining and muscle force**: It has been reported that ACL injury promotes muscle fibrosis (8). To assess fibrosis, the number of PDGFRα (fibrogenic cell marker) + cells were counted in the skeletal muscle samples. Muscle sections were fixed with 4% paraformaldehyde. PDGFRα and desmin staining were performed as previously described (6). To assess muscle performance, we measured the isometric torque of the anterior crural muscles in TG and WT mice as previously described (7). The number of PDGFRα + cells and the diameter of myotubes were counted and measured using ImageJ software**. Statistical analysis:** All results are presented as mean ± standard deviation (SD). Means from ACL injured and non-injured of WT and TG mice were compared using Student's t-test, with a significance value of $p < 0.05$.

RESULTS

Muscle-specific ERRγ activation mitigated muscle fiber atrophy after ACL rupture. As expected, mean muscle fiber size was reduced in gastrocnemius muscles (GM) from the ACL-injured limbs of WT mice compared to the contralateral, uninjured limb (**Fig. 1A** and **Fig. 1B left plot, p<0.01**). Conversely, there was significant difference in the average size of muscle fibers between ACL-injured and control limmbs for the TG mice (**Fig. 1B right plot, P>0.05**). We also observed that the muscle fibers in TG mice were significantly smaller than WT muscle fibers (**Fig. 1B right, P<0.01),** due to a greater percentage of slow-twitch muscle fibers in muscles from the TG mice**.** Desmin staining demonstrated significantly decreased myotube diameter in the MPCs isolated from WT mice after ACL injury (**Fig. 1C, D, p<0.01**). However, this ACL injury-induced myotube atrophy was not observed in TG MPCs after ACL injury (**Fig. 1C, D**). Taken together, these results indicated that ERRγ overexpression in the muscle prevented muscle fiber atrophy after ACL rupture. ***Muscle-specific ERRγ activation ameliorated muscle function loss after ACL rupture.*** Muscle torque testing revealed that muscle strength was significantly decreased in the ACL injured limbs in both male and female WT mice compared with non-injured limbs (**Fig. 2A, male, 2B, female, 2C, p<0.01**). Strikingly, after ACL injury, the hindlimb muscles in TG mice were resilient to atrophy, as muscle function was similar in the ACL-injured and control limbs (**Fig. 2. A, B and D**). ***Muscle-specific ERRγ activation prevented fibrogenic cell expansion within skeletal muscle after ACL rupture.*** The number of PDGFRα+ cells was significantly higher in the WT muscle compared to TG muscle after ACL injury (**Fig. 3A and B, p<0.05**). In summary, those results demonstrated that muscle-specific ERRγ activation mitigated muscle atrophy and appears to reduce or prevent muscle fibrosis after ACL injury.

DISCUSSION

Skeletal muscle is adversely affected by the ACL injury and recovery (even after reconstruction surgery) is often limited by muscle weakness. In the murine ACL injury model, muscle weakness is correlated with a reduction in angiogenesis and accumulation of fibrosis. It has been reported that ERRγ expression in the skeletal muscle directly correlates with vascular density, and ERRγ is highly expressed in well-vascularized muscle beds (4). This evaluated whether ERRγ overexpression can prevent muscle weakness after ACL injury, and if so whether this preventive effect would be potentially linked to increased muscle angiogenesis. We observed that the TG mice with muscle specific ERRγ activation have the capacity to mitigate muscle atrophy, as evidenced by the maintenance of muscle fiber size and muscle function after ACL injury. It is known that exercise induces muscle angiogenesis, and regular physical activity has been considered a therapeutic modality for preventing aging-related muscle wasting. Although exercise is the primary method for alleviating muscle weakness, many patients cannot achieve the exercise intensity that is necessary to prevent or reverse muscle atrophy. ERRγ belongs to the nuclear receptor superfamily, which are excellent 'druggable' targets with unique ligand-binding pockets that facilitate selective and specific drug design. Thus, future development of pharmaceuticals targeting ERRγ could provide a safe and effective therapy for improving outcomes after ACL injury and potentially other musculoskeletal disorders.

SIGNIFICANCE: The results from this study will help to develop a novel rehabilitation approach that can significantly improve outcomes after ACL injury.

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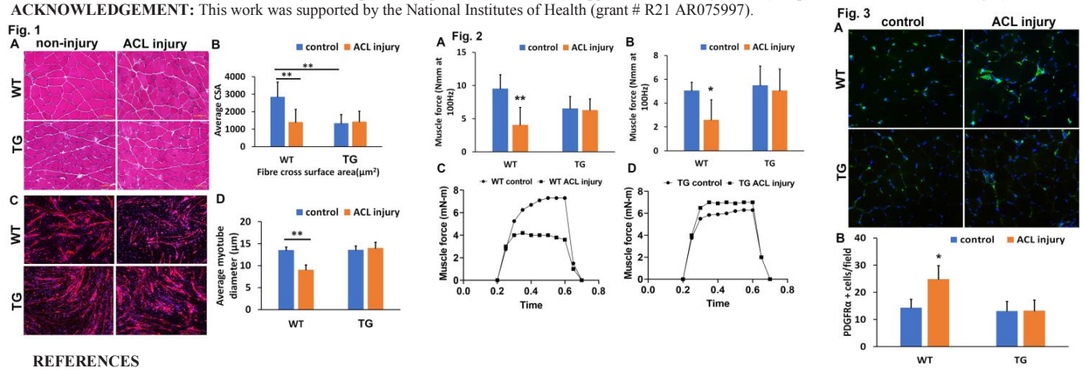

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Biomarker to Collagen X has Sensitivity to Detect Healing Difference in Long Bone Fractures

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INTRODUCTION: To date there remain no rigorously characterized biomarkers for fracture repair, limiting the possibility of exploring the quantitative effect of interventions on fracture healing. In this study we compare a novel collagen X biomarker (CXM)[1-3] with canonical bone turnover markers CTX (bone resorption) and P1NP (bone formation) in a cohort of isolated tibia and femur fracture patients. Unlike bone turnover markers, collagen X is a marker of endochondral ossification that is transiently expressed as cartilage transforms to bone during the intermediate stage of secondary bone healing. The VitaShock study was a phase II exploratory randomized trial comparing the effect of multiple vitamin D3 dosing strategies on fracture healing in patients with isolated lower extremity long bone fractures (tibia and femur) using the traditional bone turnover markers.[4] Here we perform a secondary analysis of trial patient serum to investigate CXM values in these patients allowing the first direct correlation analyses between these biomarkers. We then evaluate changes in CXM expression according to fracture healing as measured by mRUST radiographic scoring, fracture location and sex. We hypothesize that CXM may have increased sensitivity to distinguish differences in healing patterns when compared to bone turnover markers.

METHODS: 102 patients aged 18-50 receiving an intramedullary nail for a tibial or femoral shaft fracture were previously enrolled in an IRB-approved randomized controlled trial comparing VitD3 supplementation to placebo. Treatment allocation was double-blinded and included four VitD3 treatment arms: (1) 150,000 IU loading dose plus placebo daily dose; (2) 4,000 IU daily plus placebo loading dose; (3) 600 IU daily plus placebo loading dose; or (4) placebo; all dosing strategies concluded at 3 months post treatment. Serum biomarkers were measured perioperatively, at 6 weeks, and at 12 weeks post-injury using prior protocols: (1) CTX (bone resorption marker), (2) P1NP (bone formation marker), (3) CXM biomarker (endochondral ossification). Longitudinal radiographs were collected and scored using the mRUST system by a blinded fellowship trained traumatologist. Patients were grouped according to time to mRUST of 12: early healing (< 12 weeks), normal healing (13-26 weeks), and delayed healing (> 27 weeks).

RESULTS: Consistent with the previous analysis using CTX and P1NP as biomarkers[4], CXM biomarker analysis suggested VitD3 supplementation did not produce significantly different healing outcomes (data not shown). However, for the first time, we show significant correlations between CXM with P1NP ($p<0.0001$, **Fig 1A**) and CTX ($p<0.0001$, **Fig 1B**), with the most significant correlation with CTX at 6 weeks and to P1NP at 12 weeks ($p<0.0001$, **Fig 1C**). When dividing patients into early, normal, and delayed rates of healing, our data suggest that CXM in patients with early healing exhibited a significant peak in CXM at 6 weeks relative to their baseline values and that this peak value was significantly higher than those with delayed healing ($p = 0.016$, **Fig 2A**). CXM values were significantly higher in femur fractures at 6 weeks ($p=0.0415$) and 3 months ($p=0.0497$) compared to baseline, while no significant difference was seen between tibia fractures at any timepoint (**Fig 2B**). The CXM biomarker was also able to detect a higher trending level of biological healing in the femur patients at 12 weeks compared to tibia fractures ($p = 0.056$, **Fig 2B**). Sex-related differences were also noted with males producing higher CXM values than females at 6 weeks ($p=0.022$) and 3 months (**Fig 2C**) despite no sex-related differences noted in unfractured individuals ($p = 0.43$, data not shown).

DISCUSSION: There is no quantitative standard among clinicians to define the progression of fracture healing. The most utilized methodology within extremity fractures is to use radiographic images in conjunction with a validated, qualitative scoring system (mRUST). A novel serum biomarker, Collagen X (CXM), potentially presents a quantitative measure of a patient's fracture healing progression. Our data demonstrates significant correlation with biomarkers of bone turnover (P1NP and CTX), but we observed an increased sensitivity to detect differences in the rate or extent of healing with CXM. Specifically, CXM peaked in early healing fractures at 6 weeks and had a significant difference between the peak CXM value compared to delayed healers. CXM also distinguished a sex-specific difference in repair. In future studies we aim to explore whether earlier detection of CXM further increases ability of this biomarker to detect healing progression. Furthermore, sexual dimorphism of fracture repair has not been well studied at a clinical level and existing data remain inconclusive, suggesting this biomarker could offer the basis for future investigations. < >

SIGNIFICANCE: Validated quantitative biomarkers of fracture healing could be a key tool in personalizing patient fracture care, determining poor healing earlier, or better distinguishing differences in therapeutic protocols.

REFERENCES (PMID): [1]32533783, [2]35061649, [3]29212713, [4]35442058

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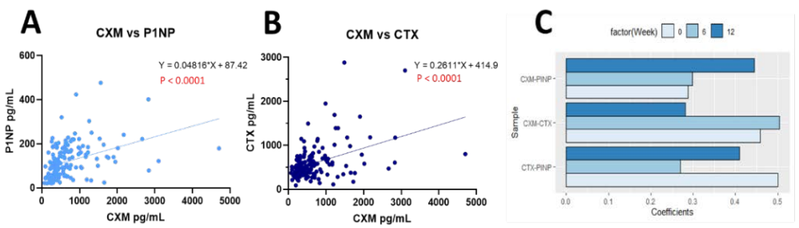

Figure 1. CXM and bone turnover markers were significantly correlated in the serum of long bone fracture patients. CXM and P1NP values **(A)** and CXM and CTX values **(B)** were significantly correlated. **(C)** Correlation coefficients were most significant at 12 weeks for CXM-P1NP, 6 weeks for CXM-CTX and 0 weeks for CTX-P1NP.

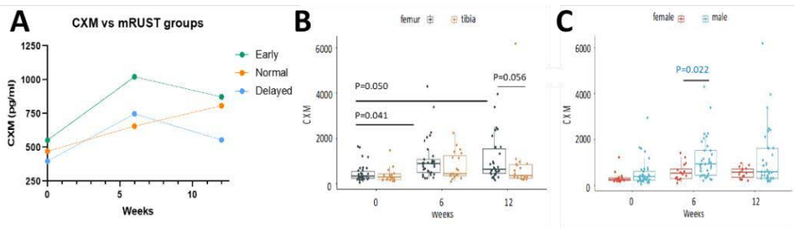

Figure 2. CXM expression vs. fracture healing as measured by mRUST radiographic scoring, fracture location and sex. **(A)** Delayed and early fracture healing exhibited a significant CXM peak at 6 weeks while normal fracture healing peaked at 12 weeks. **(B)** CXM values were significantly elevated in femurs at 6 weeks and 3 months vs. baseline. **(C)** CXM values were significantly elevated in males at 6 weeks and 3 months vs. females at baseline.

Genetic Model of Accelerated Aging Induces Elevated Systemic Senescence and Delayed Fracture Repair

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Disclosures: Johnny Huard (ORS Board of Directors, royalties Cook Myosite, Inc.) and Chelsea Bahney (ORS Section Leadership, TERMIS Board of Director, royalties Iota Biosciences), all others (N) *Co-Submitting

INTRODUCTION: Fracture healing is well established to present with significant age-related delays and increased risk for nonunion. The NIH standard for studies of age-related pathologies in mice is 24 months of natural aging, a constraint that creates a substantial time and monetary burden for studies that investigate mechanisms or therapies associated with age-related decline in bone healing. Progeria, a disease characterized by an accelerated aging phenotype, models have been utilized to research age-related diseases, but this study is the first to leverage a progeria mouse line to model fracture healing in aged mice. The *Zmpste24-/-* (*Z24*$^{-/-}$) mouse model recapitulates premature aged phenotypes of Hutchinson-Gilford progeria syndrome including genomic instability, epigenetic alterations, cellular senescence, stem cell exhaustion[1-4], and musculoskeletal deficiencies such as bone density loss, atypical skeletal geometry, sarcopenia, weight loss, osteoporosis and osteoarthritis (OA)[5]. The deregulation of lamin A/C processing in these mice leads to the destabilization of chromatin and DNA damage resulting in premature senescence, reduced osteogenesis, and increased adipogenesis[6-8] in bone. Additionally, frail individuals have reduced circulating osteoprogenitors[9] with reduced lamin A/C, providing evidence that the Z24$^{-/-}$ mouse model may have translational relevance to bone formation in the fracture setting. Given that these musculoskeletal deficiencies and aged phenotypes appear within 3-4 months of age in the Z24$^{-/-}$ mouse, we **hypothesize** that these mice will also present with delayed fracture healing when compared to age-matched wild-type (WT), C57BL/6J controls and similarly elevated senescent cell burden, chronic inflammation, and reduced fracture healing as aged WT mice.

METHODS: All procedures received IACUC approval (#1093) and followed NIH guidelines for ethical treatment of animals. Unfractured Z24$^{-/-}$ and WT mice tibiae were stained with Alizarin Red and scanned via µCT to assess bone density; blood samples were collected to assess senescent cell burden in unfractured young Z24$^{-/-}$ and WT (age=3-4 months, n=8) and old WT mice (age=18-21 months, n=8). Subsequently, Z24$^{-/-}$ mice and WT controls ages 3-4 months underwent right tibia fracture with intramedullary fixation. Mice received wet food prior to and after surgery to maintain body weight. Visual pain, movement (scale 1-10), and body weight were evaluated following surgery to ensure humane endpoints were upheld. Mice were sacrificed 9-, 14-, and 21-days post-fracture for blood and tissue collection. The following were collected to evaluate inflammation, senescence, and the rate of healing: fractured and contralateral tibias (days 14 & 21 histology, n=3/group), fracture calli (day 9 gene expression, n=3-5/group), organs (kidney, spleen, liver; day 9 n=3-5/group, day 14 n=8/group, day 21 n=3/group), and blood (day 14 n=8/group). Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood samples and frozen until processed. Inflammatory factors and cell-cycle regulator gene expression were evaluated using qRT-PCR; senescent cell burden was quantified using $C_{12}FDG$[10] (senescent cell stain), bafilomycin (reduce false positives), and DRAQ7 (live/dead stain) by flow cytometry for unfractured and subsequent post-fracture timepoints. Fracture healing was evaluated by gene expression 9- and 14- days post fracture, and functional healing was quantified from serial tissue sections stained with Hall Brundt's Quadruple stain followed by histomorphometry analysis 14- and 21- days after fracture.

RESULTS: Unfractured, Alizarin Red stained tibiae preparations and µCT showed that on average, Z24$^{-/-}$ mice have roughly 50% less bone density compared to WT mice at similar ages (data not shown, $p<0.05$). Prior to fracture, Z24$^{-/-}$ mice had significantly similar senescent PBMC burden to the aged WT mouse cohort, and both aged WT and young progeria mice had elevated senescence compared to young WT mice ($p=0.0003$ and $p=0.0126$, respectively) **(Fig. 1A-B)**. Systemic $C_{12}FDG$ senescent PBMCs ($C_{12}FDG$+) was found to be significantly higher in the Z24$^{-/-}$ mice both before ($p = 0.0126$) and 14 days following the fracture ($p = 0.0037$) **(Fig. 1C)** compared to young WT mice. 14 days after fracture, Z24$^{-/-}$ mice exhibited significantly higher expression of SASP factors *Il-1β* and *Tnf-α* in both the kidney and spleen compared to young WT mice **(Fig. 2A)**. Systemic expression of inflammatory markers *Il-4* and *TGF-β,* and senescent cell cycle regulators *p16* and *p21* all trended higher in progeria mice post-fracture and were statistically similar to inflammation markers in naturally aged mice (data not shown). Z24$^{-/-}$ mice also exhibited a significant increase in variance of expression in SASP factors *Il-1β*, *Il-4*, *Il-6*, *Tnf-α*, *Cx3cl1*, and *Mcp-1*, and cell cycle regulators *p16* and *p21* at all timepoints in both the kidney and spleen (data not shown). qRT-PCR assessment of fracture calli collected on day 9 demonstrated dysregulation in bone healing in the Z24$^{-/-}$ mice compared to their WT counterparts **(Fig. 2C)**. Lastly, histomorphometry illustrated that both 14- and 21- days (respectively) post fracture, Z24$^{-/-}$ mice had more cartilage ($p=0.0004$; $p=0.0051$) and fibrous tissue (ns; $p=0.0346$) volumes & less bone volume ($p<0.0001$) compared to young WT mice **(Fig. 3)**.

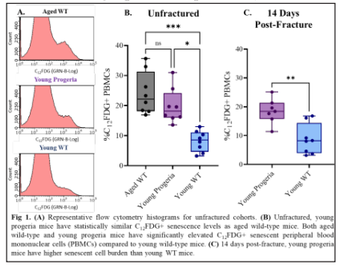

Fig 1. (A) Representative flow cytometry histograms for unfractured cohorts. **(B)** Unfractured, young progeria mice have statistically similar $C_{12}FDG$+ senescence levels as aged wild-type mice. Both aged wild-type and young progeria mice have significantly elevated $C_{12}FDG$+ senescent peripheral blood mononuclear cells (PBMCs) compared to young wild-type mice. **(C)** 14 days post-fracture, young progeria mice have higher senescent cell burden than young WT mice.

DISCUSSION: Z24$^{-/-}$ mice present compromised bone integrity and elevated PBMC $C_{12}FDG$+ senescence without fracture, supporting an age-associated skeletal pathology in the absence of an injury. After fracture, Z24$^{-/-}$ mice had increased senescent cell burden in the blood, heightened systemic SASP factor expression in the kidney and spleen, and significantly delayed endochondral fracture repair compared to age-matched WT mice. These findings support that the Z24$^{-/-}$ mouse model recapitulates not only key hallmarks of aging, but more importantly, mimics aberrant fracture repair in naturally aged mice including delayed and incomplete bone formation. Further studies are necessary to investigate fracture healing in Z24$^{-/-}$ mice at the molecular level and compare results to naturally aged mice (C57BL/6, NIA Colony) at all post-fracture timepoints

SIGNIFICANCE: This study suggests Z24$^{-/-}$ mice could function as an accelerated model for aged fracture repair, thus improving the feasibility of aged fracture studies by drastically reducing the time and monetary burden of studying novel therapeutic strategies to advance fracture healing in the elderly population. Further, given the link between senescence and age-related bone decline, this model could be leveraged to investigate senescence in the context of fracture repair and to screen senolytic therapies to develop novel treatments for age-related orthopaedic conditions.

REFERENCES: (PMID number): [1]11923874; [2]31312666; [3]23746838; [4]32710480; [5]11923874; [6]18847334; [7]21547077; [8]21982926; [9]26525092; [10]32961620

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Engineered platform for localized mRNA delivery in fracture repair

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INTRODUCTION: The long-term goal of this project is to develop an injectable and translational mRNA delivery platform specifically designed to localize therapeutic expression within a fracture site. There are currently no pharmaceutical approaches approved to accelerate fracture healing or to treat non-unions. Delivery of mRNA is an attractive strategy recently popularized by the novel coronavirus vaccine that delivers genetic material without genomic integration.[1] Liposomes are frequently employed for the delivery of mRNA to combat limitations associated with mRNA stability. However, traditional liposomes cause an innate immune response as the lipid nanoparticles are similar in size to pathogens.[3] Here, we explore using the combination of mineral coated microparticles (MCM) and lipid nanoparticles (LNPs) for mRNA delivery and their abilities to mitigate the limitations associated with mRNA delivery using liposomal complexes. MCMs have been shown to reduce cytotoxicity of non-viral vectors, improve transfection efficiency and kinetics *in vitro*.[2] In this study we aimed to test whether MCMs could be used *in vivo* to efficiently deliver mRNA to the fracture site. Further, we aimed to test whether MCMs could be used in combination with engineered nanoparticles (LNPs) that have been designed to reduce the innate immune reaction by carefully designing the lipid composition. We hypothesize that combining MCM and LNPs can prolong mRNA transfection and reduce immunogenicity seen when delivering lipid complexed mRNA alone and ultimately serve as an injectable and fully translational delivery platform.

METHODS: First, we tested MCM and LNPs separately in their capabilities to transfect chondrocytes *in vitro* and in a murine fracture model**.** MCM are comprised of a β-tricalcium phosphate core immersed in modified simulated body fluid (mSBF) to create tunable mineral coatings that can be doped with chemical compounds such as fluoride (FMCM) as described previously.[2] All *in vitro* studies were performed using the ATDC5 chondrocyte cell line cultured to hypertrophy. Ionizable LNPs were engineered using phospholipids and membrane proteins giving rise to nanoparticles around 100 nm in diameter.[3] Transfection kinetics and efficiency were studied using Firefly mRNA (TriLink) as a reporter gene and mRNA was isolated at various timepoints following delivery. Firefly luciferase (FFLuc) was used to measure the level of transfection efficiency and inflammatory markers *(IL-1β* and *TNFα* (not shown)) were probed to evaluate immune response. All *in vivo* procedures were approved through IACUC and executed according to ARRIVE guidelines. Delivery platforms were tested in a murine tibial fracture model stabilized with an intermedullary pin. mRNA platforms were injected into the fracture callus 6-8 days following fracture. IVIS imaging was used to locate and quantify firefly luciferase protein.

RESULTS SECTION: Both MCM and LNPs have previously been reported to deliver mRNA *in vitro* but have not been studied *in vivo* for fracture repair applications. Importantly, MCM and LNP have never been previously combined to use for treatment. To quantify MCMs and LNPs transfection characteristics, FFLuc mRNA was delivered to hypertrophic chondrocytes *in vitro* using the following delivery groups: 1) Lipofectamine (Lipo) alone, 2) Lipo complexed to MCM/FMCMs, or 3) in LNP's alone. Significantly more transfection was found in FMCM at 3 hours following transfection **(FIG 1A)** and significantly more transfection efficacy resulted when delivering LNP's as compared to Lipofectamine **(FIG 1C)**. Significantly less pro-inflammatory marker, IL-1β, was found when using FMCM **(FIG 1B)** and LNP **(FIG 1D)** as delivery carriers. An IVIS imaging time course shows both MCM and LNPs independently prolong and enhance mRNA delivery *in vivo* as compared to Lipofectamine **(FIG 2)**. Finally, we tested whether the combined treatments of MCM and LNPs encapsulating FFLuc mRNA had better transfection efficacy and kinetics.. *In vitro,* we found that LNPs combined with FMCM had significantly higher FFLuc expression compared to the control group 24 hours following transfection **(FIG 3A)**. Murine studies confirmed strong *in vivo* transfection across all groups but showed better localization to the fracture site with MCM platforms **(FIG 3B)**. Specifically, *ex vivo* IVIS demonstrates that LNPs alone had a propensity to travel to the liver despite tibial injection **(FIG 3C)**, yet this did not occur when coupled with MCM.

DISCUSSION: In this study, we aimed to develop a translational and localized mRNA delivery platform to improve therapeutic mRNA delivery for fracture repair. We first sought to determine the therapeutic potential of MCM and LNPs alone both *in vitro* and *in vivo*. Both platforms, MCM and LNPs, were found to supersede lipid-complexed mRNA in both transfection efficacy and prolonging kinetics. Preliminary data of the combined treatments display superior transfection capabilities *in vitro* and show reduced biodistributive properties *in vivo*. Here we show, for the first time, that combining therapies of MCMs and LNPs we can produce localized expression of mRNA within a fracture.

SIGNIFICANCE: There exists an unmet clinical need to stimulate bone regeneration through a non-surgical delivery platform. To combat the precedence of non-union and delayed healing in fractures, we are developing an injectable-based mRNA therapeutic using combined delivery platforms of MCM and engineered LNPs locally to the site of the fracture callus.

REFERENCES: 1). Sultana, N. et al. PMID: 28389322. 2). Fontana, G. et al. PMID: 31655263. 3). Molinara, R. et al. PMID: 29512198.

ACKNOWLEDGEMENTS: We gratefully acknowledge support from the Musculoskeletal Regeneration Partnership Fund by Mary Sue & Michael Shannon as well as funding through Orthoregeneration Network.

IMAGES AND TABLES:

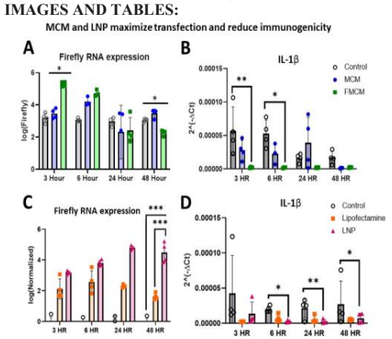

FIG 1. *Transfection efficacy and kinetics of mRNA delivery platforms* ***A).*** *MCM/FMCM and* ***C).*** *Lipofectamine and LNPs in vitro and their subsequent IL-1β activity following treatments* ***(B,D)*** *(**= p<0.03; *=p<0.05).*

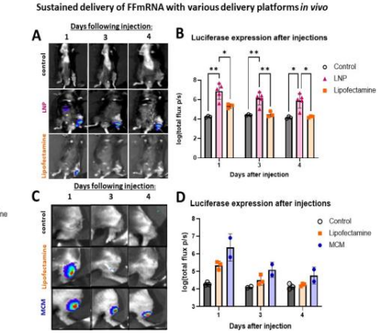

FIG 2. A,C). *IVIS images tracking transfection of Firefly luciferase using various delivery platforms.* ***B,D).*** *Quantification of IVIS images (**= p<0.01; *=p<0.03).*

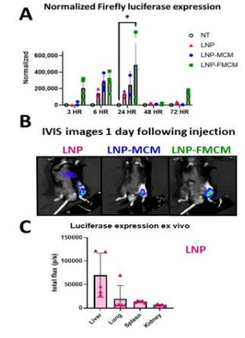

FIG 3. ***A).*** *Combined treatments maximize transfection,* ***B).*** *facilitate a localized expression and* ***C).*** *minimize biodistribution (*=p<0.05).*

DEPARTMENT OF BIOMEDICAL ENGINEERING

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SPRI'S DEPARTMENT OF BIOMEDICAL ENGINEERING is a collection of multidisciplinary laboratories including Biomotion, Robotics and Advanced Imaging. The team is comprised of scientists and researchers that apply quantitative, analytical and integrative methods to the field of orthopaedic medicine.

In 2021, the Department of Imaging Research officially joined the Department of Biomedical Engineering (BME), further integrating the labs and research projects. The team employs three full-time PhD researchers in Director Dr. Scott Tashman and Research Scientists Drs. Lauren Watkins and Colin Smith.

The BME team focuses on the role of mechanics and movement on injury and re-injury prevention as well as assessment of surgical and nonsurgical treatments for restoring musculoskeletal function. The team is dedicated to integrating clinical care, research and education, combining SPRI's engineering expertise with the resources of renowned orthopaedic surgeons from The Steadman Clinic, with the ultimate goal of improving treatment of musculoskeletal diseases and orthopaedic injuries. Biomechanics, motion analysis, advanced biomedical imaging, computational modeling and orthopaedic engineering are at the center of the department's research approach.

BME continues to collaborate closely with physicians, maintaining the imperative clinical lens that keeps its focus on high-impact research. BME's research portfolio has expanded significantly to include an essential role in nearly all of SPRI's clinical trials, providing advanced biomechanical and quantitative imaging assessments to evaluate the effectiveness of regenerative medicine treatments for restoring tissue health and function.

The department is home to a state-of-the-art Biomotion Laboratory for assessing human movement and function that includes video-motion analysis, an instrumented treadmill, force plates, a wireless EMG system, wearable IMU sensors, insole pressure sensors and a unique Dynamic Stereo X-ray system, utilizing cutting-edge technology designed by Dr. Scott Tashman, BME Director. Studies of musculoskeletal anatomy and new orthopaedic procedures are conducted in the department's Robotics Laboratory, which is one of the most advanced facilities of its kind in the world. This includes a custom robotic-arm that can recreate physiological joint motion, and video-motion analysis and 3D laser scanning technologies to capture musculoskeletal geometry and function. The BME team also conducts advanced imaging with its leading-edge 3.0 Tesla Siemens MAGNETOM Skyra and Canon Vantage Galan magnetic resonance imaging (MRI) scanners in Vail, Frisco and Basalt, Colorado, while applying imaging tools to improve patient outcomes.

The SPRI Golf Sports Medicine Program operates under the Department of Biomedical Engineering, integrating a high-tech simulator system within the Biomotion Laboratory. This unique combination of technologies enables SPRI to perform one of the most advanced biomechanical analyses of golf swings in the world, leading to a faster return to play after orthopaedic treatments, reduced injuries, and improved performance.

KEY HIGHLIGHTS

- The Biomedical Engineering team, including Robotics, Biomotion and Imaging, had an exceptional year in 2022 for disseminating work, including 38 papers accepted for presentation at major national/international meetings, including the Orthopaedic Research Society (ORS), World Congress of Biomechanics (WCB), Society for Military Orthopaedic Surgeons (SOMOS), Osteoarthritis Research Society International (OARSI), International Society for Magnetic Resonance in Medicine (ISMRM) and the American Orthopaedic Society for Sports Medicine (AOSSM). Additionally, in March 2023, SPRI's BME researchers presented 18 studies at the Orthopaedic Research Society, which is the premier conference for orthopaedic research. This is the most ever in the history of SPRI's biomedical engineering and imaging programs, representing the broad range of research conducted by this team in collaboration with our partner surgeons and fellows from The Steadman Clinic.

- The Biomotion Lab has had its busiest year ever in 2022, conducting 230 biomotion assessments for SPRI's NIH, DoD, industry and golf research studies.

- It was also a busy year for the Imaging team, who analyzed over 250 MRI scans that were acquired for SPRI's federally funded knee and hip clinical trials.

- The Robotics lab continued to develop innovative technologies to maintain its status as a leader in orthopaedic biomechanics. This included the development and validation of a unique sensor that can measure 3D forces for internal knee structures.

- The Biomotion lab added a state-of-the-art motion analysis system that uses eight cameras and machine learning/AI algorithms to perform comprehensive functional assessments and generate detailed reports on physical function in 20 minutes or less without requiring markers or special clothing.

- The SPRI Golf Sports Medicine Program currently has three active clinical trials investigating the impact on the golf swing on the shoulder, hip and spine.

ROBOTICS LABORATORY UPDATE

In the past year, the Robotics Lab has made significant strides in the field of meniscal root injuries by integrating two cutting-edge technologies: ultrasound and a 3-axis force sensor. Led by Engineers Alex Brady and Justin Hollenbeck, the lab aimed to answer two crucial questions regarding injuries to the posterior medical meniscal root.

Question 1: Effect of Meniscotibial Ligament Injury and Centralization

The first question addressed the impact of meniscotibial ligament injury and centralization on medial meniscal extrusion and three-dimensional forces at the posterior medial meniscal root. The meniscal roots are the attachment sites where the menisci connect to the tibia. Impaired function of the posterior medial meniscal root can result in knee instability, altered load distribution, reduced shock absorption and increased risk of knee joint degeneration (osteoarthritis). Because of the ligament's importance, it is critical to understand how injuries might affect knee health, and the efficacy of surgical approaches to ligament repair for restoring meniscal function. This study utilized highly innovative techniques, including ultrasound imaging to assess meniscal extrusion and a custom-designed miniature 3-axis force sensor (installed beneath the meniscal root) to measure forces.

The research team discovered that injury to the meniscotibial ligament led to increased extrusion and altered forces at the posterior medial meniscal root. Notably, an all-outside centralization surgical technique effectively restored extrusion to the native state. However, an inside-out centralization technique did not restore the native forces at the meniscal root. These findings emphasize the importance of selecting the optimal technique during meniscotibial ligament repair to minimize residual extrusion, reduce the risk of injury or reinjury to the meniscal root and preserve the long-term health and stability of the knee joint.

Building upon this work, the lab aims to investigate the relationship between meniscal extrusion and meniscal root forces in the presence of a meniscotibial ligament injury and all-outside suture repair under varying stages of cyclic loading. This ongoing research will further enhance our understanding of meniscal root injuries and inform clinical decision-making.

Question 2: Impact of Increased Posterior Tibial Slope

The second question addressed the influence of increased posterior tibial slope on forces at the meniscal root. The posterior tibial slope refers to the natural backward angle or slope of the top surface of the tibia. The lab observed that many patients with a torn posterior meniscal root also exhibited an increased posterior tibial slope. The hypothesis put forth by Hollenbeck and Brady suggested that loads on the meniscal root might be affected by the posterior tibial slope during physiologic joint loading.

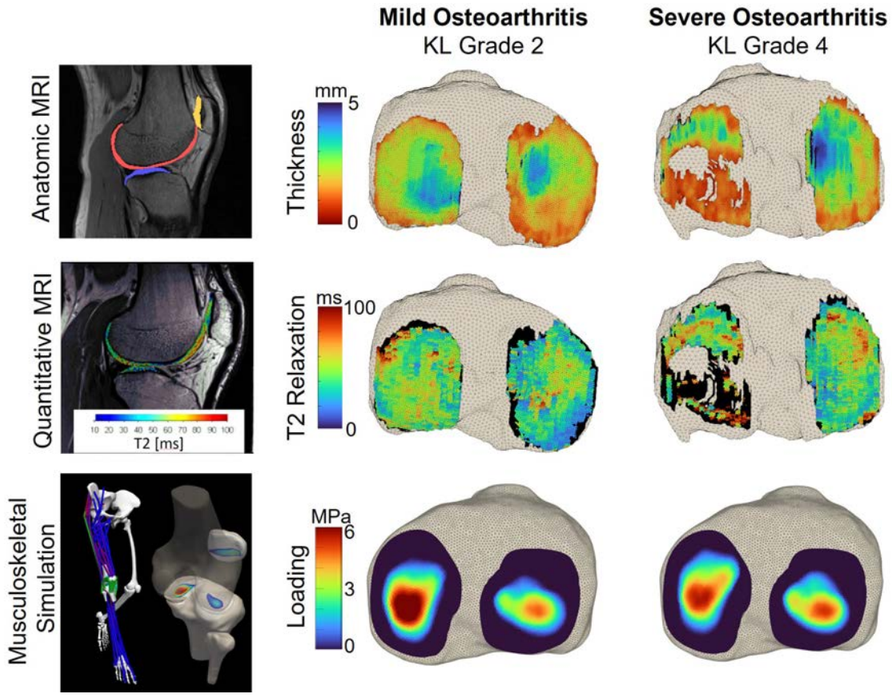
By utilizing the unique 3-axis embedded force sensor developed by the SPRI team, the research team made a significant breakthrough. They discovered that when the posterior tibial slope was increased during normal joint loading, the meniscal root experienced heightened compression and shear forces. This finding sheds light on the potential mechanism behind meniscal root failure in patients with an increased posterior tibial slope.

These results underscore the importance of performing slope-correcting osteotomies on patients undergoing a root repair in the presence of a high posterior tibial slope. The integration of the 3-axis force sensor enabled the lab to unravel the relationship between posterior tibial slope and forces at the meniscal root, thus providing valuable insights for clinicians and guiding treatment decisions.

A Successful Integration

The Robotics Lab's integration of ultrasound and the 3-axis force sensor has been instrumental in addressing these critical questions about injuries to the posterior medical meniscal root. The team's research has paved the way for a better understanding of the biomechanics involved and has practical implications for treatment strategies aimed at preserving meniscal function and patient well-being.

The Robotics Lab looks forward to continuing their groundbreaking research in the coming year, aiming to further advance our knowledge of meniscal root injuries and improve clinical outcomes for patients.



ADVANCED IMAGING AND BIOMOTION TEAMS DEVELOP NEW TECHNIQUE TO ADVANCE CLINICAL TRIALS

Last year, SPRI's Biomedical Engineering and Advanced Imaging teams fully integrated, allowing for more collaboration and synergies between the department's interdisciplinary laboratories. Scientists Lauren Watkins, PhD and Colin Smith, PhD, have exemplified this integration in their approach to SPRI's clinical trials on osteoarthritis (OA) by coupling quantitative MRI data and Biomotion measurements to investigate joint loading on OA development.

For SPRI's Department of Defense (DoD) Clinical Trial, "Senolytic Drugs Attenuate Osteoarthritis-Related Articular Cartilage Degeneration: A Clinical Trial," directed by Drs. Huard, Tashman and Philippon, Drs. Watkins and Smith developed advanced imaging and computer modeling strategies to enhance the interpretation of the extensive biomechanical and structural data collected to determine the effectiveness of the treatment. Participants completed MRI scans of the knee and a biomotion assessment at baseline (prior to their therapeutic treatment) and again at six and twelve months following treatment.

"YOU CAN SEE CHANGES IN MRI AND BIOMOTION DATA INDEPENDENTLY BUT THEY ARE MUCH MORE POWERFUL WHEN COMBINED."

— DR. LAUREN WATKINS

The knee MRI exam involved high-resolution, 3D quantitative imaging to investigate a patient's cartilage degeneration in two ways. First, the scan looked at the overall thickness of the cartilage, noting wear and tear in the tissue. Second, the quantitative scan included a technique called T2 mapping, which takes a closer look into the composition of the cartilage to assess changes to the tissue structure. From these measurements, the team could assess the severity of an individual's disease status and how their cartilage might handle loading with activities such as walking.

The Biomotion testing included video-motion capture assessments of walking, utilizing the Biomotion lab's 20-camera tracking system to measure dynamic movement of the lower-extremity joints and an instrumented treadmill to record foot-ground forces. This data was used to drive an advanced computational model developed by Dr. Smith to estimate forces internal to the knee joint. From this assessment, researchers could compare predicted cartilage contact pressure maps from the biomotion-based simulations with the measurements of cartilage degeneration from MRI. They observed trends where trial participants with more severe disease were offloading their joints as a pain response, whereas participants with more mild disease had more balanced loading patterns.

Drs. Watkins and Smith are currently analyzing data from the trial, and have already identified important findings regarding their technique. "You can see changes in MRI and biomotion data independently," shared Dr. Watkins, "but they are much more powerful when combined."

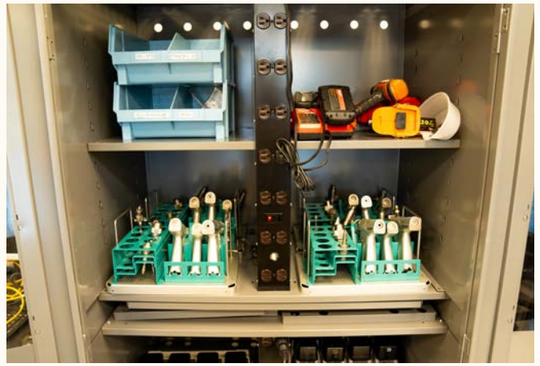
While clinical trials often incorporate either biomotion assessments or MRI, SPRI is on the forefront of combining advanced imaging and motion assessment techniques with advanced analytic approaches. "The computational prediction correlated better with MRI-based assessment of joint damage than traditional motion analysis measures like joint angles and moments," Dr. Smith said. "Our technique allows us to predict cartilage contact pressures," he continued, "and we're able to correlate that with MRI to get a clearer picture of the tissue loading environment inside the joint during functional movements and how this relates to osteoarthritis development."

As the research team finalizes the analysis on this project, they've garnered some key initial insights. "We're able to see that people who have significant degeneration on MRI and also have abnormal loading patterns in the computer model are more likely to develop painful arthritis, no matter what treatment they have," explained Dr. Watkins. "So, when we think long-term, we'd be able to save patients a lot of time and improve their treatment selection by knowing their cartilage and joint loading status in advance or even allow us to intervene earlier in their disease timeline. This ties in with our mission of personalizing patient care."

"A goal for this research," added Dr. Smith, "is to develop screening tools that can not only identify patients who may not be great candidates for biologic injections, but can also identify the candidates who are most likely to benefit from the treatment, improving outcomes as well as reducing unnecessary or ineffective treatments. The coupling of MRI and computer models can make that happen."

The research team presented their initial findings at the Summer Biomechanics, Bioengineering and Biotransport (SB^3C) conference in June 2023. Upon completing data analysis, they will publish a manuscript on the MRI and computational modeling component of the clinical trial. The team plans to apply similar techniques to SPRI's Regenerative Medicine Innovation Project (RMIP) clinical trial funded by the National Institutes of Health (NIH) and expand their technique to include the measurement of pain as it correlates to cartilage degeneration.

SURGICAL SKILLS LAB UNDERGOES SIGNIFICANT RENOVATION



One of the differentiating elements of SPRI's Biomedical Engineering Department is the Surgical Skills Laboratory. Co-located with The Steadman Clinic, the Surgical Skills lab is an essential aspect of SPRI's clinical fellowship programs. These fellows—who join SPRI out of their orthopaedic residency programs¬—hone their skills in the laboratory, where they practice techniques and treatments under the mentorship of their attending faculty, surgeons from The Steadman Clinic. The lab is a key resource for SPRI's biomechanics studies (in the adjacent Robotics lab) and it is also used as an education tool for local students participating in SPRI's educational programming, ski patrollers and other visitors.

LAB REDESIGN MAKES IMPACT

Thanks to a generous philanthropic donation, SPRI's Surgical Skills lab recently underwent a major renovation, including an overhaul of the lab's layout. Moving the arthroscopy stations to the outside walls enabled creation of a large, visually appealing open space with worktables in the center of the lab for greater collaboration during labs and learning experiences. This rearrangement of the lab allows for more than 6 stations to be used at once, while also promoting collaboration and education in the center of the work station. State-of-the-art surgical equipment was purchased so that the fellows can now train with the same instruments used in the clinical operating rooms. Along with the new instruments, medical device manufacturers sent new medical implants for use in the lab; these mimic those used in operating rooms. The final step of the renovation, installing an advanced 4k video system to facilitate international collaboration and training, is in progress, with completion expected in the summer/fall of 2023.

A CYCLE OF EDUCATION IN THE LAB

The Surgical Skills lab offers clinical fellows the opportunity to practice their techniques before they operate on patients. The Surgical Skills Lab Manager, Natalie Cortes, works closely with researchers, attendings, fellows and scientists in the lab. It's a place for asking questions and working through solutions, refining techniques and investigating how treatments work.

It's estimated that to become an expert at a procedure, it takes at least 30 times in practice. Most other clinical fellowships offer under 10 opportunities to practice on specimens in the lab. In SPRI's Surgical Skills Lab, fellows are in the lab at least 90 times in the course of their year, allowing them to have a level of repetition that leads to confidence in the operating room when they graduate from fellowship. This level of access to hands-on training is unique to the TSC/SPRI clinical fellowship program. Between this valuable resource and the skills and knowledge of the attending surgeons at The Steadman Clinic, patients seeing a recent SPRI fellowship alumnus can feel confident that their surgeon is exceptionally well trained and prepared to treat them with the latest, most effective surgical techniques.

SEEING THINGS IN A NEW WAY

For The Steadman Clinic surgeons, the lab provides the opportunity to investigate sports medicine pathologies, practice surgical techniques and develop innovative treatments and techniques. For SPRI researchers, the lab enables recreation of any surgical technique for advanced biomechanical studies. Local ski patrollers, coaches, athletic trainers and other sports professionals who visit the lab get a unique perspective on the injuries they assess on the hill or the field. And for young students, participating in simulated scopes with model joints can ignite a spark for a future in STEM.

LOCATED AT THE NORTHEAST CORNER OF THE STEADMAN CLINIC—AND NOW OUTFITTED WITH THE LATEST EQUIPMENT AND INSTRUMENTATION—

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BME 2023 ORTHOPAEDIC RESEARCH SOCIETY (ORS) ABSTRACTS

Gait Evaluation of a 3-Layer Carbon-Fiber Orthotic Device for Post-operative Ankle Fusion Patients

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Introduction: Despite the high success rate of ankle fusion (AF) for patients with degenerative ankle arthritis, many patients still experience functional limitations during daily activity, including reduced sagittal ankle range of motion, plantarflexion moment and power, and greater gait asymmetry[1,2]. Several consumer orthotics and footwear options are available to AF patients, including rocker-bottom shoes, heel lifts, and solid ankle cushion heels, though none have been shown to restore normal gait biomechanics[3]. Recently, carbon-fiber insoles placed in high-performance running shoes to increase longitudinal bending stiffness have been shown to increase running economy, up to 3% [4]. To our knowledge, no studies have been performed investigating the use of carbon-fiber orthotic insoles for AF patients. This study aimed to determine whether a 3-layer carbon-fiber orthotic insole could improve gait biomechanics for AF patients. Our central hypotheses were that 1) gait asymmetry in joint kinematics and kinetics, 2) ground reaction force (GRF) at heel strike, and 3) muscular activation would each be reduced in AF patients while wearing the orthotic insole device.

Methods: Seven participants (2 females; age: 55 ± 9.9 years; weight: 84.7 ± 19.9 kg; height: 1.78 ± 0.11 m) who were at least 29 months removed from single-leg total ankle fusion surgery (mean 77.1 ± 27.9 months) were recruited from the patient population at The Steadman Clinic (Vail CO). This study was IRB-approved and all subjects gave their written informed consent prior to data collection. Three footwear conditions were evaluated, each in standardized shoes: a control condition with no orthotic (NO), a regular orthotic condition (RO), and a stiffened orthotic condition (SO). To create the SO condition, a compressible foam wedge was inserted behind the middle and bottom layers of the device, providing increased resistance to compression of the orthotic. Gait analysis was performed in each condition on a split-belt instrumented treadmill with a 16-camera motion-capture system and surface EMG at two different walking speeds: a self-selected pace (Normal: 1.01 ± 0.11 m/s), and a speed that was 20% faster (Fast: 1.22 ± 0.13 m/s). Twenty-six retroreflective markers were placed on the shoes, legs, hips, and trunk of each participant for motion capture. Participants were fitted bilaterally with wireless EMG sensors on their medial gastrocnemius. Nine gait cycles were selected as representative of each condition for analysis, and statistical tests evaluating the effect of the orthotics were performed independently for the Normal and Fast walking speeds. All kinetics, kinematics, and EMG data were normalized to 0 to 100% of stance phase and filtered with a zero-phase low-pass fourth-order 6Hz Butterworth filter. Joint moments and powers were normalized to the product of each participant's height and weight, and ground reaction forces were normalized to weight. Paired t-tests using Statistical Parametric Mapping (SPM) were implemented in MATLAB using open-access SPM1D functions to assess the effect of each orthotic condition on the data of the affected leg[5]. Gait asymmetry was quantified using a symmetry ratio of affected versus contralateral leg swing time, as a percentage of cycle time[6]. All statistical tests were performed with a significance level of 0.05.

Results: SPM paired *t*-tests showed statistically significant reductions in ankle plantarflexion moment, knee extension moment, and gastrocnemius activity during midstance (~30-70% stance) in the SO condition when compared to the control. Significant effects were still seen in the RO condition compared to the control, though to a lesser degree than SO. Significant reduction in impact force occurred at heel-strike (0-10% stance) in each insole condition, with greater reductions seen in the SO condition than the RO condition. Non-parametric paired *t*-tests showed significant reductions in gait asymmetry at both walking speeds for the SO condition, decreasing gait asymmetry by between 1.1% and 2.6% across all comparisons.

Discussion: The stiff orthotic (SO) condition showed the most statistically significant changes in gait compared to the control. Reductions in sagittal knee and ankle moments and gastrocnemius muscle activity during mid-stance suggests savings in muscular effort for the participants. Ground reaction forces were also reduced at heel-strike in both orthotic conditions, indicating a level of energy absorption by the orthotic device at impact. The SO condition, with the additional foam wedge inserted between the carbon-fiber spring system, acts as a better shock absorber than the carbon-fiber on its own, dampening the impact of the foot at heel-strike. Vertical GRFs were also reduced in terminal stance in both RO and SO as compared to the control, near the point of peak push-off force, indicating that the energy absorbed at heel-strike was not released at toe-off. Previously discussed reductions in knee and ankle moments and gastrocnemius EMG indicate the energy may have been returned during mid-stance instead, although the viscoelastic properties of the foam may lead to overall loss of some of the energy absorbed at impact, resulting in less energy returned overall. Lastly, reductions in patient gait asymmetry in the SO condition can be seen as a positive impact of the device. Further research is needed on the optimal stiffness of the orthotic device, and on patient comfort and quality of life with long-term use.

Significance: No options are available to ankle fusion patients that can restore normal gait biomechanics post-surgery. Though this study only investigates the effects of an orthotic device on a small sample size, significant results in specific gait metrics are promising for future fusion patients.

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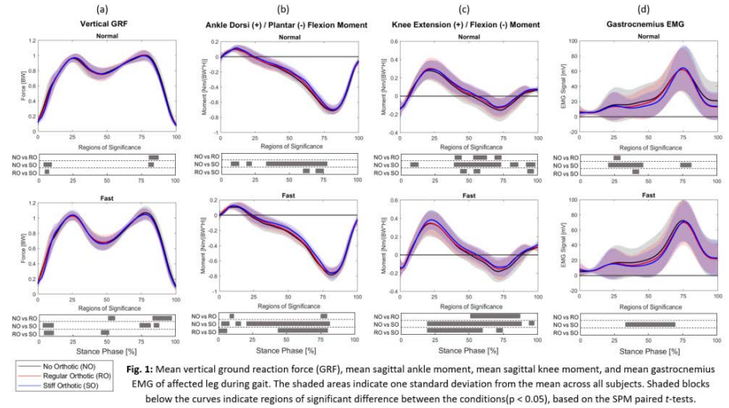

Fig. 1: Mean vertical ground reaction force (GRF), mean sagittal ankle moment, mean sagittal knee moment, and mean gastrocnemius EMG of affected leg during gait. The shaded areas indicate one standard deviation from the mean across all subjects. Shaded blocks below the curves indicate regions of significant difference between the conditions($p < 0.05$), based on the SPM paired *t*-tests.

Diffusion Weighted Imaging Metrics are Related to Muscle Strength in Asymptomatic and Osteoarthritic Thigh Muscles

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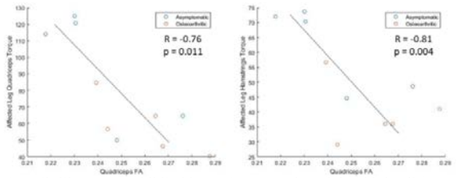
Introduction: Altered muscle physiology leading to poor muscle function is one of the greatest contributing factors to ongoing disability following musculoskeletal diseases such as osteoarthritis. Muscle health and quality are often measured using subjective criteria that are not well correlated to functional outcomes. Quantitative MRI techniques such as diffusion weighted imaging (DWI) show promise for objective evaluation of structural alterations including fatty infiltration, edema, atrophy, and muscle fiber disruption[1-4]. However, DWI measures vary throughout literature, in part due to differences in b-values selected for the diffusion sequence[5-7]. In this preliminary study, we examine differences between DWI measures in the muscles of asymptomatic volunteers and patients with knee osteoarthritis calculated with different b-values and investigate the links between DWI measures and muscle function.

Methods: Asymptomatic volunteers and patients with knee osteoarthritis scheduled for a total knee arthroplasty were recruited for the study.. Participants were excluded if they had a history of a muscular disorders, cardiovascular disease, diabetes, or surgery in the study leg within the past year. Demographic information was collected prior to a muscle scan on a 3T MRI (Skyra-Fit, Siemens Medical Solutions USA) using body and spine coils. Asymptomatic and osteoarthritic participants had a scan of the dominant leg or affected leg in the mid-thigh area, respectively. Sequences included a 2-point Dixon sequence for morphological imaging and quantification of muscle fat fraction (TR = 4.06 ms, TE = 1.3 ms, voxel volume = 1.18 mm x 1.18 mm x 3 mm). A multi-slice spin-echo planar imaging (EPI) diffusion-weighted sequence with fat saturation (TR = 1700 ms, TE = 60 ms, voxel volume = 2.58 mm x 2.58 mm x 4 mm; MDDW mode) was used to calculate the diffusion tensor for b-value pairs of 0 s/mm^2 (2 averages, 1 direction) and either 375, 500, 625, 750, or 875 s/mm^2 (6 averages, 6 directions) using the MuscleDTI Toolbox for MATLAB[8,9]. Quadriceps and hamstring muscles were segmented as regions of interest from Dixon images using ITK-SNAP and applied to diffusion images to calculate the mean diffusivity (MD) and fractional anisotropy (FA) for each b-value pair. Finally, muscle strength was assessed using a HUMAC NORM isokinetic testing system (Computer Sports Medicine Inc.) with participants performing 3 sets of 15 repetitions of maximum-effort flexion and extension. The average total work and torque in the hamstrings and quadriceps were compared to the MD and FA in those muscle groups using a Pearson's correlation coefficient. Differences in hamstrings and quadriceps MD and FA between groups and between b-value pairs were compared with a two-way ANOVA test.

Results: Matched five asymptomatic participants (66 ± 6 years, BMI 24.3 ± 2.5 kg/m^2, 2 females) and five osteoarthritic participants (66 ± 4 years, BMI 27.2 ± 5.7 kg/m^2, 2 females) were included in the study. The mean fat fraction for the asymptomatic group was 8.5 ± 1.7% in the quadriceps and 16.2 ± 1.5% in hamstrings; in osteoarthritic participants the mean fat fraction was 14.4 ± 4.6% in the quadriceps and 20.8 ± 6.9% in the hamstrings. There were significant differences between asymptomatic and osteoarthritic hamstrings FA ($p = 0.005$), quadriceps FA ($p = 0.012$), and hamstrings MD ($p < 0.001$), but not quadriceps MD ($p = 0.128$) (Table). MD calculated with b-value pairs of 0-375 and 0-500 resulted in significantly greater hamstrings FA ($p = 0.003$), hamstrings MD ($p < 0.001$), and quadriceps MD ($p < 0.001$) compared to those calculated with a b-value pair of 0-875. Quadriceps FA was strongly negatively correlated with the quadriceps work in the affected and unaffected legs, quadriceps torque in the unaffected leg, and hamstrings torque in both legs for 0-375, and also with the affected leg quadriceps torque for 0-500 and 0-625 pairs (Figure). In the 0-750 pair, quadriceps FA was correlated with quadriceps torque in both legs and quadriceps work in the unaffected leg; quadriceps FA was only correlated with unaffected leg quadriceps torque in the 0-875 pair. There were no significant correlations between muscle work and torque and hamstrings FA, quadriceps MD, or hamstrings MD.

		Asymptomatic		Osteoarthritic	
		Hamstrings	Quadriceps	Hamstrings	Quadriceps
Fat fraction (%)		16.2 ± 1.5	8.5 ± 1.7	20.8 ± 6.9	14.4 ± 4.6
Mean Diffusivity (x10^{-3} mm^2/s)	0-375	1.65 ± 0.07	1.57 ± 0.06	1.52 ± 0.12	1.55 ± 0.08
	0-500	1.58 ± 0.04	1.51 ± 0.05	1.46 ± 0.13	1.49 ± 0.08
	0-625	1.54 ± 0.03	1.46 ± 0.05	1.40 ± 0.13	1.43 ± 0.07
	0-750	1.46 ± 0.04	1.41 ± 0.04	1.34 ± 0.14	1.38 ± 0.06
	0-875	1.40 ± 0.05	1.35 ± 0.05	1.28 ± 0.15	1.32 ± 0.06
Fractional Anisotropy	0-375	0.35 ± 0.05	0.24 ± 0.02	0.32 ± 0.05	0.26 ± 0.03
	0-500	0.33 ± 0.05	0.24 ± 0.02	0.30 ± 0.06	0.26 ± 0.02
	0-625	0.31 ± 0.04	0.24 ± 0.03	0.29 ± 0.06	0.26 ± 0.02
	0-750	0.30 ± 0.02	0.24 ± 0.03	0.27 ± 0.06	0.25 ± 0.03
	0-875	0.28 ± 0.02	0.23 ± 0.01	0.24 ± 0.03	0.24 ± 0.02
Total Work (ft-lb)	Affected Leg	62 ± 14	95 ± 35	40 ± 10	59 ± 17
	Unaffected Leg	53 ± 20	80 ± 22	40 ± 23	61 ± 20
Total Torque (ft-lb)	Affected Leg	44 ± 14	74 ± 31	37 ± 9	57 ± 13
	Unaffected Leg	45 ± 12	74 ± 18	39 ± 22	58 ± 16



Discussion: There were significant differences in quadriceps and hamstring FA and hamstring MD between asymptomatic and osteoarthritic groups. Only quadriceps FA showed significant correlations with muscle strength, particularly for FA calculated using a b-value a pair of 0 and 500 s/mm^2. These trends agree with prior literature, which suggest that an upper b-value between 400-500 s/mm^2 yields improved diffusion tensor accuracy in muscle tissue[10]. Muscles in the osteoarthritic group exhibited lower torque and work but higher FA values compared to asymptomatic muscles. Higher FA values in osteoarthritic muscles may suggest greater muscle atrophy in this group but may also be subject to bias from elevated muscle fat fraction compared to asymptomatic muscles. Overall, these preliminary findings suggest that DWI metrics are related to muscle changes associated with osteoarthritis and are related to muscle strength.

Significance/Clinical Relevance: This study investigates the links between muscle DWI MRI measures calculated using different b-values and muscle function between asymptomatic volunteers and those with knee osteoarthritis. DWI shows promise for objective, non-invasive evaluation of structural alterations in muscle including fatty infiltration, atrophy, and muscle fiber disruption related to osteoarthritis.

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Average Femoral Cartilage T2 Relaxation Times are Related to Cellular Senescence in Peripheral Blood

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INTRODUCTION: Osteoarthritis (OA) is the most common form of degenerative joint disease and causes debilitating pain in many adults (1). Although OA is one of the greatest causes of disability, factors leading to its initiation and progression are still not fully understood, and currently there are no disease-modifying therapies to treat OA. The accumulation of senescent cells is a fundamental property of aging (2, 3, 4) and may be a contributing factor to age-related diseases such as OA. These cells produce pro-inflammatory cytokines/chemokines, proteases, and other factors, otherwise known as the senescence-associated secretory phenotype (SASP) (4). The accumulation of senescent cells may be linked with OA pathogenesis, and OA severity has been reduced by the removal of senescent cells in pre-clinical models (5). In this study, we aim to investigate the relationship between cellular senescence in peripheral blood and the severity of osteoarthritis-related cartilage degradation in the knee, as measured using T2 relaxation times from MRI.

METHODS: Twenty four individuals with knee OA (13 female, aged 61 ± 9 y, BMI 26.5 ± 4.9 kg/m^2, Kellgren-Lawrence grade II-IV) participating in an IRB-approved, ongoing clinical study investigating senolytics for the treatment of OA (NCT04210986) were included in this study. Only baseline data, prior to any study therapy, are presented. The affected knees were scanned on a 3T MRI system (SkyraFit, Siemens Healthineers USA) implementing a double echo in steady state (DESS) sequence for cartilage segmentation and a multi-echo spin echo (MESE) sequence for calculation of T2 relaxation times. Femoral cartilage was first manually segmented from DESS images using Mimics software (Materialize). T2 relaxation time images were created using MapIt software and registered to DESS images in Mimics. Femoral, tibial, and patellar cartilage masks were applied to T2 images, and the average T2 relaxation time was calculated for each cartilage surface. At the same visit, a peripheral blood sample was taken, and flow cytometry was used to quantify senescence levels in the isolated peripheral blood mononuclear cells (PBMCs) and T-cell subsets using the analyte $C_{12}FDG$. Correlations between femoral cartilage T2 relaxation times and the percentage of bright PBMCs and T-cells on flow cytometry were assessed using a Pearson correlation test ($\alpha = 0.05$).

RESULTS: The average femoral cartilage T2 relaxation time was significantly correlated with the percentage of bright T-cells ($R = 0.522$, $p = 0.011$, Figure 1) and the percentage of bright PBMCs ($R = 0.590$, $p = 0.005$, Figure 1). There were no significant correlations between the average tibial T2 relaxation time and bright T-cells and PBMCs ($R = 0.305$, $p = 0.179$ and $R = 0.150$, $p = 0.539$ respectively), nor between the average patellar T2 relaxation time and bright T-cells and PBMCs ($R = 0.522$, $p = 0.546$ and $R = 0.266$, $p = 0.339$ respectively).

DISCUSSION: There were significant correlations between the average femoral cartilage T2 relaxation time and both the percentage of bright T-cells and the percentage of bright PBMCs. Higher average femoral T2 relaxation times, related to a greater degree of cartilage degradation, were associated with higher cellular senescence in peripheral blood as characterized by elevated levels of bright PBMC and T-cell peripheral blood populations. PBMCs function as an accessible cell population that can be used to determine systemic senescence. Additionally, high senescence levels in T-cells are associated with frailty, rheumatoid arthritis, and bone loss (6). Quantifying senescence in both cell populations will be useful in determining the clinical utility of blood-based and MRI-based markers of OA severity. While the sample size is small in this exploratory study, both average femoral cartilage T2 relaxation times and cellular senescence in peripheral blood will continue to be monitored over the course of the trial to provide further information about the relationship between them.

SIGNIFICANT CLINICAL RELEVANCE: Cellular senescence, a contributor to pro-inflammatory and age-related degeneration, is related to the extent of cartilage degeneration as assessed using T2 MRI. Monitoring the changes in both measures in response to treatment may provide complementary information about the efficacy of new OA treatments.

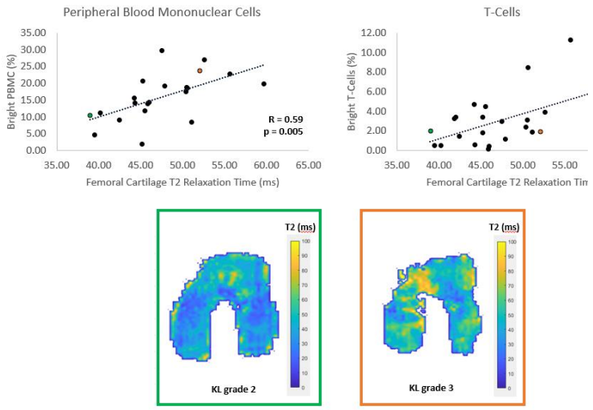

Figure 1: Average femoral T2 relaxation times were positively correlated with the percentage of bright PBMCs and T-cells in peripheral blood, suggesting that cellular senescence is related to cartilage degeneration in osteoarthritic knees. The femoral cartilage T2 relaxation time map is shown for a participant with low cellular senescence (green) and high cellular senescence (orange).

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Ultrasound Detects Increased Vascularity Following Rotator Cuff Tears Compared to Asymptomatic and Repaired Rotator Cuff Tendons

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Disclosures: Canon Medical Systems, Inc. Provided use of the ultrasound system used for this work at no cost to the institution.

INTRODUCTION**:** Tears to the rotator cuff tendons are among the most common clinical tendon complications in the aging population [1]. Tendons are dense, highly organized, and relatively avascular tissues; however, tendon structural integrity and vascularity decrease with age and may be related to increased injury risk with age [1-3]. Further, tendon vascularity may change in the presence of tendon pathology consistent with acute inflammatory and healing processes such as in response to rotator cuff tears [4,5]. The aim of this study is to examine the microvasculature of the supraspinatus tendon (SSP) and long head of the biceps tendon (LHBT) using an ultrasound technique for superb microvascular imaging (SMI) in asymptomatic, torn, and repaired rotator cuff tendons.

METHODS**:** This prospective study was approved by the institutional IRB and included 18 volunteers with asymptomatic shoulders (55 ± 18 y BMI 22.6 ± 3.0 kg/m^2, 7 males), 6 with an unrepaired rotator cuff tear involving the SSP (64 ± 3 y, BMI 24.6 ± 4.1 kg/m^2, 5 males, mean 28 months since tear), and 6 who had undergone a rotator cuff repair and biceps tenodesis surgery at least 4 months prior (57 ± 10 y, BMI 25.5 ± 1.5 kg/m^2, 5 males, mean 7 months since repair). An ultrasound examination was performed using an Aplio i800 system and an i18LX5 linear array transducer (PLI-1205BX/FS, Canon Medical Systems, Inc.). Two orthopedic surgeons with 6 years of experience with musculoskeletal ultrasonography measured microvasculature in the SSP and proximal LHBT with the SMI mode. The area of the vascular signal (cm^2) within the tendons was recorded in both transverse and longitudinal orientations. Correlations between tendon vascular signal and age were assessed for asymptomatic subjects using a Pearson's correlation coefficient. Differences between asymptomatic, tear, and repair cohorts and between readers were compared using a two-way ANOVA statistical test with a Tukey post-hoc analysis.

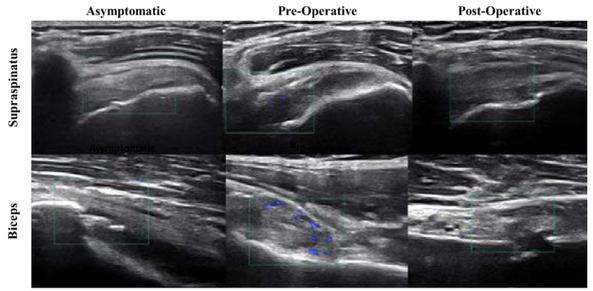

Figure 1: Representative images showing SMI microvascular signal (blue) overlaid with B-mode ultrasound images of the supraspinatus (top) and biceps tendon (bottom) in the longitudinal plane for three 65-year-old individuals in the asymptomatic, pre-operative, and post-operative cohorts.

RESULTS**:** There was significantly greater vascular signal in the longitudinal direction of torn supraspinatus (p = 0.036, Figures 1 and 2) and biceps tendons (p = 0.002, Figure 1) compared to asymptomatic and post-operative tendons. There were no differences between groups when vascularity was measured in the transverse direction (SSP p = 0.728, biceps p = 0.264). Correlations could not be found between the age of asymptomatic participants and the vascular signal within the SSP (R = 0.1732, p = 0.5063) and LHBT (R = -0.3594, p = 0.1566) in the longitudinal direction, nor in the transverse direction for the SSP (R = -0.2903, p = 0.2583) or LHBT (R = 0.2498, p = 0.3335).

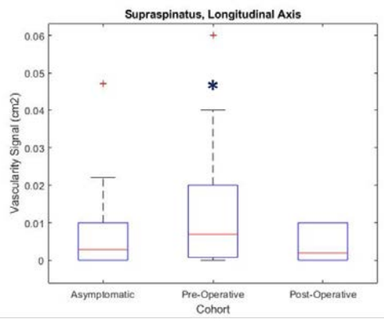

Figure 2: Tendon vascularity was elevated in torn supraspinatus and biceps tendons, and was not significantly different from asymptomatic tendons 4-8 months post-operatively. * p = 0.036.

DISCUSSION**:** There was minimal vascular signal within asymptomatic tendons. The vascularity signal increased in individuals with unrepaired SSP tears. Approximately 4-8 months following SSP repair and biceps tenodesis, tendon vascularity was not significantly different from asymptomatic tendons. Overall, results suggest that ultrasound techniques that evaluate tendon microvasculature in the absence of contrast agents may represent a low-risk and reliable method to monitor rotator cuff tendons following injury and during recovery. Further investigation will examine if the increased vascularity in the torn state could be a potential target for medication to increase healing rates.

SIGNIFICANCE/CLINICAL RELEVANCE**:** Ultrasound examination provides a low-risk method of examining microvascular changes seen in tendons after injury and during recovery. This study investigates the potential for utilization in future treatment and recovery monitoring.

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Ultrasound-Assessed Medial Meniscal Extrusion is Greatest in a Weight-Bearing Stance in Asymptomatic Knees

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Disclosures: Canon Medical Systems, Inc. provided use of the ultrasound system used for this work at no cost to the institution

INTRODUCTION**:** Meniscal injuries are among the leading risk factors for the development of knee osteoarthritis[1,2]. The current standard for assessing meniscal injuries is MRI. Recently, meniscal displacement, or extrusion has gained interest as it may indicate altered meniscal function. Current literature defines more than 3 mm of meniscal extrusion relative to the tibial plateau as pathologic[3]. However, meniscal extrusion measured in a supine, unloaded position may not accurately reflect normal or pathologic meniscal behavior during activities of daily life. Alternative techniques that enable measurement of meniscal function during weight-bearing, such as ultrasonography (US), can relate meniscal pathology to altered joint mechanics[4], which may help inform clinical decisions regarding clinical management of meniscal root tears or diseases like osteoarthritis Ultrasound allows for evaluation of meniscal extrusion during loading and flexion and may help relate meniscal behavior observed in supine positions to what is experienced during weight-bearing activities. However, little is known about how different flexion angles and weightbearing status affect the meniscus movement over the average life span. The aim of this study was to examine physiologic medial meniscal extrusion (MME) in both unloaded and loaded states as well as between different knee flexion angles in asymptomatic volunteers of different age groups.

METHODS**:** This prospective study was approved by an IRB and included twenty volunteers with asymptomatic knees and no history of knee injury or surgery (33 ± 12 years, BMI 23.0 ± 2.3 kg/m2, 9 females). Ultrasound imaging of the medial meniscus of both knees was performed by an orthopedic surgeon with 6 years of experience with musculoskeletal ultrasonography using an Aplio i800 system (Canon Medical Systems USA) with an 18 MHz linear array transducer (PLI-1205BX/FS, Canon Medical Systems USA). The transducer was placed in the coronal plane parallel to the medial collateral ligament to measure medial meniscal body extrusion in supine and weight-bearing positions using the medial collateral ligament as a landmark for standardized images of the meniscus. Ten retroreflective markers were placed on the distal fibula, joint line, and greater trochanter on the lateral side of both legs, as well as on the lateral right thigh, for real-time assessment of joint angles using a 16-camera video-motion capture system (Oqus 700+ series and QTM Connect MATLAB plugin, Qualisys). Three ultrasound images were taken in each position and joint angles were captured simultaneously. Participants were first examined in the supine position at individual neutral (0-5°), 20°, and 45° flexion. Then, participants were examined while standing on two legs and instructed to balance their weight between legs as they stood in a neutral position (0-10° flexion) and squatted to 20° and 45° flexion. This procedure was repeated with participants standing on a single leg at a time, alternating between left and right legs (Figure 1). MME was measured on each image using a custom MATLAB script and averaged for each position (Figure 1). Meniscal extrusion was compared between legs, stance, and knee angle using a general linear model with age as a covariate and differences between group means were compared using a Tukey post-hoc assessment.

Figure 1: Ultrasound images of the medial meniscus were acquired in supine and 1- and 2-leg standing positions (left). A motion-capture system was used to accurately record joint angles as participants stood in a neutral stance (0-5°) and squatted to 20° and 45° flexion (center). Medial meniscal extrusion was measured for 3 images for each stance and position using a custom MATLAB script (right) and then averaged.

RESULTS**:** There were significant relationships between MME and age (p < 0.001), stance (p < 0.001), and knee flexion angle (p < 0.001) but not between right and left legs (p = 0.257). Increasing knee flexion angle was associated with significantly lower MME (Figure 2), with the greatest extrusion in a neutral angle compared to 20 and 45 degrees of flexion. Further, MME was significantly lower in the supine position compared to MME in single- and double-leg stances but there was no difference between the two weight-bearing stances.

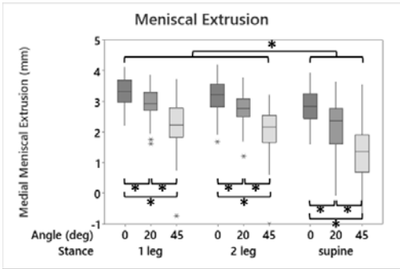

Figure 2: Medial meniscal extrusion was greatest in neural flexion compared to 20 and 45 degrees of flexion. Extrusion was lowest in the supine position but there were no differences between either 1- or 2-leg standing positions.* p < 0.001

DISCUSSION**:** Weight-bearing on 1 or 2 legs increased meniscal extrusion compared to the supine, unloaded position. Meniscal extrusion measurements are highly correlated between MRI and ultrasound in a supine, unloaded position[5,6]; however, this position may not allow for the best assessment of meniscal function. These findings suggest that MME may be most evident in an upright weight-bearing position with the knees in a neutral position. While the 1-leg stance trended toward higher MME in each position, both a 1- and 2-leg stance may provide similar information about meniscal behavior under load. Standing on both legs allows for even weight distribution and provides an alternative position for patients with difficulties in weightbearing or painful knees, such as in cases with meniscal tears.

Significance/ Clinical Relevance: This study investigates the utility of ultrasound in analyzing meniscal extrusion in weight-bearing states rather than the traditional supine position. Meniscal extrusion measurements made under weight-bearing conditions using ultrasound may provide more useful information about meniscal function compared to a supine MRI.

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Ultrasound Evaluation of Massive Rotator Cuff Tears is Comparable to MRI Measurements

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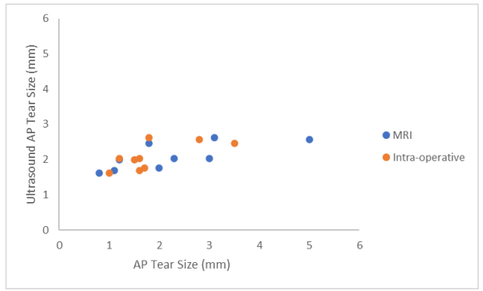
Disclosures: Canon Medical Systems, Inc. Provided use of the ultrasound system used for this work at no cost to the institution.

INTRODUCTION: Massive rotator cuff tears represent a surgical and diagnostic challenge. These tears are most commonly diagnosed using MRI; however the tear size and muscle quality assessed on MRI may not be predictive of its surgical repairability, resulting in surgeons being confronted with a non-reparable rotator cuff intraoperatively[1]. Ultrasound is a proven and studied technique which has a high sensitivity to detecting rotator cuff tears (RCTs). Ultrasound has multiple advantages over MRI including a lower cost barrier, instant access to images, and the ability to perform a dynamic rather than static exam to evaluate tendon flexibility and retraction. The ability to improve pre-operative planning based on pre-operative ultrasound findings would significantly decrease costs to the healthcare system as well as overall time in the operating room. In addition, changes over time in the tear pattern cannot be detected with one-time MRI examination. Often, only the intraoperative assessment of tear size and the lateral mobility of the musculotendinous unit defines repairability. In this preliminary study, we compared ultrasound measures of tear size, area, and retraction to MRI and intra-operative measures in patients with massive rotator cuff tears.

METHODS: This prospective study was approved by the institutional IRB and included 9 volunteers with an MRI diagnosis of a massive rotator cuff tear scheduled for a primary repair surgery (57 ± 8 y, BMI 25.3 ± 3.95 kg/m^2, 4.6 ± 1.6 months between symptom onset and surgery dates, 8 males). An ultrasound examination was performed using an Aplio i800 system and an i18LX5 linear array transducer (PLI-1205BX/FS, Canon Medical Systems, Inc.) within 24 hours of the scheduled repair. The exam included measurements of tear size, area, and thickness, and classifications for tendon retraction (1 = bony insertion, 2 = level of humeral head, 3 = glenoid level), muscle atrophy (1 = no, 2 = mild, 3= moderate, 4 = severe) and fatty infiltration (1 = no, 2 = present). Shear wave elastography was used to measure stiffness in the supraspinatus muscle belly by averaging the shear modulus for three 1-mm regions of interest placed in areas of the muscle with homogenous shear wave velocity. Tear size, muscle atrophy, and muscle fatty infiltration were measured from clinical MRI exams. Finally, tear size and retraction were measured intra-operatively. Correlations between tear size measurements taken with ultrasound were compared to tear sizes measured on MRI and intra-operatively as well as with measures of muscle quality and tendon retraction using a Pearson's correlation test (α= 0.05).

RESULTS: There were significant positive correlations between the anterior-posterior (AP) tear size measured MRI and the AP tear size measured using ultrasound (R = 0.72, p = 0.029), and trends with intra-operative measurements of AP tear size (R = 0.66, p = 0.054) (Figure 1). Tendon tear area on ultrasound was significantly correlated with the antero-posterior (AP) tear size on MRI (R = 0.73, p = 0.026), muscle atrophy on MRI (R = 0.80, p = 0.009), and the Patte classification for tendon retraction (R = 0.72, p = 0.029). The intra-operative assessment of tear elasticity was also significantly positively correlated with tear thickness on ultrasound (R = 0.71, p = 0.033) and the Patte classification on ultrasound (R = 0.69, p = 0.038). Trends between the supraspinatus muscle shear modulus and muscle atrophy and fatty infiltration did not reach significance.



DISCUSSION: In this pilot study, we observed that pre-operative measurements of tear size and area on ultrasound were strongly correlated with measures taken intra-operatively and on MRI, although ultrasound tended to underestimate the size of larger tears. Tear sizes on ultrasound were also strongly linked with the degree of tendon retraction and the extent of muscle atrophy. Future work will further explore links between ultrasound measures of muscle and tendon quality in patients with different tear sizes and in relationship to tendon repairability.

SIGNIFICANCE/CLINICAL RELEVANCE: This study investigates the ability to improve preoperative planning based on ultrasound findings, as this would significantly decrease costs to the healthcare system as well as overall time in the OR.

REFERENCES: [1] Liang W et al. Med Ultrason. 2020 May 11;22(2):197-202

Biomechanical Evaluation of Posterior Shoulder Instability with a Clinically Relevant Posterior Bone Loss Model

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INTRODUCTION: Existing biomechanical studies of posterior glenoid bone loss and labral pathology are limited by their employment of anterior instability models which differ in both orientation and morphology and have been performed in only a single, neutral arm position. The purpose of this study was to evaluate the biomechanical effectiveness of a posterior labral repair in the setting of a clinically relevant posterior bone loss model in various at-risk arm positions.

METHODS: Ten fresh-frozen cadaveric shoulders were tested in 7 consecutive states using a 6-degrees-of-freedom robotic arm:: (1) native, (2) posterior labral tear (6-9 o'clock), (3) posterior labral repair, (4) mean posterior glenoid bone loss (7%) with labral tear, (5) mean posterior glenoid bone loss with labral repair, (6) large posterior glenoid bone loss (28%) with labral tear, and (7) large posterior glenoid bone loss with labral repair. Bone loss was created using 3D printed computed tomography model templates. Biomechanical testing consisted of 75N of posterior-inferior force and 75N of compression at 60 and 90 degrees of flexion and scaption. Posterior-inferior translation, lateral translation, and peak dislocation force were measured for each condition. To match the repeated measures study design, a 2-factor linear mixed effects model was created for each test using labrum state (torn vs. repaired) and bone loss (small, medium, large). All pairwise comparisons were made using Tukey's method to adjust for multiple tests.

RESULTS: Labral repair significantly increased dislocation force independent of bone loss state or arm position with mean +12.1 ± 2.0N across all arm positions. Dislocation force significantly decreased between no bone loss and small bone loss (mean -12.4 ± 0.7N) and between small bone loss and large bone loss (mean -11.8 ± 2.1N). Labral repair significantly decreased posterior-inferior translation compared to labral tear states by a mean 1.71 ± 0.54mm. In the native state, the shoulder was most unstable in 60° scaption with 29.9 ± 6.1mm posterior-inferior translation.

DISCUSSION: Posterior labral repair improves stability of the glenohumeral joint, and even in smaller to medium amounts of posterior glenoid bone loss the glenohumeral stability is maintained with labral repair. However, a labral repair with large bone loss could not improve stability to the native state. This study shows that larger amounts of posterior glenoid bone loss of >25% may require bony augmentation for adequate stability.

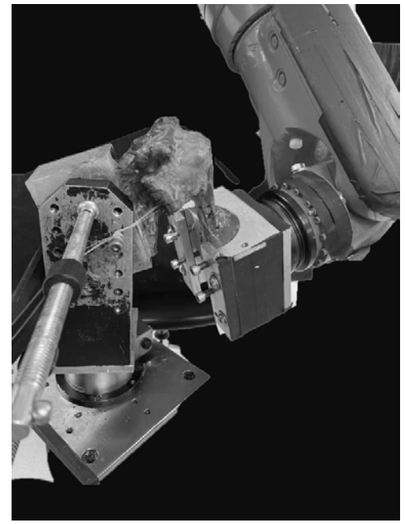

Figure 1: Experimental Robotic Setup

CLINICAL RELEVANCE: This study evaluates the effectiveness of labral repair in increasing severity of bone loss, which can help surgeons in their decision-making process when treating a patient with posterior instability. The use of a clinically relevant bone loss model in combination with physiological testing positions makes this study more relevant to patient care than previous literature.

Position	Elevation	Measure	*Signif Factors in LME Models*			
			Labrum State	Bone Loss State		
			tear->repair	none->small	small->large	none->large
Scaption	60	Post-Inf Trans	↓2.29mm	n.s.	n.s.	n.s.
		Lat Trans	↑1.08mm	↓1.53mm	↓1.87mm	↓3.40mm
		Disloc Force	↑13.2N	↓10.7N	↓12.9N	↓23.5N
	90	Post-Inf Trans	↓1.79mm	n.s.	n.s.	n.s.
		Lat Trans	n.s.	↓2.03mm	↓1.63mm	↓3.66mm
		Disloc Force	↑11.3N	↓11.9N	↓10.6N	↓22.4mm
Flexion	60	Post-Inf Trans	↓1.78mm	n.s.	n.s.	n.s.
		Lat Trans	↑1.69mm	↓1.82mm	↓2.01mm	↓3.82mm
		Disloc Force	↑9.87N	↓10.9N	↓10.8N	↓21.7N
	90	Post-Inf Trans	↓0.99mm	n.s.	n.s.	n.s.
		Lat Trans	↑0.90mm	↓1.37mm	↓1.20mm	↓2.57mm
		Disloc Force	↑9.06N	↓10.8N	↓8.5N	↓19.2N

Table 1: Summary of the statistically significant changes between states, based on a linear mixed effects statistical model. An upwards arrow represents a significant increase and a downwards arrow represents a significant decrease. n.s = non-significant.

The Biomechanical Role of the Deltoid Ligament on Ankle Stability: Injury, Repair and Augmentation

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Disclosures: C. Kreulin: 1; Arthrex, Restor3D. 3B; Restor3D, Arthrex, Synthes. 5; Arthrex, Wright Medical, Envois/Medshape. T. Haytmanek: 3B; Arthrex, Gemini Mountain Medical, Integra, Bioventis, Exatech, Stryker. 5; Stryker. T.O. Clanton: 1; Athrex, Stryker. 2; Arthrex, Wright Medical, Stryker. 3B; Arthrex, BICMD, Stryker, SubioMed. 4; SubioMed. 5; Arthrex, Stryker, Wright Medical. 8; Foot and Ankle International

INTRODUCTION: Injuries to the deltoid ligament occur in association with ankle fractures, syndesmotic injuries as well as in isolation. Both operative and nonoperative treatment have been utilized with little consensus as to optimum treatment. This is related to the multiple components of the deltoid ligament, different injury methodologies, and limited versus complete injuries. The contribution of the anterior and posterior bundles of the deltoid ligament to the stability of the ankle continues to be debated as is the optimal surgical repair method. The purpose of this study was to determine the biomechanical role of the native anterior and posterior deltoid ligament in ankle stability and to determine the efficacy of simple suture vs augmented repair.

METHODS: Ten cadaveric ankles (50.9 mean age, male) were mounted on a six-degrees-of-freedom robotic arm. Each specimen underwent biomechanical testing in eight states: 1) intact, 2) anterior deltoid cut, 3) anterior repair, 4) tibiocalcaneal augmentation, 5) tibiotalar augmentation, 6) posterior deltoid cut, 7) posterior repair, 8) complete deltoid cut. Testing consisted of: 1) anterior drawer, 2) eversion, 3) external rotation (ER), each run at 0° and 25° plantarflexion. A 1-factor random-intercepts linear mixed effect model was created, and all pairwise comparisons were made between testing states.

RESULTS: Cutting the anterior deltoid introduced ER and eversion laxity (+6.2 degrees eversion, $p<0.0001$). The anterior deltoid repair restored native ER, but not eversion. The tibiocalcaneal augmentation further reduced eversion laxity without restoring native, but the tibiotalar augmentation provided no additional benefit. The posterior deltoid tear showed no increase in laxity. However, the complete anterior and posterior tear introduced massive anterior translation, ER and eversion laxity (+7.6 mm translation and +33° eversion $p<0.0001$). The results of eversion testing at 25 degrees of plantarflexion are shown on figure 1.

DISCUSSION: The most important finding of this study is that a complete deltoid tear (anterior + posterior) caused major instability of the ankle joint. Therefore, it is strongly recommended to surgically repair the deltoid ligament in this situation. In the setting of a complete deltoid tear, the anterior repair with tibiocalcaneal and tibiotalar augmentation was the optimal treatment, with no additional benefit provided by repairing the posterior deltoid. In the setting of an isolated anterior deltoid tear, the ankle joint was significantly less stable than native. The simple repair restored native ER stability and reduced eversion laxity but did not restore native eversion stability. The addition of the tibiocalcaneal augmentation further reduced eversion laxity, but the addition of the tibiotalar augmentation provided no additional benefit in eversion, and overconstrainined the joint in ER. Therefore, the optimal treatment for an anterior tear was the anterior repair with tibiocalcaneal augmentation. Our study design was not able to determine if the tibiocal-caneal augmentation alone was sufficient to stabilize a complete tear, or if the additional tibiotalar augmentation was useful in that setting, as these augmentations were only tested separately in the setting of an isolated anterior tear. Further research is necessary to determine the potential benefit of the tibiotalar augmentation in the setting of a complete tear and the potential benefit of the tibiotalar augmentation alone in the setting of an anterior tear.

CLINICAL RELEVANCE: Deltoid repair with augmentation may reduce or avoid the need for postoperative casting and encourage accelerated rehabilitation, preventing stiffness and promoting earlier return to preinjury activity.

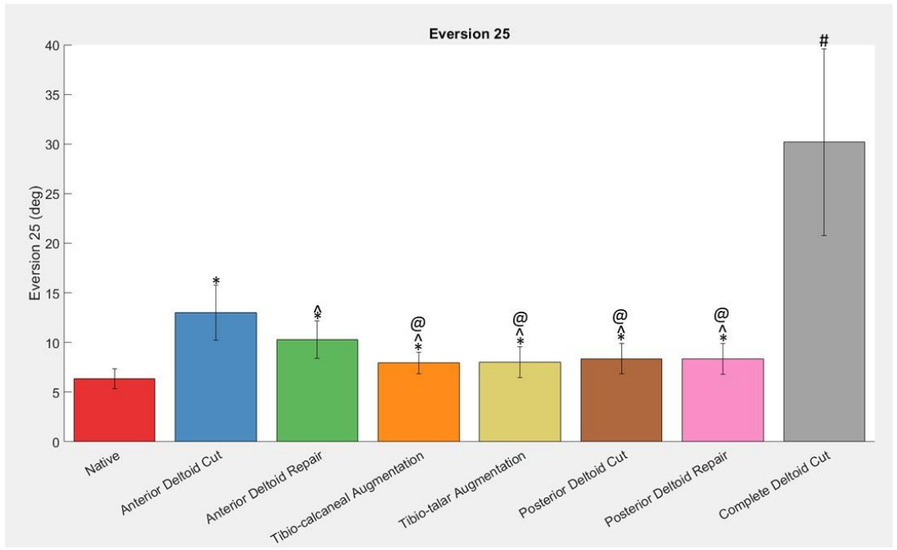

Figure 1: Eversion at 25 degrees of plantarflexion in each state. *: significantly different from native; ^ significantly different from deltoid cut; @ significantly different from anterior deltoid repair; #: significantly different from all other states.

Direct Measurement of Three-Dimensional Forces at the Posterior Medial Meniscal Root: A Validation Study

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INTRODUCTION: The posterior medial meniscal root (PMMR) experiences variable and multiaxial forces during loading. Current methods to measure these forces are limited and fail to adequately characterize the loads in all three dimensions at the root. Our novel technique resolved these limitations with the installation of a 3-axis sensing construct that we hypothesized would not affect contact mechanics, would not impart extraneous loads onto the PMMR, would accurately measure forces, and would not deflect under physiological loads.

METHODS: Six cadaveric specimens were dissected to the joint capsule and a sagittal-plane, femoral condyle osteotomy was performed to gain access to the root. A high-grade stainless steel, environmentally sealed three-axis sensor that determines force measurements in three orthogonal directions, was placed below the PMMR and was validated across four tests: contact mechanics, tibial displacement, load validation, and bone plug deflection.

RESULTS: The contact mechanics test demonstrated a contact area precision of 44 mm^2 and a contact pressure precision of 5.0 MPa between the pre-installation and post-installation states. The tibial displacement test indicated an average bone plug displacement of < 1mm in all directions. The load validation test exhibited average precision values of 0.7 N in compression, 0.5 N in tension, 0.3 N in anterior-posterior shear, and 0.3 N in medial-lateral shear load. The bone plug deflection test confirmed <2 mm of displacement in any direction when placed under a load.

DISCUSSION: This is the first study to successfully validate a technique for measuring both magnitude and direction of forces experienced at the PMMR. This validated method has applications for improving surgical repair techniques and developing safer rehabilitation and postoperative protocols that decrease root loads.

CLINICAL RELEVANCE: In knees undergoing a meniscus procedure, the position of the repair or transplant can be placed in the optimal orientation to place the least amount of force on the posterior medial meniscal root. In addition, rehabilitation maneuvers and postoperative protocols can be tailored to decreasing the force seen at the meniscal root.

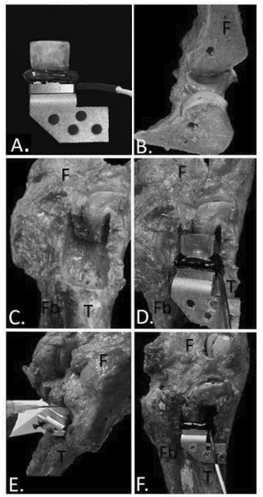

Figure 1. Sensor installation. F – femur, T – tibia, Fb - fibula

Table 1. Validation test results.

		Tibial Plateau Displacement Test						Bone Plug Deflection Test	
		Displacement (mm)			Δ Orientation (°)				
		AP	ML	SI	AP	ML	SI	AP (mm)	ML (mm)
Average		0.38	0.42	0.21	0.96	1.30	2.66	0.90	0.67
Std Dev		0.34	0.31	0.14	0.67	1.39	2.09	0.60	0.20
Upper Interval		1.04	1.04	0.48	2.26	4.03	6.75	2.07	1.06
Lower Interval		-0.29	-0.19	-0.05	-0.35	-1.43	-1.44	0.26	0.27

		Contact Mechanics Test			
		Medial Pressure (MPa)		Contact Area (mm^2)	
		Pre-Installation	Post-Installation	Pre-Installation	Post-Installation
Average		84.11	89.63	872.83	870.83
Std Dev		41.22	39.44	236.06	195.65
Upper Interval		164.90	166.93	1,335.52	1,254.31
Lower Interval		3.31	12.32	410.15	487.36

		Load Validation Test								
		Tension (N)					AP Shear (N)			
Applied Load		0	10	20	30	40	0	5	10	15
Measured Load	Average	-0.03	9.78	19.99	29.97	40.13	-0.07	5.00	9.71	14.37
	Std Dev	0.32	0.47	0.54	0.75	1.12	0.47	0.38	0.23	0.29
	Upper Interval	0.59	10.70	21.04	31.44	42.32	0.85	5.74	10.17	14.95
	Lower Interval	-0.65	8.86	18.94	28.51	37.93	-0.99	4.26	9.25	13.81

		Compression (N)						ML Shear (N)			
Applied Load		0	10	20	30	40	50	0	5	10	15
Measured Load	Average	-0.03	9.70	19.54	29.36	39.17	48.92	0.02	4.76	9.27	13.88
	Std Dev	0.32	0.30	0.52	0.83	1.11	1.53	0.06	0.19	0.33	0.54
	Upper Interval	0.59	10.28	20.56	30.98	41.26	51.91	0.15	5.12	9.93	14.93
	Lower Interval	-0.65	9.11	18.52	27.73	36.99	45.93	-0.10	4.39	8.61	12.83

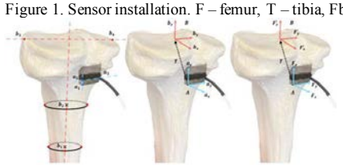

Figure 2. Force vectors.

Taking the Load Off: Effect of Varus Producing Distal Femoral Osteotomy and High Tibial Osteotomy on Compartment Pressures and Contact Area in Varying Stages of Knee Flexion

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INTRODUCTION: Genu valgum causes mechanical pressure to be transferred to the lateral compartment. In patients with valgus alignment and a diseased lateral compartment, both lateral opening wedge distal femoral osteotomy (DFO) and high tibial closing wedge osteotomy (HTO) can be used to unload a diseased lateral compartment of the knee. To the best of our knowledge, there are no biomechanical studies investigating how a DFO and HTO affect lateral compartment contact pressure and area in varying states of flexion. Prior anatomic studies have shown that, in valgus knees, the tibial wear is posterior. Additional, biomechanical studies have indicated that DFO exerts the greatest effect in full extension but it's effect is decreased as flexion angle rises.

METHODS: Lateral opening wedge DFO and medial closing wedge HTO was performed, correcting a mean of 8°, in 10 cadaveric knees using plate fixation. Tibio- femoral contact pressure of the medial and lateral compartment was measured in 0°, 30°, 60°, and 90° of knee flexion before and after osteotomy using thin electronic sensors (K-scan Model 4000, 1500 psi; Tekscan Inc, South Boston, MA) and load applied through an Instron device (Instron, Norwood, MA). Peak contact pressure (PCP), average contact pressure (ACP), and contact area (CA) were measured for each condition. Mixed-effects regression analyses were used to compare the change in variables before and after osteotomy and between flexion angles.

RESULTS: The lateral ACP was significantly decreased in the HTO state when compared to native in 30° (p=0.015), 60° (p=0.0199), and 90°(p<0.0001)of flexion. The lateral ACP was also significantly decreased in the HTO state when compared to the DFO state in 60° (p=0.0093) and 90°of flexion (p<0.0001). Following DFO, the lateral MCP returned to that of the native state in 60°(p=1.000) and 90° (p=1.000) of flexion. The medial MCP was significantly increased when compared to both the native and HTO state in 60° (p<0.001; p=0.015) and 90° (p<0.0001; p=0.0435) of flexion. The lateral PCP decreased for all test states in all degrees of flexion; the HTO state was significantly decreased when compared to the native state in 60° (p<0.0001) and 90° (p<0.0001). The medial compartment contact area was significantly increased in the HTO state when compared to both the native and DFO state in 30° (p<0.0001; p<0.0001), 60° (p<0.0001; p<0.0001), and 90° (p<0.043; p=0.0282).

DISCUSSION: With varus corrections of <10°, medial closing HTO is more effective at unloading the lateral compartment than lateral opening DFO. This effect is amplified as the knee flexion angle increases.

CLINICAL RELEVANCE: In knees undergoing a varus producing osteotomy for a correction of <10*, medial closing HTO was more effecting at both unloading the lateral compartment and transferring contact area to the medial compartment than the lateral opening DFO.

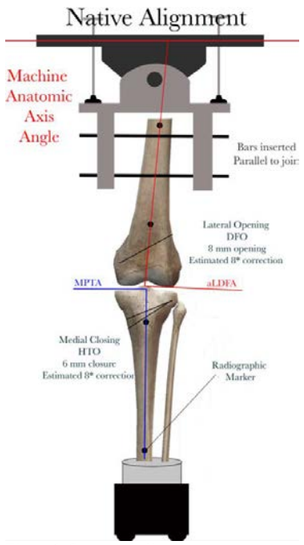

Figure 1. Set up and alignments.

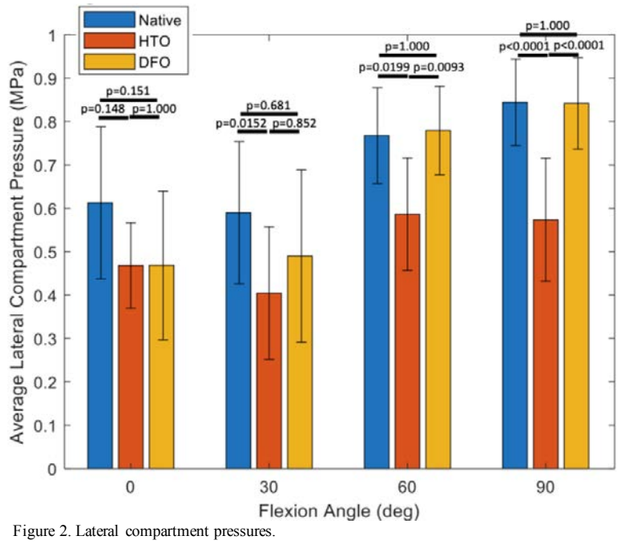

Figure 2. Lateral compartment pressures.

Effect of Posterior Tibial Slope and Flexion Angle on Posterior Medial Meniscal Root Forces

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INTRODUCTION: Although the biomechanical and clinical consequences of medial meniscal posterior medial meniscal root tears (PMMRT) have been previously reported, these have not been fully evaluated in terms of overall bony morphology. It is well documented that higher flexion angles leads to higher postero-medial pressure, with many groups proposing that PMMRTs are caused by elevated compression and shear forces at the root when the loaded knee is at high flexion angles Additionally, several authors have advocated that increasing tibial plateau slope (PTS) is an anatomical risk factor for PMMRTs, due to the higher posterior shear forces at the root insertion site. Thus, the purposes of this study are to evaluate the forces across the posterior medial meniscal root (PMMR) utilizing a novel three-dimensional forces sensor with varying posterior tibial slopes and flexion angles. We hypothesized that an increased flexion angle and/or posterior tibial slope will result in increased posterior shear forces acting on the PMMR.

METHODS: Ten fresh-frozen cadaveric knees (53.2 mean age, all male) were tested in all combinations of the three states of posterior tibial slope (5º, 10º, 15º) and the four states of varying flexion angles (0º, 30º, 60º, and 90º). A novel three-axis sensor that determines force measurements in three orthogonal directions was installed below the posterior tibial plateau, with the specimen being mounted to a load frame (Figure 1) which applied a 500-N axial load. A 5-Nm internal rotational (IR) torque was then applied. After the IR torque, a 5-Nm external rotational (ER) torque was applied. The amount of compression-tension and shear forces acting on the PMMR were measured.

RESULTS: Increased tibial slope significantly decreased tension and significantly increased compression of the PMMR (5°→10°: p = 0.0368, 5°→15°: p < 0.0001, 10°→15°: p < 0.0001) when the joint was loaded in compression. Increased tibial slope significantly increased anterior shear of the PMMR (5°→10°: p < 0.0001, 5°→15°: p < 0.0001, 10°→15°: p < 0.0001) when the joint was internally rotated. Increased tibial slope significantly decreased compression of the PMMR (5°→10°: p = 0.0188, 5°→15°: p < 0.0001) when the joint was externally rotated. Increased flexion angle significantly increased medial shear forces of the PMMR (0°→30°: p = 0.0362, 0°→60°: p = 0.0005, 0°→90°: p < 0.0001, 30°→90°: p = 0.0434) when the joint was loaded in compression. 90° of flexion significantly increased tension of the PMMR (0°→90°: p = 0.0438, 30°→90°: p < 0.0001, 60°→90°: p = 0.0005) when the joint was internally rotated. 30° of flexion angle significantly increased compression of the PMMR (0°→30°: p = 0.0004, 30°→60°: p = 0.0118, 30°→90°: p < 0.0001) when the joint was externally rotated.

DISCUSSION: Increased PTS results in an increase in compression forces acting on the posterior horn of the medial meniscus when the knee joint is loaded. Increases in flexion angles displays an increase in medial shear forces seen at the PMMR under a load. This increase in force may place the PMMR at increased risk of stress and potential failure after repair. This study begins to provide clinicians with information to create safer protocols to decrease the forces experienced at the PMMR after injury or postoperatively.

CLINICAL RELEVANCE: This increase in force may place the PMMR at increased risk of stress and potential failure after repair. This study begins to provide clinicians with information to create safer protocols to decrease the forces experienced at the PMMR after injury or postoperatively.

Figure 1. Set up.

Biomechanical Analysis Evaluating Meniscal Extrusion Following Knotless Suture Anchor Fixation for Segmental Meniscus Transplantation

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INTRODUCTION: Meniscal tissue excision following a meniscal tear subsequently increases the contact stress seen on articular cartilage in the tibiofemoral joint, which leads to degenerative changes such as osteoarthritis. The most common fixation methods used for securing meniscal allograft transplantation (MAT) tissue are suture-only fixation, double plug fixation and the keyhole technique. To present, there have been no biomechanical evaluations comparing a suture-only technique augmented with knotless suture anchors. The purpose of this study was to evaluate the degree of meniscal extrusion and biomechanical function of segmental meniscal transplants performed with meniscocapsular sutures alone versus the augmentation with the knotless suture anchor fixation. We hypothesized that the segmental medial MAT augmentation with intracapsular knotless suture anchor fixation will show significantly improved restoration of meniscal extrusion, contact area and contact pressure to the native state when compared to an MAT performed without use of suture anchors.

METHODS: Ten fresh frozen cadaveric knees underwent central midbody medial meniscectomy and subsequently segmental medial MAT. Knees were loaded in a dynamic tensile testing machine to 1000 N for 20 seconds at 0°, 30°, 60°, and 90° of flexion. Four conditions were tested: (1) intact, (2) segmental defect, (3) inside-out segmental repair, and (4) anchors plus inside-out segmental repair of the MAT. Meniscal extrusion was measured for each test state using ultrasound imaging by three independent observers. Submeniscal medial and lateral pressure-mapping sensors assessed the mean contact pressure, peak contact pressure, mean contact area, and pressure mapping. Assessment of inter- and intraobserver reliability of measurements were done using intraclass correlation coefficients. A two-way repeated measures ANOVA was computed in order to evaluate whether there is a significant interaction between meniscal states and knee flexion angles on the outcome variables. If P value ≤ 0.05, a pairwise comparison of the means was determined using the Bonferroni method.

RESULTS SECTION: At 0° of knee flexion, the segmental defect demonstrated the statistically significant higher degree of meniscal extrusion (2.08 mm $\pm$ 0.78 mm) compared with intact (1.02 mm $\pm$ 0.86 mm, P = 0.039) and anchor plus inside-out segmental repair (1.44 mm $\pm$ 0.76 mm, P = 0.044). Segmental defect state also showed higher degree of meniscal extrusion compared with other states at 90° of knee flexion (P < 0.01). The degree of meniscal extrusion in the intact state was no significant difference than that at the inside-out repair or anchor plus inside-out segmental repair in all knee flexion angles (P > 0.05). Three observers showed good-to-strong intra-rater and moderate-to-strong inter-rater reliabilities for extrusion measurements. There was no significant difference in the mean contact pressure and peak contact pressure among four states in all knee flexion angles for both medial and lateral compartment except for that at 0° of knee flexion, there was significant lower of peak pressure at medial compartment after anchor plus inside-out segmental repair compared with the segmental defect state (P = 0.048). The medial contact area of the intact state was significantly higher than the other states at 0° of knee flexion (P < 0.05). However, the lateral contact area after anchor plus inside-out repair was no significant difference compared with the intact state (P = 0.13).

DISCUSSION: The results suggest that the addition of knotless suture anchor fixation in the segmental medial MAT does not reduce the amount of meniscal extrusion compared with standard inside-out segmental repair. Segmental medial MAT performed with meniscocapsular sutures alone or the augmentation with the knotless suture anchor fixation restored degree of meniscal extrusion and mean and peak medial compartment pressure to values measured in the intact state. Extrusion of the transplanted meniscal allograft suggests a loss of proper mechanical function and has also been associated with poorer outcomes.

SIGNIFICANCE/CLINICAL RELEVANCE: Meniscocapsular sutures alone or with knotless suture anchor fixation for segmental medial MAT both repaired the meniscus to a level not significantly different to native state biomechanics. Meniscocapsular repair augmented with knotless suture anchor provides an alternative fixation method for segmental medial MAT.

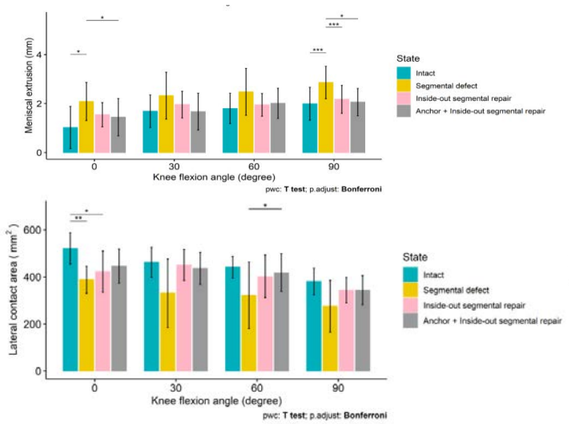

Fig 1: Meniscal extrusion among different meniscal states and each knee flexion angle

Fig. 2: Lateral contact area at different meniscal states and each knee flexion angle

The Effect of Meniscotibial Ligament Disruption and Repair on Posterior Medial Meniscal Root Forces

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INTRODUCTION: Meniscal injuries occur in as many as 1% of active people, and while outcomes for tears at the posterior medial meniscal root (PMMR) are well cited, the risk factors for injury have room for greater understanding. Incidence of disruption to the meniscotibial ligament (MTL) is correlated to occurrence of PMMR tears, suggesting that disruption to the MTL may increase forces at the PMMR and put the PMMR at greater risk of injury. The effect of an MTL disruption and repair on three-dimensional forces at the PMMR is unknown. The purpose of this study was to determine if MTL insufficiency alters forces at the PMMR., if a tenodesis procedure can restore PMMR forces to that of an MTL intact state, and to determine how knee flexion angle impacts PMMR root forces in the MTL intact and cut states. We hypothesize that all shear forces will increase following MTL disruption.

METHODS: Ten fresh-frozen cadaveric knees (Average age: 53.2 years) were tested in three conditions (Intact, MTL Cut, MTL Tenodesis). Specimens were dissected down to the knee capsule with ligaments intact. A 3D load cell construct (Figure 1) was installed inside the tibia such that 3D forces of the PMMR could be measured when the joint was loaded. Each specimen was mounted to a materials testing machine (Instron) via a custom fixture that allowed the specimen flexion angle to be changed in 30 degree increments. The specimen was first loaded to 500 N of compression with 0 N-m of torque, then to 5 N-m of internal torque with 50 N of compression, and finally to 5 N-m of external torque with 50 N of compression. 3D forces at the PMMR were recorded for all loading sequences, and the process was repeated for each flexion angle (0°, 30°, 60°, and 90°). PMMR forces along each axis (compression-tension, anterior-posterior shear, and medial-lateral shear) were compared across MTL testing states across flexion angles using linear mixed modelling.

RESULTS: When the joint was loaded in compression, MTL Cut state significantly increased compression of the PMMR (p = 0.0368), and the Tenodesis state did not significantly restore tension-compression forces of the PMMR (Intact→Tenodesis: p = 0.008)(Figure 1). When the joint was loaded in external rotation, the MTL Cut State significantly increased compression (p < 0.0001), significantly decreased anterior shear (p = 0.0003), and, in high flexion angles, significantly decreased ML shear forces of the PMMR (Figure 1). The Tenodesis state did not significantly restore tension-compression (Intact→Tenodesis: p < 0.0001) or AP forces (Intact→Tenodesis: p = 0.0002) of the PMMR (Figure 1).

DISCUSSION: The key finding of this study is that MTL disruption increases compression forces and decreases AP shear forces seen at the root when the joint is loaded in compression and external rotation. These findings indicate that the intact MTL protects the PMMR from compression and shear loads during movements where the joint is loaded in compression and external rotation, movements that are clinically reported to put the knee joint at risk of PMMR injury. The authors believe these relationships are observed because the MTL may play a role in maintaining meniscal hoop stresses, which converts joint compression loads into tension and shear forces along the meniscal attachment points. When the MTL is disrupted, the other meniscal attachment points, the PMMR included, see elevated compression loads as a result. This is the first study to measure 3D forces at the PMMR, a necessary capability to assess PMMR injury risk. It is notable that the tenodesis procedure performed in this study did not restore PMMR forces. Future studies should develop improved centralization or tenodesis procedures to better restore PMMR forces and decrease likelihood for PMMR injury after MTL disruption.

CLINICAL RELEVANCE: These findings indicate that MTL disruption alters forces at the PMMR when the joint is loaded in compression and external rotation. This work can help clinicians understand PMMR injury etiology, develop tenodesis techniques that best restore PMMR mechanics, and design rehabilitation protocols that do not put a patient at risk of reinjury after MTL disruption or root repair.

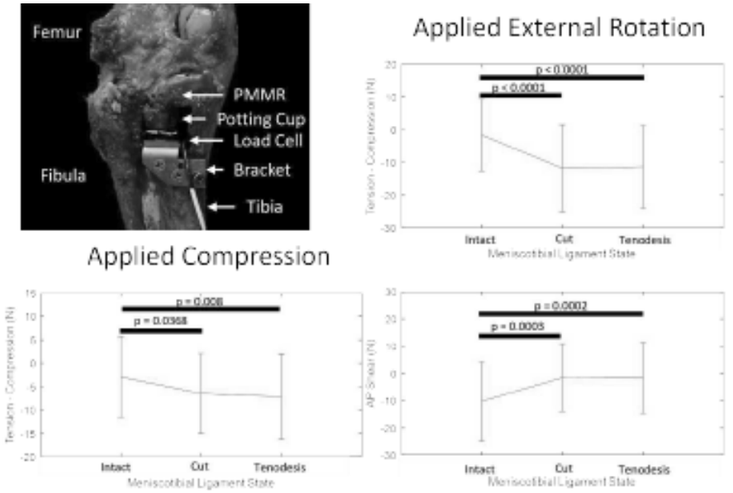

Fig 1. Mechanical testing set up (top left). Plots of tension-compression force (top right) and AP shear force (bottom right) under an applied external rotation. Plot of tension-compression force under an applied compression load (bottom left).

Biomechanical Analysis of Meniscotibial Ligament Tenodesis to Treat Meniscal Extrusion

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INTRODUCTION: There has been an increased focus on the significance of meniscal extrusion in medial meniscal root repairs, as meniscal extrusion is often still present following a successful medial meniscal root repair. If the meniscus is extruded, the function of the meniscus as a shock absorber and secondary knee stabilizer can be compromised. There is no literature evaluating tenodesis of the meniscotibial ligament (MTL) to the tibia, which theoretically reduces meniscal extrusion by tethering the capsule to the tibia. The purpose of this study was to evaluate an MTL tenodesis for treating meniscal extrusion in the setting of medial meniscal root tears and repair. We hypothesized that tenodesing the medial tibial capsule to the tibia will decrease meniscal extrusion in the setting of a medial meniscal root tear and repair along with improving the medial cartilage contact pressures in a cadaveric model.

METHODS: Ten fresh-frozen cadaveric knees (Average age: 50.5 years) were tested in five conditions (Intact, MTL Tear, MTL + Root Tear, Root Repair + MTL Tear, Root Repair + MTL Tenodesis). Tekscan pressure sensors were inserted submeniscally between the femoral condyles and the superior surface of the medial and lateral tibial compartments. Specimens were mounted in full extension to a load frame (Instron) which applied a 1000 N axial load to the specimen (Figure 1). Average and peak contact pressures and contact area were recorded. Medial meniscal extrusion was measured using an ultrasound machine with a 18MHz transducer (Canon I-series). Contact pressures, contact mechanics, and extrusion were compared across states.

RESULTS: The Root Tear state alone yielded significantly elevated average ($p = 0.0423$) and peak contact pressures ($p = 0.0028$) and reduced contact area ($p =$ in the medial compartment compared to the intact state. Notably, meniscotibial ligament disruption (MTL Tear state) had no effect on medial contact pressure or contact area. Medial meniscal extrusion significantly increased from the intact state with both an MTL tear ($p = 0.035$) and root tear ($p < 0.0001$). Root repair alone was able to restore extrusion ($p < 0.0001$), average pressure ($p = 0.0276$), and peak pressure ($p = 0.0169$) to the intact state. MTL tenodesis did not significantly improve the extrusion, contact pressures, or contact area from the root repair state.

DISCUSSION: There are two key findings of this study. First, MTL disruption increased medial meniscal extrusion but did not change contact pressures or contact area, which supports prior clinical and biomechanical observations. Meniscal root tear alone increased contact pressures and decreased contact area. Secondly, meniscal root repair alone restored meniscal extrusion and contact pressures back to native. Further, we reject our hypothesis in that the meniscotibial ligament tenodesis had no effect on restoring contact pressures, contact area, and extrusion. Further clinical studies are needed to evaluate post operative clinical outcomes as well as measurable meniscus extrusion following MTL tenodesis. This is one of the first studies to measure extrusion by ultrasound and the first study to measure extrusion both in the setting of root tear and meniscotibial ligament disruption.

CLINICAL RELEVANCE: These findings indicate that, in the setting of a root tear and an MTL disruption, MTL tenodesis is unnecessary as root repair alone is sufficient in restoring intact mechanics and extrusion. Clinicians can consider these observations when planning treatment for patients with concomitant root tear and meniscotibial ligament disruption.

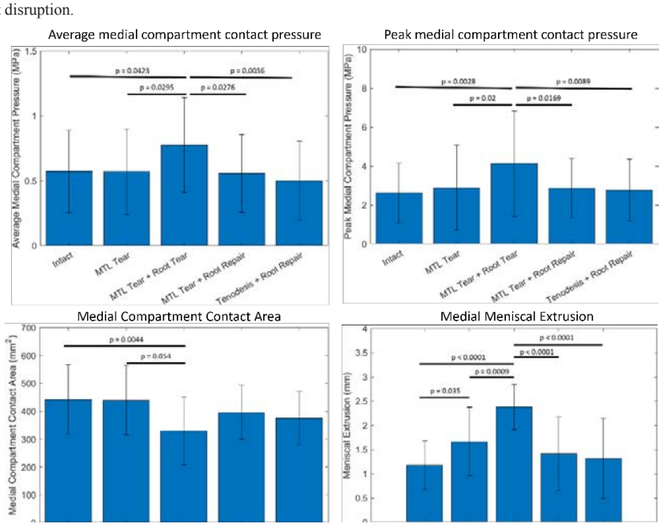

Fig 1. Mechanical testing set up (left). Bar graphs of average contact pressure (top center), peak contact pressure (top right), contact area (bottom center), and extrusion (bottom right).

Biomechanical Comparison of UCL Reconstruction with Single-Tunnel Proximal Endobutton Fixation versus Modified Docking Technique

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Introduction: Despite technical advance, elbow ulnar collateral ligament (UCL) reconstruction is a challenging procedure limited by challenging tunnel placement and potential injury to the ulnar nerve. Furthermore, current techniques for reconstruction and repair are inferior clinically and biomechanically compared to native UCL tissue. A modified docking technique utilizing single-tunnel proximal endobutton fixation may reduce complications and provide a biomechanically superior technique for UCL reconstruction. The purpose of this study was to evaluate and compare the biomechanical performance two elbow UCL reconstruction techniques: (1) standard docking technique (SD) and (2) a proximal single tunnel (PST) technique using endobutton fixation. We hypothesized that the PST technique would be biomechanically superior to the SD technique.

Methods: Twelve matched pairs of cadaveric elbows were dissected and fixed at 70˚ of flexion for biomechanical evaluation (Figure 1). Gracilis allografts were used for a docking reconstruction and the modified reconstruction with a single tunnel proximal endobutton fixation. A cyclic valgus torque protocol was used to precondition specimens with either reconstruction technique, and ulnohumeral gapping was then assessed. After gapping measurements, post-surgical specimens underwent a valgus rotation applied at a rate of 5°/s until the anterior band of the UCL failed or fracture occurred, and ultimate load to failure, stiffness, and mode of failure were recorded.

Results: There were no statistical differences between the two groups. Mean rotational stiffness of the SD (2.3 ± 0.6 Nm/deg) compared to the SPT (1.9 ± 0.7 Nm/deg) ($p = 0.41$) (Figure 2) and mean ultimate failure torque of the SD (30.5 ± 9.2 Nm) compared to the SPT (30.9 ± 8.6 Nm) ($p = 0.86$) (Figure 3) were similar. There was also no statistical difference ($p=0.83$) between the native UCL ulnohumeral gapping (6.0 ± 2.0 mm) compared to the mean ulnohumeral gapping of the standard docking reconstruction (6.0 ± 1.8 mm) (Figure 4).

Discussion: This study compares the biomechanical strength of elbow UCL reconstructions performed using the SP technique to that of a PST technique. Among all tested parameters, including ultimate failure torque, stiffness and ulnohumeral gapping, there were no statistically significant differences between the two techniques.

Clinical Relevance: This study validated that the PST technique is equivalent to the standard docking technique. These results give surgeons a second, equivalent option for UCL reconstruction.

Fig 1. Biomechanical setup with the humerus secured to a custom-designed fixture

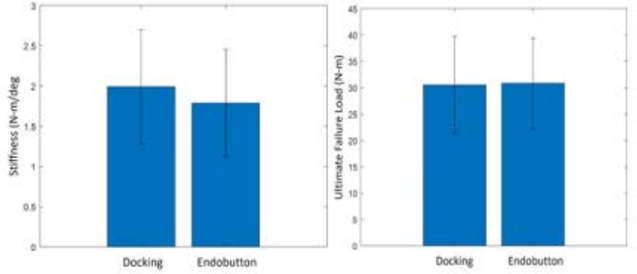

Fig 2. Plots comparing the docking technique and the endobutton fixation technique. Stiffness (left), Ultimate failure load (right) Differences between groups were not significant.

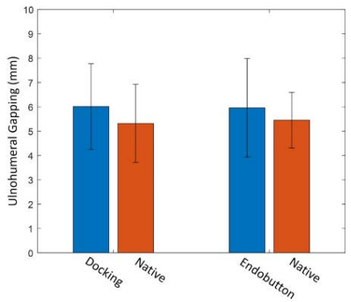

Fig 3. Plot comparing ulnohumeral gapping between the native UCL, the docking technique, and the endobutton fixation technique. Differences between groups were not significant.

Utility of Talus Osteochondral Allograft Augmentation for Varying Size of Hill-Sachs Lesion: A Cadaveric Study

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INTRODUCTION: Recurrent anterior glenohumeral instability remains a growing concern, largely due to its complex pathology of osseous defects. Hill-Sachs lesions (HSL) are a compression fracture of the postero-superolateral humeral head that tends to be associated with anterior shoulder instability or dislocation. The incidence of HSL is not known with certainty, with reports of it being present 40-90% for patients presenting with anterior shoulder instability. For patients with recurrent anterior shoulder instability events, it may be nearly 100%. HSL pose a significant risk for recurrent anterior glenohumeral instability that is not readily responsive to surgical treatment. Because of this, it is important to address HSL quickly and treat properly when indicated. This study aims to study the surface geometry of Talus OCA Augmentation for management of anterior shoulder instability with varying size of HSL. We hypothesize that Talus OCA Augmentation can restore the bone thickness and surface area of the shoulder relative to the native state.

METHODS: Eight fresh-frozen cadaveric shoulders were tested in this study. The humeral head will be digitized using the Romer Arm (SimVITRO, Cleveland, OH). - A 3D plastic model was created using the CT scan of a representative humeral head with varying sizes of HSL as small-, medium-, and large-sizes: (1) Native state, (2) Small HSL, (3) After talus OCA augmentation for small HSL, (4) Medium HSL, (5) After talus OCA augmentation for medium HSL, (6) Large HSL and (7) After talus OCA augmentation for large HSL. Processing of the laser scan data was performed using a custom MATLAB (version R2021a, The MathWorks Inc., Natick, MA) script. Surface congruency was calculated as the average absolute error and root mean squared error in distance between registered points from the non-native specimen to the native specimen. A one-way repeated measures ANOVA followed by post-hoc test was performed to evaluate the effects of the HSLs size differences and associated talus OCA on surface congruency and HSL surface area.

RESULTS: All HSLs and the large OCA state had significantly larger surface areas than the native state ($p < 0.0001$). The medium and small OCA states did not have significantly different surface areas than the native state. All OCA sizes yielded significantly smaller surface areas than their respective HSL sizes ($p < 0.0001$). All OCA sizes were significantly more congruent to the native state surface than HSLs (small $p = 0.0378$, medium $p = 0.0044$, large $p = 0.0088$).

DISCUSSION: Talus OCA augmentation restored surface area to an insignificant difference in small and medium HSLs and resulted in significant improvement in congruency in all HSLs. The results indicate that Talus OCAs can aid in treating patients with HSLs and recurrent anterior glenohumeral instability.

SIGNIFICANCE/CLINICAL RELEVANCE: Talus OCA provides a viable option for restoring bone thickness and surface area of the shoulder in patients with recurrent anterior glenohumeral instability in small and medium HSLs and provides significant improvement in patients with large HSLs.

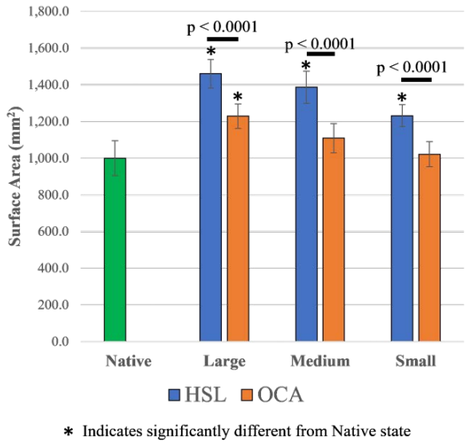

Figure 1. Plot of surface area between testing states.

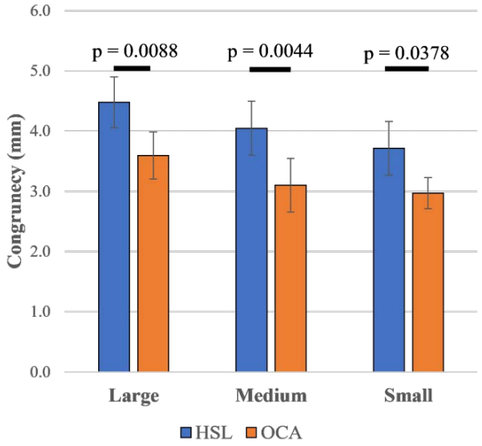

Figure 2. Plot of congruency between testing states.

The Anterior Labral Circumferential Onlay Technique (ALCOT) serves to reconstruct the anterior labrum and biomechanically restores anterior glenohumeral joint stability

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INTRODUCTION: The standard of care for treating anterior shoulder instability caused by a labral detachment is the Bankart repair. In the case of a deficient labrum with significant antero-inferior glenoid bone loss, the latarjet technique can be used instead. However, when the labrum is deficient and the bone loss is minimal, labral reconstruction has been recently proposed as an alternative. A new technique called Anterior Labral Circumferential Onlay Technique (ALCOT) was developed, which reconstructs the labrum using the long head of the biceps tendon. The purpose of this study was to biomechanically evaluate the efficacy of the ALCOT to stabilize the shoulder joint against anterior dislocation in the setting of a deficient labrum with no glenoid bone loss.

METHODS: Ten fresh-frozen cadaveric shoulders were tested in 5 consecutive states using a 6-degrees-of-freedom robotic arm: (1) Native, (2) Capsular Repair, (3) Labral Tear (4) ALCOT (5) Latarjet. The capsule repair state used as a control because the joint capsule needed to be opened and repaired to perform the other states. This state ensured that any differences were due to the surgical procedure being tested, and not because of any iatrogenic damage to the capsule. The Labral Tear was performed by detaching the labrum from the glenoid between 3-6 o'clock and removing it from the joint. For the ALCOT, the biceps tendon was shortened at the distal portion and pulled into the joint. Three knotless all suture anchors were placed at 3, 4:30 and 6 o'clock position on the glenoid rim, and the biceps tendon was secured to the suture anchors using mattress stitches. For the latarjet, a coracoid autograft was cut 25 mm from the tip, keeping the conjoint tendons intact, and secured to the glenoid using two screws. In the native state, each specimen underwent an initial test to determine the appropriate amount of anterior and inferior displacement for future tests. In this test, a 50N compressive load was maintained while an 80N force was applied in the sagittal plane at a 45° angle between the anterior and inferior axes. The corresponding anterior and inferior displacements were recorded. Then, in each state including native, a dislocation test was performed. In this test, a 50N compressive load was maintained while the joint was driven in position control to the previously recorded positions on the anterior and inferior axes. The amount of force needed to displace the shoulder and the lateral displacement of the humeral head were recorded throughout the motion. Higher lateral translation was considered more stable, because it corresponded to a larger obstacle to overcome during the dislocation. To account for variation in the amount of compressive load during the test, antero-inferior dislocation load was normalized by compressive load to create a force ratio. This metric accounts for the fact that the amount of force required to dislocate depends on the amount of stabilizing compressive load provided by the robot in vitro, and by muscle tension in vivo.

RESULTS: No significant differences were found between the native and capsular repair states. Compared to native, the labral tear significantly decreased the lateral translation of the humeral head during dislocation from 6.5mm to 5.4mm ($p<0.001$) and decreased the force ratio from 1.8 to 1.1 ($p=0.002$), corresponding to a decrease from 90N to 55N at 50N of compressive load. The ALCOT restored these values to 6.4 mm and 1.4, respectively, showing no significant difference from native. The latarjet restored the force ratio to 1.3 (not significant from native) but failed to restore lateral translation with a value of 5.6 mm ($p=0.003$ from Native, not significantly different from the labral tear).

DISCUSSION: The ALCOT is a novel technique for labral reconstruction that can be considered as a treatment option for anterior instability in the setting of a deficient labrum. Compared to the latarjet technique, the ALCOT involves less morbidity and represents a more anatomic glenoid surface by replacing the labrum without changing the bony morphology. In this study, the ALCOT also showed superior stabilization by restoring native force ratio and lateral humerus translation. Further research is necessary to clinically validate this technique, and possibly to expand indications to small degrees of glenoid bone loss.

CLINICAL RELEVANCE: This study evaluates the effectiveness of ALCOT compared to Latarjet in the setting of anterior instability with a deficient labrum. It proposes, and biomechanically validates, a novel surgical technique for labral reconstruction that may be used by surgeons to treat patients with anterior instability.

CENTER FOR OUTCOMES-BASED ORTHOPAEDIC RESEARCH

FACULTY AND STAFF

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DIEGO GARCIA
Summer Research Intern 2023

THE CENTER FOR OUTCOMES-BASED ORTHOPAEDIC RESEARCH (COOR) is the longest standing department at SPRI. The team has been tracking and studying patient outcomes for over thirty years, and is now tracking over 48,000 surgeries in the COOR database. The team has collected over 195,000 subjective patient questionnaires and has over 170,000 unique patients included in the database.

COOR tracks patient outcomes following their orthopaedic treatments. In studying these outcomes, the team is able to validate the efficacy of surgical techniques, therapies and other medical treatments. COOR partners closely with The Steadman Clinic physicians, who use the database to aid in diagnosis and treatment selection. Patients also benefit from the database, as physicians can offer recovery expectations based in concrete data. One of COOR's primary responsibilities is producing long-term outcomes studies, including studies on the pioneering techniques of SPRI's late founder, Dr. J. Richard Steadman and Chairman, Dr. Marc J. Philippon.

COOR is involved in each of SPRI's clinical trials and is integral in the publication of SPRI's papers. The outcomes data in COOR's database supports The Steadman Clinic's mission of practicing evidence-based medicine—treatments and techniques that are backed by research. Physicians from all over the world look to COOR's evidence-based medicine research to inform their clinical decision making.

As a partner to the U.S. Olympic & Paralympic Committee (USOPC), COOR has been instrumental in organizing the past seven Injury Prevention Symposium events. The annual event connects leading scientists, researchers and clinicians from all over the world to engage on topics related to injury prevention and protecting the health of athletes.

48,154
SURGERIES BEING TRACKED

197,942
SUBJECTIVE PATIENT QUESTIONNAIRES RECORDED

109,515
OBJECTIVE CLINICAL EXAMS

19,962
MRIS INTERPRETED

170,563
UNIQUE PATIENTS INCLUDED IN DATABASE

PUBLICATIONS

- **43 COOR** primary publications since July 2022
- **24 publications** utilizing Patient Outcomes Database
    - 8 publications in the *American Journal of Sports Medicine*
    - 5 publications in *Arthroscopy*
- **69%** of SPRI's publications since 2021 included a COOR author
- **100%** of COOR primary publications were performed in conjunction with the Clinical Fellows and/or International Scholars

KEY HIGHLIGHTS

- COOR was instrumental in securing the International Olympic Committee (IOC) Center for Excellence designation for the next Olympic cycle (2023–2026). Currently, SPRI and its partners within the U.S. Coalition for the Prevention of Illness and Injury in Sport represent the United States as one of 11 countries with the IOC research center designation.
- Outcomes research from COOR helped to secure Chairman Dr. Marc J. Philippon's Clinical Research Award from the Orthopaedic Education and Research Foundation (OREF). Congratulations to Karen Briggs (COOR emeritus), Grant Dornan and the entire outcomes team on this achievement.
- The COOR team was an integral part of the planning and execution of the 7th Annual Injury Prevention Symposium, co-hosted with the U.S. Olympic & Paralympic Committee. The event was broadcast from the USOPC training center in Colorado Springs, CO.
- COOR continued its support of SPRI's clinical trials, including database support and retrospective studies for Department of Defense (DoD)-funded clinical trials.

SPRI Continues Global Commitment to Athlete Health

AS A PARTNER TO THE U.S. OLYMPIC & PARALYMPIC COMMITTEE (USOPC), SPRI AND THE STEADMAN CLINIC HAVE BEEN SUPPORTING TEAM USA ATHLETES THROUGH MEDICAL TREATMENT, INJURY PREVENTION RESEARCH AND INJURY SURVEILLANCE PROGRAMS.

With the University of Utah, SPRI and the USOPC form the U.S. Coalition for the Prevention of Illness and Injury and Sport, which was designated by the International Olympic Committee (IOC) as one of 11 research centers worldwide, focused on injury prevention and the protection of athlete health. In this capacity, SPRI's research not only has an impact on the United States, but also globally. With the Olympic spirit in mind, these centers form an international network of connected scientists and clinicians—experts who share their discoveries and findings to help athletes worldwide.

The United States as a Center for Excellence

Last summer, SPRI—alongside its U.S. Coalition partners—submitted an application to the IOC to continue its status as an IOC Research Centre of Excellence, highlighting SPRI's educational conferences, fellowship and residency programs, international research scholarships, laboratories and distinctive research focused on injury prevention. SPRI's efforts were led by the Center for Outcomes-Based Orthopaedic Research.

In October, the IOC announced that the U.S. Coalition would be recognized as a member of the IOC Medical Research Network for the next Olympic Cycle: 2023–2026. In its designation, the IOC indicated that the U.S. Coalition demonstrated scientific, clinical and educational expertise in sport and exercise medicine worthy of the IOC Medical Research Network.

Looking Ahead

As SPRI continues its work with its U.S. Coalition partners, the organization looks ahead to the Summer Games in Paris, France (2024) and the Winter Games in Milan and Cortina d'Ampezzo, Italy (2026) as the next games of the 2023–2026 Olympic Cycle. For SPRI's partners at The Steadman Clinic, that means taking care of Team USA and helping athletes return to competition. At SPRI, it's focusing on injury surveillance and re-injury prevention research—a vital component of injury prevention efforts.

ACCELERATING THE POTENTIAL OF OUTCOMES RESEARCH WITH ARTIFICIAL INTELLIGENCE

AS ARTIFICIAL INTELLIGENCE (AI) HAS BECOME MORE MAINSTREAM WITH THE COMMERCIAL LAUNCH OF LARGE LANGUAGE MODELS LIKE CHATGPT AND GOOGLE'S BARD, ORGANIZATIONS ARE MORE OPENLY EMBRACING AI APPLICATIONS TO IMPROVE EFFICIENCY AND PRODUCTIVITY. AT COOR, THE TEAM HAS BEEN USING SEVERAL AI APPLICATIONS LIKE PREDICTIVE ANALYTICS AND MACHINE LEARNING FOR YEARS, AND IS CURRENTLY INVESTIGATING SOME FUTURE-LOOKING AI APPLICATIONS THAT GO BEYOND THEIR PRECEDING TECHNOLOGIES.

The Turing Test

Originally called "the imitation game" by its inventor Alan Turing in 1950, the Turing Test tests a machine's ability to exhibit intelligent behavior that is equal to that of a human. When ChatGPT was launched, the COOR team decided to complete its own Turing Test: could the department's director, Grant Dornan, distinguish the scientific abstract that was written by ChatGPT?

SPRI International Research Scholar, Dr. Marco Rupp, was tasked with writing an abstract for a scientific paper, including references; ChatGPT was given the same assignment. Both abstracts began with the following statement: "In recent years, the understanding of correctly diagnosing and treating septic arthritis (SA) of the knee following anterior cruciate ligament reconstruction (ACLR) has steadily increased."

Grant Dornan read each abstract three times to complete the Turing Test. When he chose the human-composed abstract, he indicated that he had **51% certainty.** While he guessed correctly, it was clear that ChatGPT was passable for human, showing that models like it could make evidence aggregation and scientific processes more efficient—According to *Nature*, at least four scientific papers have cited ChatGPT as a co-author, leading to a debate among scientists and researchers.

Current AI Solutions in Practice

SPRI has been using **predictive analytics** for years. Utilizing patient-reported outcomes from the COOR database, the team can use patient demographic information to predict their recovery from surgical treatment. To achieve this, the predictive analytics

application runs all of the data points compiled on similar patients who have received treatment. This data helps the physician make a treatment selection while educating the patient on expectations.

Machine Learning is commonly used in medical applications, specifically in medical imaging. The algorithm looks for patterns that indicate a particular disease, disorder or injury pattern. At SPRI, our advanced imaging research team uses this type of technology to automate anatomical segmentation and the results are significant: a task that manually takes up to eight hours to complete is completed in minutes with this automated technology.

MRIs to Model Joints

SPRI Fellow Dr. Ali Noorzad used a machine learning software to take MRI scans and create 3D models. These models can be printed, allowing a clinician to physically hold the model joint and explore the patient's unique anatomy before operating. 3D-printed models could also be used by clinicians for the purpose of patient education, offering a new way to show the patient their injury, repair techniques and anticipated recovery.

SPRI scientist Dr. Lauren Watkins is using a similar software, but the program she uses is designed to specifically investigate the quality of tissue in the shoulder. One of the biggest issues a surgeon faces with rotator cuff repair is the quality of a patient's tissue. Prior to the development of this software application, the only way to assess tissue quality was after the surgeon had begun the repair. This technology will enable surgeons to assess tissue quality and know what kind of repair will be most effective for the patient before they are in the operating room. And, adding a predictive analytics element to this technique, the surgeon will be able to compare tissue quality across different patients, furthering the understanding of what treatments are most effective for each patient. Dr. Watkins and her team have submitted a grant to the National Institutes of Health (NIH) to fund additional research into this application.

Future AI Applications, Within Reach

As an innovative, forward-looking organization, SPRI is currently investigating AI tools that are in development, exploring how they might enhance or improve clinical practice and scientific research. Two of the applications currently being investigated are **Explainable AI** and **Causal AI.**

Explainable AI

Explainable AI is a burgeoning field. It takes artificial intelligence and builds trust, as many AI/Machine Learning tools are highly powerful in terms of predictive accuracy, but remain opaque with respect to human interpretation for a particular prediction or individual patient. This limits the trust of physicians and patients when making difficult treatment decisions. Explainable AI takes an extra step to better understand the key factors that drive predictions of good or poor outcomes for individual patients. In medical applications, this is the epitome of personalized medicine.

Explainable AI effectively "shows the work," and prioritizes the factors that matter. This includes how the same injury may lead to different outcomes based on data, and how the same outcomes may result from different reasons.

AS AN INNOVATIVE, FORWARD-LOOKING ORGANIZATION, SPRI IS CURRENTLY INVESTIGATING AI TOOLS THAT ARE IN DEVELOPMENT, EXPLORING HOW THEY MIGHT ENHANCE OR IMPROVE CLINICAL PRACTICE AND SCIENTIFIC RESEARCH.

Causal AI

An additional possible source of mistrust for current AI/Machine Learning models arises from the much-discussed distinction between causation and correlation. Correlations are common in modern big datasets. Yet, understanding causation is what healthcare decision-makers truly need, and it's not yet available. The fields of causal inference and causal AI offer a paradigm shift for identifying causal relationships for situations where a randomized clinical trial could be cost prohibitive, impractical or unethical.

COOR completed a Causal Inference AI study with SPRI Chairman Dr. Marc Philippon, which looked at a clinical issue with different opinions:

- Dr. Philippon's position: Whenever possible, repair the labrum versus reconstruction or removal
- Other clinical opinion: Reconstruction is the correct course of treatment.

Similar to a randomized clinical trial, the causal inference AI application reviewed nearly 1,200 of Dr. Philippon's past cases, examining all of the available data points to estimate the results. The result of this exercise was that Dr. Philippon's approach to repair the labrum whenever possible was the best course of treatment. This is an example of how AI can help a clinician answer critical clinical questions when there are conflicting opinions and approaches in the field.

The team completed a manuscript following this study and plan to submit for peer review.

Harnessing AI is Necessary

The arrival of large language models and AI-powered assistants makes it clear that people are open to accepting artificial intelligence into their daily lives. As the field continues to evolve into new applications with significant clinical impact, it's important for organizations like SPRI to harness these tools, helping to enhance the patient experience, backed in research.

DEPARTMENT OF EDUCATION

FACULTY AND STAFF

MARC J. PHILIPPON, MD
Chairman, Steadman Philippon Research Institute
Co-Director, Sports Medicine Fellowship; Co-Director,
Hip Preservation & Reconstruction Fellowship

MATTHEW T. PROVENCHER, MD, MBA
Co-Director, Sports Medicine Fellowship

JOEL M. MATTA, MD
Co-Director, Hip Preservation & Reconstruction Fellowship

C. THOMAS HAYTMANEK JR, MD
Director, Foot & Ankle Fellowship

THOMAS R. HACKETT, MD
Faculty, Sports Medicine Fellowship

PETER J. MILLETT, MD
Faculty, Sports Medicine Fellowship

ARMANDO F. VIDAL, MD
Faculty, Sports Medicine Fellowship

LESLIE B. VIDAL, MD
Faculty, Sports Medicine Fellowship

JONATHAN A. GODIN, MBA, MD
Faculty, Sports Medicine Fellowship

LIZ PYKE
Fellowship Program Coordinator

ALEX BRADY, MS
Education and Public Outreach Program Co-Manager

PATRICIA MCNAMARA
Education and Public Outreach Program Co-Manager

Educating Tomorrow's Orthopaedic and Sports Medicine Leaders

CLINICAL FELLOWSHIPS

SPRI's Department of Education is focused on educating top orthopaedic surgeons following their residency. Each year, SPRI hosts these surgeons for a one-year intensive at SPRI and The Steadman Clinic. These programs include the ACGME-accredited Sports Medicine Fellowship, Foot & Ankle Fellowship and Hip Preservation & Reconstruction Fellowship. Over the past year, SPRI has hosted eight sports medicine fellows, one foot & ankle fellow and one hip preservation & reconstruction fellow. These physicians work with attending faculty from The Steadman Clinic and each of SPRI's research teams to have a comprehensive clinical fellowship that is based in research and the practice of evidence-based medicine.

Each year, two clinical fellows are selected by the Tang Family as named fellows, sponsored by the Tang Family Education Endowment.

The clinical fellowship alumni network now includes nearly 260 surgeons practicing in communities around the world. These alumni often hold academic positions at leading universities, maintain chief positions at hospitals and work at elite practices. Several of the programs' former fellows have been recruited back to The Steadman Clinic as physicians, including Drs. Randall W. Viola, Peter J. Millett, C. Thomas Haytmanek, Jr, Jonathan A. Godin, Jared T. Lee and Joseph J. Ruzbarsky. Three of these former fellows now serve on the faculty for these clinical fellowships—Drs. Millett, Haytmanek and Godin.

INTERNATIONAL SCHOLARSHIPS

SPRI is proud to host international physicians and scientists each year to participate in research and learn from SPRI's renowned scientists. As practicing surgeons in their native countries, these scholars focus on research while at SPRI and observe The Steadman Clinic's attending physicians. This year, SPRI hosted international scholars from Japan, Thailand, Germany and Italy.

The goal of both the fellowships and scholarships is to provide a comprehensive, immersive education experience for surgeons during their time in Vail. They are able to participate in research in their areas of interest, from biomechanics and biologics to clinical outcomes research. Fellows and scholars work closely with surgeons and scientists, refining their orthopaedic skills in the Surgical Skills Laboratory. They investigate the causes, prevention and cures of degenerative diseases alongside the treatment and prevention of joint injuries. When they complete their time in Vail, these fellows and scholars go on to practice orthopaedic and sports medicine care that is backed in research, ensuring that patients around the world have the opportunity to receive the best evidence-based medical care for their needs.

2022–2023 TANG-FAMILY-ENDOWED SPORTS MEDICINE FELLOWS

These fellows are generously funded by the Tang Family Education Endowment.

JOHN (YIANNI) APOSTOLAKOS, MD, MPH
Undergraduate: University of Connecticut
Medical School: University of Connecticut
Residency: Hospital for Special Surgery

CLAIRE RYAN, MD
Undergraduate: Northwestern University
Medical School: Weill Cornell Medical College
Residency: University of Texas at Austin

2022–2023 SPORTS MEDICINE FELLOWS

RICHARD AMENDOLA, MD
Undergraduate: University of Iowa
Graduate: University of Iowa
Medical School: University of Iowa Medical School
Residency: SUNY Upstate Medical University

MATTHEW DEASEY, MD
Undergraduate: University of Virginia
Medical School: Temple University
Residency: University of Virginia

MATTHEW ANDERSON, MD
Undergraduate: Duke University
Medical School: Icahn School of Medicine at Mount Sinai
Residency: Columbia University

JONATHAN HASKEL, MD
Undergraduate: Duke University
Medical School: Rutgers Robert Wood Johnson Medical School
Residency: NYU Langone Orthopedic Hospital

PETER CHANG, MD
Undergraduate: Arizona State University
Medical School: University of South Dakota School of Medicine
Residency: Washington University in St. Louis

ALI NOORZAD, MD
Undergraduate: George Mason University
Medical School: Northeast Ohio Medical University
Residency: Cedars-Sinai Medical Center

2022–2023 FOOT & ANKLE FELLOW

GARRET GAROFOLO, MD
Undergraduate: University of Michigan
Medical School: AUC School of Medicine
Residency: Maimonides Medical Center
Fellowship: Southern California Orthpedic Institute

2022–2023 HIP PRESERVATION & RECONSTRUCTION FELLOW

BENJAMIN KUHNS, MS, MD
Undergraduate: Colgate University
Graduate School: Northwestern University
Medical School: Case Western Reserve University
Residency: University of Rochester Medical School

2022–2023 INTERNATIONAL SCHOLARS

MARCO ADRIANI, MD
Medical School: University of Tor Vergata
Residency: University of Brescia
Visiting Fellowship: University of Bradenburg (Germany)
Italy

PHOB GANOKROJ, MD
Medical School: Chulalongkorn University
Residency: The Medical Councils of Thailand
Fellowship: Police General Hospital, Thai Orthopaedics Society for Sports Medicine, Ewha Womans University Medical Center
Thailand

HARUKI NISHIMURA, MD, PHD
Undergraduate: University of Occupational and Environmental Health
Medical School: University of Occupational and Environmental Health, Japan School of Medicine
Residency: University of Occupational and Environmental Health, Fukuoka Shin-Mizumaki Hospital
Japan

MARCO-CHRISTOPHER RUPP, MD
Medical School: Technical University of Munich
Residency: Department of Orthopaedic Sports Medicine, University Hospital Rechts-der-Isar, TU Munich
Visiting Fellowship: Midwest Orthopaedics at Rush
Germany

KOHEI YAMAURA, MD, PHD
Undergraduate: Kobe University School of Medicine
Medical School: Kobe University School of Medicine
Residency: Rokko Island Konan Hospital, Kobe University Hospital, Himeji St. Mary's Hospital, Steel Memorial Hirohata Hospital, Hyogo Prefectural Central Rehabilitation Hospital
Japan

The 2023 Class of Clinical Fellows celebrated their year of fellowship at the July 27th Hail and Farewell event at Donovan Pavilion in Vail

YOUTH EDUCATION SPARKS PASSION FOR SCIENCE AND MEDICINE

As education is intertwined with SPRI's mission, the organization is proud to offer programs to local Colorado students. Since 2011, SPRI has offered fifth grade science tours through SPRI's state-of-the-art laboratories—Biomotion, Regenerative Medicine, Robotics and Surgical Skills—interactive sessions at local middle school and science fair judging and immersive experiences for high school students.

SPRI offers a year-long program for local high school students in which juniors and seniors perform hands-on research alongside SPRI scientists and researchers. In addition to the science club, SPRI hosts a week-long summer course called the Summer Scholars Program, in which dozens of high school students participate in an immersive STEM course. All of SPRI's youth education programs are made possible by SPRI researchers, engineers and scientists, who volunteer their time to work with local students.

SPRI expanded its program offerings this year to include a collaborative program with Keystone Science School's Girls in STEM, welcoming students in grades 4–8 to explore SPRI's laboratories and meet with a panel of women scientists, physicians, and researchers.

(TOP) In June, 41 students from Colorado mountain communities participated in the 2023 SPRI Summer Scholars program

(BOTTOM) This year, SPRI collaborated with Keystone Science School's Girls in STEM program, inviting students in grades 4-8 to explore SPRI's laboratories

EVENTS

Johnny Huard, PhD and Marc J. Philippon, MD welcome guests to the 7th Annual Vail Scientific Summit

SPRI's Events Connect Scientists, Clinicians and Researchers

SPRI's commitment to collaboration extends beyond the team science performed at the institute—from establishing research networks to hosting and co-hosting major academic meetings, SPRI is dedicated to participating in important conversations in orthopaedic and sports medicine science and research. These events welcome clinicians, scientists and researchers from all over the world to join in key discussions around topics like regenerative medicine, therapeutics, injury prevention and more.

VAIL SCIENTIFIC SUMMIT

The 7th Annual Vail Scientific Summit was held August 20–24, 2022 at the Hythe Resort in Vail. The theme of the meeting was "Advances in Translational Science," and the presenting sponsor of the event was Canon Medical.

The conference included a keynote address from Samuel Stupp, PhD, of Northwestern University, entitled, "New Supra-Therapies for Healthier and Longer Lives." The opening evening also included a talk from Rear Admiral Bruce L. Gillinham, MD, Surgeon General of the U.S. Navy, that discussed SPRI's U.S. Military Collaboration.

This year's summit included cross-disciplinary speakers including scientists, physicians, veterinarians and researchers. The event featured sessions focused on musculoskeletal repair after injury and disease; innovative technologies for musculoskeletal repair; interventional strategies for healthy aging; biomedical engineering; orthopaedic surgery and military performance; bone and articular cartilage regeneration and repair; biologics and more.

As in past years, the summit included presentations from world-renowned physicians, scientists and researchers. Some of these presenters included Dr. Susan Bakata of UC San Diego Health; Dr. Constance Chu of Stanford University, Dr. Micky Collins of the University of Pittsburgh Medical Center, Dr. Farshid Guilak of Washington University in St. Louis; Dr. James

Kirkland of the Mayo Clinic; Drs. William Murphy and Mark Markel of the University of Wisconsin; Dr. Linda Sandell of Washington University School of Medicine; Dr. Samuel Stupp of Northwestern University; and many other esteemed scientists and clinicians. Several of The Steadman Clinic's physicians—Drs. Philippon, Millett, Evans, Provencher, Godin, Sachar, Anderson, Ruzbarsky and Gallizzi—and SPRI's scientists—Drs. Huard, Bahney, Gao, Guo, Lu, Nakayama, Nishimura, Tashman and Watkins also presented at the Summit.

The 8th Annual Vail Scientific Summit will be held August 20–23, 2023 at The Hythe Resort in Vail, and will feature updates on the latest musculoskeletal innovations including basic science and clinical applications, amongst other topics.

INJURY PREVENTION SYMPOSIUM

The 7th Annual Injury Prevention Symposiums was presented via live webinar June 7–8, 2023, following great success from the previous years' virtual formats.

Hosted by SPRI and its partner the U.S. Olympic & Paralympic Committee (USOPC), the Injury Prevention Symposium was broadcast from the USOPC Headquarters in Colorado Springs.

The event included a keynote addresses each day from Drs. Kay Crossley and Claudia Reardon from the Amsterdam Collaboration on Health and Safety in Sports. Dr. Crossley discussed the prevalence of knee injuries in female athletes and Dr. Reardon focused on the necessity of prioritizing athlete mental health. This year's symposium featured a live demonstration by athletic trainer Marc Ryan, who serves as the rehabilitation coordinator for Dr. Marc Philippon. Ryan conducted the Vail Hip Sport Test, which is a functional test to measure a patient's readiness to return to sport following hip surgery.

SPRI's onsite team included members of the Media and Production Department, Events and the Center for Outcomes-Based Orthopaedic Research (COOR), who collaborated with the USOPC team in Colorado Springs to produce another successful conference.

The 7th Annual Injury Prevention Symposium was live broadcast from USOPC headquarters in Colorado Springs, CO.

Athletic Trainer Marc Ryan conducted a live demonstration of the Vail Hip Sport Test at this year's Injury Prevention Symposium

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