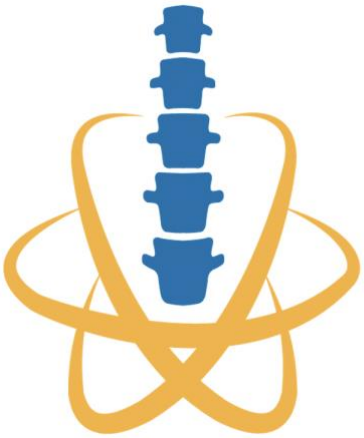
9th Annual Skeletal Research Symposium



Monday, May 6, 2024
8:00 AM – 4:15 PM

Mass General Brigham
Assembly Row
MGB Assembly Row, 399 Revolution Dr, Somerville, MA 02145

WELCOME

Welcome to the eighth annual Symposium sponsored by the Center for Skeletal Research (CSR). This symposium is an important part of the Center's activities, which have the purpose of fostering and serving the community of bone scientists in the Boston and New England region. The symposium features two world-leading investigators in bone science: Alison Boyce from the National Institutes of Health and Sundeep Kholsa from the Mayo Clinic College of Medicine and Science.

The Center grew out of a seminar series sponsored by the Endocrine Unit at the MGH and the Harvard School of Dental Medicine that has now met monthly for twelve years.

Central to the day's activities is a poster session with 59 scientific posters. You can see that these posters include participants from throughout Boston from seventeen different institutions.

The core facilities will only succeed if they are providing services that investigators want. A central mission of the cores is to be innovative and provide new services that allow the sharing of new ideas and procedures. Please let the core directors know your ideas and wish list for further offerings when you see them today or subsequently by e-mail.

Be sure to visit our website, http://csr-mgh.org. This site provides news about bone science in Boston and information about the core facilities, including how to order the Core Facilities' products, and updates on CSR activities.

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9:00 – 9:05 am	Welcome & Introduction
9:05 – 9:10 am	Introduction to CSR Mentoring Program
9:10 – 10:10 am	**Selected oral abstract session #1** • 9:10 - 9:25 - Jared Whitlock • 9:25 - 9:40 - Ridhima Seth • 9:40 - 9:55 - Jakob Hoeppner • 9:55 - 10:10 - Byron Chan
10:10 – 10:25 am	Break
10:25 – 11:25 am	**Keynote Speaker**: Alison Boyce - *"Fibrous Dysplasia/McCune-Albright Syndrome: Interplay of osteoclast/osteoblast function"*
11:25 – 11:30 am	Update from P50
11:30 – 1:00 pm	Poster session • 11:30 – 12:15 – Even Poster Presenters • 12:15 – 1:00 – Odd Poster Presenters
1:00 – 2:00 pm	Lunch & Networking/Early Stage Investigators lunch with invited speakers
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3:00 – 4:00 pm	**Keynote Speaker**: Sundeep Kholsa - *"Sympathetic tone and cellular senescence: Leveraging integrative physiology for new osteoporosis treatments"*
4:00 – 4:10 pm	Awards & Closing Remarks

CSR SYMPOSIUM COMMITEE

Julia Charles, MD, PhD
Chair, CSR Symposium Committee
Assistant Professor of Orthopaedics & Medicine, Harvard Medical School
Associate Physician, Brigham and Women's Hospital

Jingting Yao, PhD
Chair, Abstract Review Subcommittee
Postdoctoral Fellow in Radiology, Massachusetts General Hospital

Michael Albro, PhD
Assistant Professor, Mechanical Engineering
Materials Science & Engineering, Biomedical Engineering

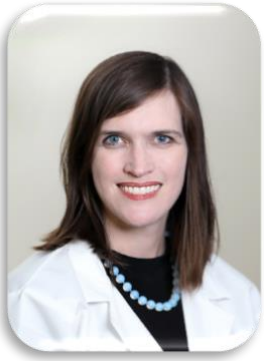
Shannon R. Emerzian, PhD
Postdoctoral Research Fellow
Center for Advanced Orthopaedic Studies, Department of Orthopedic Surgery, BIDMC

Emily Moore, PhD
Postdoctoral researcher (Vicki Rosen Lab)
Developmental Biology at the Harvard School of Dental Medicine

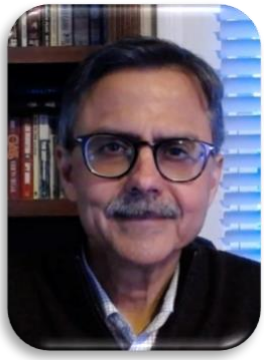
Li Zeng, PhD
Associate Professor
Tufts University School of Medicine, Dept. Immunology

Marc Wein, MD, PhD
Associate Professor of Medicine, Harvard Medical School
Massachusetts General Hospital, Endocrine Unit
Associate Member, Broad Institute of MIT and Harvard
Principal Faculty, Harvard Stem Cell Institute

INVITED SPEAKERS



Alison Boyce, MD is a pediatric endocrinologist and Chief of the Metabolic Bone Disorders Unit in the National Institute of Dental and Craniofacial Research, National Institutes of Health. The goal of her work is to enhance health and well-being for children with skeletal disorders by developing novel tools and treatments informed by pathophysiologic studies. She leads investigations in fibrous dysplasia/McCune-Albright syndrome, a rare disease affecting the bone and endocrine systems, and has characterized many aspects of this disorder and its treatment. Dr Boyce is a faculty member in the NIH Endocrinology fellowship training program. She is a member of the Fibrous Dysplasia/McCune-Albright Syndrome International Consortium and serves as Chair of the Medical Advisory Committee to the FD/MAS Alliance.



Dr. Sundeep Kholsa is the Dr. Francis Chucker and Nathan Landow Research Professor of Medicine and Physiology and a Mayo Foundation Distinguished Investigator. Dr. Khosla received his A.B. degree from Harvard College and his M.D. from Harvard Medical School. He was subsequently a resident in Internal Medicine and a fellow in Endocrinology at the Massachusetts General Hospital. In 1988 he moved to Mayo Clinic, where his research has focused on mechanisms of age-related bone loss and osteoporosis. Dr. Khosla has served as Director of the Center for Clinical and Translational Science and Dean for Clinical and Translational Science at Mayo Clinic. He has received numerous awards and honors for his work, including the Frederic C. Bartter Award for Clinical Investigation and the William F. Neuman Award for Outstanding Scientific Contributions from the ASBMR, the Outstanding Clinical Investigator Award and Plenary Lecture from the Endocrine Society, and election to the ASCI and AAP.

ORAL ABSTRACT PRESENTATIONS

Jared Whitlock
NICHD, Section on Membrane Biology
Presenting: *Getting to the "wrong" place at the right time: A Redox switch directs La protein to the osteoclast surface, promoting multinucleation and bone resorption.*

Ridhima Seth
Harvard School of Dental Medicine, Department of Oral Medicine, Infection, and Immunity, Division of Bone and Mineral Research
Presenting: *PTH signaling in Ctsk+ lineage cells is critical for skeletal homeostasis and tooth formation and eruption*

Jakob Hoeppner
Mass. General Hospital, Endocrine Unit
Presenting*: Unraveling the Pathophysiology of Eiken Syndrome: Deciphering the Role of the PTH Receptor's C-Tail in Enchondral Ossification and Bone Mineral Homeostasis*

Byron Chan
Endocrine Unit, Massachusetts General Hospital, Harvard Medical School"
Presenting: Ebf3-expressing osteoblast progenitors contribute to homeostatic bone remodeling and intermittent parathyroid hormone-induced bone formation

Sihan Liu
Tufts University, Department of Immunology
Presenting: *Macrophage-derived Wnt5a Promotes Osteoarthritis by Disrupting T cell Balance*

Gabrielle Gilmer
MGB, Physical Medicine and Rehabilitation
Presenting: *Loss of estradiol and progesterone with menopause drives cartilage degeneration via chondrocyte senescence and extracellular matrix disassembly*

9th Annual Skeletal Research Symposium

Poster Presentations

MGB Assembly Row, 399 Revolution Dr, Somerville, MA 02145

11:30 – 12:15pm – Even Poster Presenters
12:15 – 1:00pm – Odd Poster Presenters

Poster Number	First Author	Abstract Title
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2	Ridhima Seth	PTH signaling in Ctsk+ lineage cells is critical for skeletal homeostasis and tooth formation and eruption.
3	Jakob Hoeppner ***Poster & Oral presentation**	Unraveling the Pathophysiology of Eiken Syndrome: Deciphering the Role of the PTH Receptor's C-Tail in Enchondral Ossification and Bone Mineral Homeostasis.
4	Byron Chan ***Oral presentation only**	Ebf3-expressing osteoblast progenitors contribute to homeostatic bone remodeling and intermittent parathyroid hormone-induced bone formation
5	Sihan Liu ***Oral presentation only**	Macrophage-derived Wnt5a Promotes Osteoarthritis by Disrupting T cell Balance
6	Gabrielle Gilmer ***Poster & Oral presentation**	Loss of estradiol and progesterone with menopause drives cartilage degeneration via chondrocyte senescence and extracellular matrix disassembly
7	Tadatoshi Sato ***Oral presentation only**	Recombinant AAV9-mediated gene editing for Osteogenesis Imperfecta
8	Beth Bragdon	Defining Stem Cell Activation following Fracture
9	Wenhui Li	Overexpression of Wnt5a in Macrophages Promotes Osteoarthritis Progression without Inducing Macrophage Polarization
10	Andria Fremaint	Electrical Impedance Myography and Muscle Health in Older Adults with Type 1 Diabetes
11	Chloe Scanlan	Body Composition and Bone Health in Adults with Type 1 Diabetes
12	Emily R. Moore	The role of BMP2 in appositional bone growth
13	Camryn Berry BS	Dissecting the Heterogeneity of Craniofacial Lesions in Patients with Fibrous Dysplasia/McCune-Albright Syndrome

14	Jason Marvin	The Role of the Innate Immune Response in Regeneration of the Adult Zebrafish Enthesis
15	Sachin Chaugule	Essential Roles of Ubiquitin Specific Protease (USP8) in Postnatal Skeletal Development
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18	Marcela Granados	Femoral Cortical Bone Material Properties in Older Adults with Type 2 Diabetes
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20	Tianbai Wang	A Bio-inspired Latent TGF-β Conjugated Scaffold to Improve Cartilage Regeneration
21	Carolyn Chlebek	In Obese C57Bl6 Mice, Weight Loss due to Caloric Restriction Reduced Bone Morphology but Improved Components of Systemic Metabolism
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Poster Abstracts

Presenter: Jared Whitlock
Institution: NICHD **Department:** Section on Membrane Biology

Poster Number: 1 ***Oral presentation only***

Title: Getting to the "wrong" place at the right time: A Redox switch directs La protein to the osteoclast surface, promoting multinucleation and bone resorption.

Co-Authors: Jarred M. Whitlock, Evgenia Leikina, Kamran Melikov, Luis Fernandez de Castro, Wendy Zhang, Michael P. Bachmann, Benjamin Geiger, Ari Elson, Alison Boyce, Michael T. Collins, Leonid V. Chernomordik

Abstract:
Bones are living tissues, continuously remade on-site by teams of multinucleated osteoclasts that resorb old bone and osteoblasts that deposit new bone. Multinucleated osteoclasts form by the fusion of mononucleated precursors, and the number of nuclei within an osteoclast determines its resorption capacity (i.e., more fusion leads to more bone resorption). Many skeletal pathologies (e.g., fibrous dysplasia, osteopetrosis, osteoporosis, metastatic bone disease) are underpinned by perturbations in the number/size of osteoclasts, resulting in skeletal dysfunction in >200 million individuals world-wide. Our work demonstrates that osteoclasts have repurposed a nuclear RNA chaperone – lupus la protein (La)- as a fusion manager at the surface of osteoclast precursors, setting osteoclast size and biological activity. During osteoclast formation, we find that the nuclear localization sequence that retains La in the nuclei of all eukaryotic cells is cleaved and that reactive oxygen species oxidize critical cystine residues diverting La from the cytosol to the osteoclast surface. Cleaved, oxidized, surface La promotes osteoclast membrane fusion, multinucleation, and subsequent resorptive function. When osteoclasts reach a mature size, cleaved, oxidized, surface La is degraded and replaced by a full-length reduced species, which returns to in the nuclei of mature multinucleated osteoclasts. Moreover, our data suggest that targeting cleaved, oxidized, surface La modulates osteoclast formation and function in preclinical models of fibrous dysplasia of bone and malignant infantile osteopetrosis – two pediatric skeletal diseases with no currently approved pharmacological therapy. We hypothesize that osteoclast La represents a paradigm shifting target in our efforts to modulate catabolic skeletal metabolism in metabolic bone disease – from ablating osteoclasts to tuning their size and function in a tailored effort to address unmet needs in pediatric skeletal disease.

Presenter: Ridhima Seth
Institution: HSDM **Department:** Department of Oral Medicine, Infection, and Immunity, Division of Bone and Mineral Research

Poster Number: 2

Title: PTH signaling in Ctsk+ lineage cells is critical for skeletal homeostasis and tooth formation and eruption.
Co-Authors: Dorothy Hu, Dhairya Raval, Shawn Berry, Roland Baron and Francesca Gori

Abstract:
PTH signaling is of primordial clinical importance in the regulation of skeletal development and homeostasis, as well as in tooth formation and eruption. Both skeletal and dental mesenchymal cells express Pth1r and are target of PTH signaling. Our lab has been investigating the function of a recently identified periosteal stem cell (PSC) population labeled by Cathepsin K (Ctsk) in the regulation of cortical bone homeostasis. Ctsk+ lineage PSCs, which fulfil stemness criteria, express high levels of the Pth1r and respond to iPTH treatment. Ctsk is also expressed in dental pulp cells, dental follicle cells, and the periodontal ligament which are known to play a key role in tooth development and eruption.

Whether PTH signaling in Ctsk+ lineage cells is required for proper periosteal bone formation and tooth

development and eruption is not known. We, therefore, undertook a study to investigate whether PTH signaling in the Ctsk expressing PSCs and dental mesenchymal cells regulates these processes. To this end, we generated mice lacking Pth1r, specifically in Ctsk+ cells, using the CtskCre mice and analyzed their cortical bone and teeth.

Eight-week-old CtskCre;Pth1rfl/fl (CtskPth1r) male and female mice are significantly smaller than their control littermates (Pth1rfl/fl). mCT and bone histomorphometry analyses revealed a significant decrease in cortical bone volume (%) (p=0.04), cortical thickness (p=0.002), and periosteal MAR (p=0.02) and increased cortical porosity (p=0.01) in CtskPth1r mice compared to Pth1rfl/fl littermates. Notably, CtskPth1r male and female mice present with failure of molar eruption and impaired incisor eruption. mCT analyses and histological examination revealed several abnormalities in 8-week-old CtskPth1r mice, including truncated molar roots, loss of the periodontal ligament, root ankylosis, reduced cementoblasts, and markedly decreased alveolar bone. Severe dental anomalies were also seen in CtskPth1r mice at postnatal day 12 and 19. LysM-CrePth1rmice display normal cortical bone and tooth eruption, confirming that Pth1r is not expressed in osteoclast-lineage cells.

While our findings confirm the significance of PTH signaling in both periosteal bone formation and tooth development and eruption, they reveal for the first time a crucial role for Ctsk+ lineage cell-dependent PTH signaling within the periosteum and dental mesenchyme. The periosteum is a significant source of stem cells and progenitors contributing to bone growth and homeostasis, regeneration and response to anabolic drugs. Investigating the signaling molecules and pathways regulating periosteal stem cells offers an opportunity to advance our understanding of the mechanisms involved in these processes and may open novel and targeted therapeutic approaches for human diseases associated with bone fragility and impaired bone regeneration. Similarly, a comprehensive understanding of distinct subsets of dental mesenchymal cell populations and unravelling their regulation is of significance for the effective pursuit of novel dental regenerative strategies.

Presenter: Jakob Hoeppner

Institution: MGH **Department:** Endocrine Unit

Poster Number: 3

Title: Unraveling the Pathophysiology of Eiken Syndrome: Deciphering the Role of the PTH Receptor‘s C-Tail in Enchondral Ossification and Bone Mineral Homeostasis.

Co-Authors: Jakob Höppner, Ignacio Portales-Castillo, Monica Reyes, Thomas Dean, Patrick Hanna, Prem S. Yadav, Ashok Khatri, Thomas J. Gardella & Harald Jüppner

Abstract:
Eiken Syndrome (ES) is a rare disease, characterized by delayed bone mineralization and evidence of PTH resistance in some patients. The disease is caused by non-lethal homozygous mutations in the parathyroid hormone (PTH)/PTH-related peptide (PTHrP) receptor (PTH1R) which plays a key role in skeletal development and in bone/mineral homeostasis. Truncation of the PTH1R C-tail at arginine 485 (R485X), as occurs in one family with ES, removes serine phosphorylation sites involved in βarrestin binding. In HEK293 cells, R485X-PTH1R exhibits deficient βarrestin recruitment, increased basal cAMP signaling as well as ligand-biased hyper-responsiveness towards PTHrP.

To study how this PTH1R C-tail truncation results in delayed bone mineralization, we generated humanized R485X-PTH1Rhomo knock-in mice. Skeletal whole mounts of R485X-PTH1Rhomo mice (P3) revealed delayed bone mineralization as observed in ES patients and histology of their growth plates showed delayed chondrocyte maturation. Tails of these mice were ~50% shorter than those of WT littermates, reminiscent of mice overexpressing PTHrP via the Col2 promoter (Weir et al. PNAS 1996). These findings suggest that the skeletal phenotype in R485X-PTH1Rhomo mice is at least partly due to PTHrP hyper-responsiveness. To test this hypothesis, we generated R485X-PTH1Rhomo/PTHrPf/f/Col2-Cre(tg) mice. The resulting PTHrP-

ablation in proliferating chondrocytes normalized tail length as well as growth plate histology, supporting a mechanism by which PTH1R C-tail truncation causes excess cAMP signaling in response to PTHrP thus delaying chondrocyte differentiation and bone mineralization. In-utero treatment with a PTHrP(7-36) antagonist/inverse agonist (Liu et al. JACS 2024) dramatically improved bone mineralization and accelerated chondrocyte maturation in P0 R485X-PTH1Rhomo mice. Adult R485X-PTH1Rhomo mice were found to have profoundly increased serum PTH levels while total serum calcium remained low-normal and 1,25(OH)2-vitamin D was significantly decreased. Moreover, treatment with PTH(1-34) failed to increase serum calcium as well as urinary cAMP excretion, thus providing evidence for PTH resistance as also observed in some ES patients. R485X-PTH1R expression in transiently transfected HEK293 cells was significantly reduced, as was expression of another C-terminal truncated PTH1R, E546X. Treatment with the proteasome inhibitor Lactacystin led to an increase in surface expression of both mutants, pointing towards a critical role of the PTH1R C-tail in protein maturation and surface membrane transport.

Overall, our data provide insight into plausible mechanisms underlying the skeletal and PTH resistance phenotypes in ES patients, as well as the role of the PTH1R C-tail in modulating signaling and surface expression in vital target cells. Further, our data demonstrate that a PTH antagonist/inverse antagonist can effectively target this hypersensitive PTH1R, thus providing therapeutic options for other diseases associated with PTH1R overactivity.

Presenter: Byron Chan

Institution: MGH **Department:** ENDOCRINE UNIT

Poster Number: 4

Title: Ebf3-expressing osteoblast progenitors contribute to homeostatic bone remodeling and intermittent parathyroid hormone-induced bone formation

Co-Authors: Majd George, Marc Wein, Henry Kronenberg

Abstract:
Background: Parathyroid hormone (PTH) analogs serve as effective bone anabolic agents for osteoporosis treatment. Osteoblasts originate from early progenitor cells of the mesenchymal lineage, and presumably, a major mechanism responsible for the bone anabolic action of PTH is mediated by osteoprogenitors and their response. However, osteoprogenitors are heterogenous and little is known about the effects of PTH on specific subsets of these cells. A distinct group of osteoprogenitors residing in the bone marrow stroma known as Cxcl12-abundant reticular (CAR) cells, functions to support hematopoiesis and gives rise to osteoblasts. CAR cells are thought to be the major source of osteoblasts responsible for homeostatic bone remodeling in adult life. Early B-cell factor 3 (Ebf3) is a transcription factor expressed in CAR cells and has been shown to be a crucial regulator of osteoblast differentiation. The hypothesis of this study is that Ebf3-expressing cells are an important population of osteoblast progenitors contributing to increased bone formation seen following intermittent PTH treatment. The objectives of this study are to track the fates of Ebf3-expressing cells and their descendants during the course of intermittent PTH treatment and to discern the transcriptional profile of these cells at the single cell level.

Methods: Ten-week-old Ebf3-creER; tdTomato reporter mice were injected with tamoxifen (2 mg via intraperitoneal injection) to induce Cre-mediated tdTomato expression in Ebf3-expressing cells and their progeny. Concomitantly, these mice were treated with PTH 1-34 (100 mg/kg via subcutaneous injection) or vehicle once per day for 2 days or 8 weeks. At sacrifice, humeri were isolated for histology in which tdTomato-positive (tdT+) cells were counted in distinct bone regions in a blinded manner. All other long bones were pooled, subjected to serial digestion, and tdT+ positive cells were isolated by fluorescent activated cell sorting (FACS) for single cell RNA-sequencing using the 10x Genomics platform.

Results: Histology showed that numbers of tdT+ cells in the central marrow, trabecular, endocortical, and periosteal surfaces at the metaphyseal and diaphyseal regions were increased at 8 weeks compared to 2

days post-tamoxifen. At 2 days post-tamoxifen, intermittent PTH treatment did not affect numbers of tdT+ cells in all surfaces examined except the endosteal surface. At 8 weeks post-tamoxifen, intermittent PTH treatment resulted in significantly increased tdT+ cells in trabecular, endocortical, and periosteal surfaces at the metaphyseal and diaphyseal regions. No significant difference in tdT+ cells were observed in the central marrow at both metaphyseal and diaphyseal regions with PTH versus vehicle treated groups. Single cell-RNA sequencing cluster analysis of tdT+ cells revealed 11 cell clusters, including 3 CAR cell clusters, 2 osteoblast cell clusters, 1 immature osteocyte cluster, 1 mesenchymal progenitor cell cluster, and 4 endothelial cell clusters.

Conclusions: Lineage tracing analysis confirmed the involvement of Ebf3-expressing cells and their descendants in homeostatic bone remodeling over time in skeletally mature mice. In response to intermittent PTH treatment, Ebf3-lineage cells increase in number in trabecular, endocortical, and periosteal surfaces, but not in the central marrow. Based on single cell RNA-sequencing analysis, several distinct CAR clusters were identified from FACS sorted Ebf3-labelled tdT+ cells. Further investigation regarding the transcriptional changes of these cell clusters in response to PTH is ongoing. This will facilitate improved understanding of the molecular mechanisms involved in intermittent PTH action on distinct subsets of osteoblast progenitors, which will allow for the development of superior therapies for osteoporosis.

Presenter: Sihan Liu

Institution: Tufts University **Department:** Department Immunology

Poster Number: 5 ***Oral presentation only***

Title: Macrophage-derived Wnt5a Promotes Osteoarthritis by Disrupting T cell Balance

Co-Authors: Sihan Liu, Wenhui Li, Jingshu Liu, Matthew Gordon, Daniel Sun, Li Zeng

Abstract:
INTRODUCTION: Osteoarthritis (OA) is a prevalent chronic joint disease with no FDA-approved disease modifying therapy. An increased ratio of Th17 (a pro-inflammatory T cell subtype) vs. Treg (an anti-inflammatory T cell subtype) was found in the serum of OA patients. However, the mechanisms that control the balance of Th17 vs. Treg in OA specific inflammatory environment remain unknown. Here, we explored the role of Wnt5a in shifting the balance of Th17 vs. Treg, thus promoting local inflammation and joint damage in OA.

METHODS: We performed ACL transection (ACLT) and Sham surgeries under approved IACUC protocols and evaluated joint damage by histology and T cell levels by FACS. Lentiviral Wnt5a and GFP were generated to infect bone marrow-derived macrophages (BMDMs). BMDMs expressing Wnt5a (Mφ-Wnt5a) or GFP (Mφ-GFP) were intraarticularly injected (5x10E5 cells/joint) into the mice at 5 weeks post-surgery. Mice were harvested 6 weeks post-surgery. Human peripheral T cells were subjected to in vitro differentiation of Th17 or Treg cells. Lentiviral Aurora kinase B was generated for inducting overexpression in T cells. Proteomic analysis was obtained by Reverse Phase Protein Array (RPPA).

RESULTS: 1. Increased Th17/Treg ratio in ACLT-OA mice. ACLT resulted in increased joint damage (Fig. 1A), increased number of effector T cells (Fig. 1B), and an elevated Th17/Treg ratio Fig. 1C) in ACLT-OA knee joint draining lymph nodes. 2. Wnt5a is increased in OA synovium and is expressed in Macrophages. By analyzing scRNA-seq and DNA array databases from OA patient samples, we found that Wnt5a is the most elevated among all Wnts (data not shown). Immunostaining confirmed the increase of Wnt5a in ACLT-OA synovium (Fig. 2A) and demonstrated that Wnt5a is predominantly expressed in synovial macrophages (marked by F4/80, Fig. 2B). 3. Macrophage-derived Wnt5a promotes OA severity and Th17/Treg ratio in vivo. Adoptive transfer of Mφ-Wnt5a increased synovitis and the ratio of Th17/Treg in ACLT-OA (Fig. 3A-B). 4. Wnt5a acts through Aurora kinase B (AUKB) to exert its effect on T cells. Treating Th17 and Treg T cells with Wnt5a led to increased Rorγt (Th17 marker) and decreased Foxp3 (Treg marker), supporting the role of Wnt5a in altering Th17/Treg balance (Fig. 4A). Wnt5a induced IL-17A (Th17 signature cytokine) expression

in Treg cells (Fig. 4B). Proteomic analysis suggested that Wnt5a significantly inhibited Aurora kinase B in Treg cells (Fig. 4C). Lentivirus mediated Aurora kinase B overexpression inhibited Wnt5a's activity on Treg cells (Fig. 4D).

CONCLUSION and SIGNIFICANCE: Our data suggests that macrophage derived Wnt5a promotes Th17 and suppresses Treg in OA and acts through Aurora kinase B, whereby altering the OA inflammatory microenvironment. This study is the first to investigate the regulating mechanisms of T cell subtype balance in OA unique inflammatory microenvironment and the first to determine the function of Wnt5a and Aurora kinase B in altering Th17/Treg ratio. The success of this work will provide important insights into OA treatment strategies.

Presenter: Gabrielle Gilmer

Institution: MGB **Department:** Physical Medicine and Rehabilitation

Poster Number: 6

Title: Loss of estradiol and progesterone with menopause drives cartilage degeneration via chondrocyte senescence and extracellular matrix disassembly

Co-Authors: Hirotaka Iijima, Zachary Hettinger, Natalie Jackson, Juliana Bergmann, Allison C. Bean, Nafiseh Shahshahan, Ekaterina Creed, Rylee Kopchak, Hannah Houston, Michael Calderon, Claudette St Croix, Rebecca C. Thurston, Christopher Evans, Fabrisia Ambrosio

Abstract:
Knee osteoarthritis (KOA) is twice as likely to occur in post-menopausal women than in men. However, few preclinical studies appropriately model menopause, as female rodents rejuvenate their ovarian follicles in middle-age. This gap in knowledge likely contributes to the lack of disease modifying therapies for KOA. The purpose of this study was to implement a chemically-induced menopause model in middle-aged female mice, characterize the trajectory of cartilage degeneration, and systematically test potential treatment modalities. Middle-aged female C57/BL6N mice were randomized to receive intraperitoneal injections of either an ovarian-specific toxin (4-vinylcyclohexene diepoxide, VCD; "menopause group") or sesame oil ("non-menopause group"). Knees were collected from menopause and non-menopause groups across the perimenopause and menopause transition and were prepared in paraffin blocks, sectioned, and stained with Safranin-O/Fast green. A validated, semi-quantitative scoring system was used to assess the degree of cartilage degeneration by a blinded scorer. The menopause group had significantly more cartilage degeneration compared to the non-menopause group (Figure 1). Next, using mass spectrometry proteomics on cartilage across groups, we found that menopause triggers cellular senescence and extracellular matrix disassembly. These cascades were predicted to be driven by a loss estradiol and progesterone. To validate these findings in vivo, we randomized menopausal mice to one of the following treatment groups: non-hormonal control, estradiol (E2), progesterone (P4), or combined estradiol+progesterone (E2+P4)). We found E2 and E2+P4 decreased cartilage degeneration (Figure 2). To evaluate the translational potential of this murine finding, we isolated post-menopausal human chondrocytes and exposed them to sex hormone conditions in vitro. Here, E2+P4 decreased markers of senescence (Figure 3) and increased markers of chondrogenicity (Figure 4). These findings demonstrate that (1) a loss of E2 and P4 with menopause propagates cartilage degeneration by increasing chondrocyte senescence and extracellular matrix disassembly and (2) supplementation of these hormones protects cartilage against the deleterious effects of menopause-induced KOA.

Presenter: Tadatoshi Sato

Institution: UMass Med **Department:** Department of Medicine

Poster Number: 7 ***Oral presentation only***

Title: Recombinant AAV9-mediated gene editing for Osteogenesis Imperfecta

Authors: Yeon-Suk Yang, Sachin Chaugule, Zhihao Chen, Agustina Rodriguez, Hong Ma, Jun Xie, Guangping Gao, Jae-Hyuck Shim

Abstract:
Background: Osteogenesis Imperfecta (OI) is the most common rare skeletal disease characterized by bone fragility. The incidence is approximately 1 in 25,000-50,000 in the US. Up to 85% of OI patients have autosomal dominant mutations in the COL1A1 or COL1A2 gene. The treatments of OI are improving bone strength, mitigating fracture risk and pain, and preventing long-term complications. However, treatment options show limited success because they cannot correct the causes of collagen mutations.
Methods/results: This study utilized OIM mice harboring single guanine (G) nucleotide deletion at position 3983 within the Col1a2 gene to model the dominant form of human OI type III. As a result, OIM mice displayed smaller body sizes, multiple non-union bone fractures, and pelvic bone deformity. µCT analysis demonstrated a significant decrease in trabecular bone mass in the long bones of these mice. We examined CRISPR/Cas9-mediated editing efficiency of the Col1a2 gene in immortalized OIM osteoblasts. The gene-editing efficiency in these cells was substantially increased when CRISPR/Cas9 was coupled with a donor AAV9 vector containing a promoterless partial mouse Col1a2 complementary DNA sequence (GeneRide designed the optimized Condons for avoiding OIM mutation gRNA targeting, Figure 1) confirmed by NGS (next generation sequencing) analysis. This approach effectively reversed the dysregulation of osteogenic differentiation by a Col1a2 mutation in vitro and in vivo. Furthermore, systemic administration of dual AAV vectors lowered bone matrix turnover rates by reducing osteoblast and osteoclast development while improving the cellular network of mechano-sensing osteocytes embedded in the bone matrix. This strategy significantly improved bone architecture/mass/mineralization, skeletal deformities, grip strength, and spontaneous fractures. Conclusion: These findings demonstrate that AAV-mediated gene editing effectively corrects a collagen mutation in OI osteoblasts and reverses skeletal phenotypes in OIM mice.

Presenter: Beth Bragdon

Institution: Boston University Chobanian & Avedisian School of Medicine **Department:** Orthopaedic Surgery

Poster Number: 8

Title: Defining Stem Cell Activation following Fracture

Co-Authors: Yu Liu, Simon Lu, Yuchen Liu, Louis Gerstenfeld, Chao Zhang

Abstract:
Mesenchymal stem cells (MSCs), more recently named skeletogenic stem cells (SSCs) are terms for the stem/progenitor cells that differentiate to the chondrogenic, osteogenic, and/or adipogenic lineages and are involved with bone homeostasis and early phases of fracture repair. One such stem/progenitor cell population expresses Prx1 (paired-related homeobox 1). These cells have been identified in various locations including the periosteum and skeletal muscle of limbs, however it is not clear how these cell populations are similar or respond to injury. In this study, we assessed the activation and early commitment of the Prx1 cells derived from the periosteum and muscle following fracture using single cell RNA sequencing.

Use of mice (male and female age 8-12 weeks) were approved by IACUC. The tamoxifen inducible Prx1 reporter mouse (Prx1CreER;Rosa26tdTomato;Rag) was used to fluorescently tag the Prx1 cell population. Tamoxifen induction occurred three days prior to surgery. Mice received a stabilized femoral fracture. Non-operated mice served as day 0. Three days post fracture, the fracture callus and surrounding skeletal muscle were harvested and enzymatically digested, followed by cell sorting for fluorescence. Single cell RNA sequencing was performed on the positive dTomato cell populations. Seurat V4 R Tool kit for single cell genomics and the Moncocle software tool for pseudotemporal ordering to project cell lineage fates were

used.

At homeostasis (day 0), both the periosteum and muscle derived Prx1 cell populations mark similar mesenchymal cell types such as MSC/SSCs, tenocyte-like cells and low proliferative progenitors. However, the periosteum consisted of more proliferative progenitors, differentiated osteoblasts, fibroblasts, and chondrocytes while the muscle Prx1 cells consisted of greater numbers of endothelial and smooth muscle cells. Following fracture injury the periosteal derived Prx1 cells showed a quick response of the MSC/SSCs, becoming active and shifting to the proliferating progenitors and osteoblasts. In the muscle, the Prx1 derived cells also showed activation, however, the shift is primarily from the MSC/SSCs to an intermediate transitional stem/progenitor cell population. Pseudotemporal analysis identified different trajectories of the Prx1 cells derived from the two tissue compartments.

These results suggest the periosteal Prx1 SSC/progenitors cells are highly sensitized to injury response and contain a population that rapidly shift to the proliferating progenitor stage as an early repair response. It was more unexpected that the proliferative cell population did not include the differentiation stages. The muscle Prx1 stem cells appear to first go through a transitional phase or the activation process is delayed in the muscle. This is consistent with prior studies that suggested that the stem/progenitor cells in the muscle are in a more quiescent state needing more stimuli to become activated and contribute to repair.

Acknowledgements: We want to thank the Boston University Chobanian and Avedisian School of Medicine Flow Cytometry Core and Single Cell Sequencing Core for their assistance. This research was supported by grants from NIH NIAMS K99/R00 AR068582 and from BU CTSI 1UL1TR001430

Presenter: Wenhui Li

Institution: Tufts University **Department:** Pharmacology and Drug Development

Poster Number: 9

Title: Overexpression of Wnt5a in Macrophages Promotes Osteoarthritis Progression without Inducing Macrophage Polarization

Co-Authors: Sihan Liu, Li Zeng

Abstract:
Introduction: Osteoarthritis (OA) is a common disease characterized by synovium inflammation, cartilage degradation, and bone remodeling. Currently, there is no available disease-modifying medication for OA. Elevated level of Wnt5a has been observed in human OA synovium, and we found it was primarily expressed by macrophages. Here, we explore the effect of Wnt5a overexpression on macrophages and the function of Wnt5a-overexpressing macrophages in OA.

Methods: Bone marrow derived macrophages (BMDMs) were infected with Lentiviral-GFP or Wnt5a. In vitro assays were performed to test the effect of Wnt5a overexpression on the function of macrophages. C57BL/6 wild type mice were subjected to ACL transection (ACLT) surgery under IACUC protocol. Mice were intraarticularly injected with Lenti-Wnt5a infected BMDMs (M**φ**-Wnt5a) or M**φ**-GFP at 5 weeks post-surgery. Whole knee joints were harvested 1 week after injection. Safranin O (Saf. O), Immunofluorescence (IF), and Immunohistochemistry (IHC) staining were performed to assess pathological changes and cellular features. Cells were counted using ImageJ. Statistical analysis: Student's t-test or ANOVA modeling. Error bars, SEM.

Results: 1. Wnt5a positive cells are increased in ACLT+M**φ**-Wnt5a synovium. IHC staining showed that the number of Wnt5a positive cells was significantly increased in ACLT+M**φ**-Wnt5a group (Fig.1). 2. M**φ**-Wnt5a promotes OA progression in ACLT mice. Saf. O staining and OA scoring results showed that after adoptive transfer of lentiviral-infected BMDMs into mouse knee joint, the ACLT mice injected with M**φ**-Wnt5a (ACLT+M**φ**-Wnt5a) developed more severe synovitis and osteophyte (Fig.2). 3. Overexpression of Wnt5a does not change the phenotype of macrophages in vivo. There was no significant difference between the

numbers of M1 (F4/80+iNOS+, pink arrows) and M2 (F4/80+CD206+, red arrows) macrophages in sham or ACLT group (Fig.3, yellow arrows: F4/80+ only), which is consistent with the in vitro result that Wnt5a overexpression did not influence the phenotype of macrophages. 4. Overexpression of Wnt5a in BMDMs promotes cytokine expression in vitro. The ratio between the RNA levels of iNOS (M1 marker) and Arg1 (M2 marker) was not significantly changed (Fig.4a), while elevated levels of both pro-inflammatory IL-6 and anti-inflammatory IL-10 were observed in Mφ-Wnt5a under LPS-treated condition by ELISA (Fig.4b). Cytokine array also showed similar trends (data not shown), implicating that Wnt5a promoted macrophage activation but did not induce its polarization.

Discussion: Ectopically expressing Wnt5a in Mφ increased OA severity without altering the proportion of M1 and M2 macrophages in mouse knee joints, suggesting that Wnt5a exacerbating OA is not due to promoting macrophage polarization. However, Wnt5a could augment the cytokine production from macrophages, which will be further examined. This study provides important insights into the role of Wnt5a in controlling inflammatory microenvironment in OA.

Presenter: Andria Fremaint

Institution: MGH **Department:** Endocrine Unit

Poster Number: 10

Title: Electrical Impedance Myography and Muscle Health in Older Adults with Type 1 Diabetes

Authors: Grace Jung, Mary Bouxsein, Ph.D, Elaine Yu, MD

Abstract:
Recent studies indicate that individuals with Type 1 diabetes mellitus (T1D) develop skeletal muscle health impairments which might contribute to falls and explain their high fracture risk. A novel handheld Electrical Impedance Myography (EIM) device (mScan model 1301, Myolex) has been developed to assess muscle quality by calculating tissue impedance. We hypothesized that mScan could identify individuals with worse functional outcomes and lower muscle mass among older adults with and without T1D.

Using data from the ongoing the T1D Bone Health Connection (BEACON) Study, we examined patients aged 50+ who had undergone EIM testing (n=60 T1D, n=48 non-diabetic controls). We compared the muscle strength of T1D patient and controls by looking at the 100 kHz EIM phase data at the non-dominant biceps brachii. A total of six muscles (biceps, wrist extensors, lumbar paraspinals, medial gastrocnemius, and tibialis anterior) were studied unilaterally in each individual. In healthy muscles, the phase angle—a ratio of reactance to resistance calculated as Phase = arctan(reactance/resistance)—typically increases, whereas lower phase values indicate poorer muscle health. Non-dominant max grip strength from a handheld dynamometer was correlated with EIM upper muscle data, and appendicular lean mass/height2 (ALM/ht2) from whole-body DXA scans with all assessed muscle groups. For lower limbs, correlations were drawn with the Short Physical Performance Battery (SPPB) scores and MET values from self-reported physical activity. Pearson's correlations were used for all statistical analyses.

Mean age (63±7 years) and sex (54% female) were similar between T1D and control groups. Adults with T1D had tendency towards lower muscle quality at the biceps brachii than controls, although not statistically significant (9.3±5.6 vs 11.4 ±6.4 degrees, p=0.062). Higher muscle quality at the biceps brachii in T1D group was associated with higher grip strength (r=0.40, p <0.001) as well as greater ALM/ht2 values (r=0.29, p=0.03) Improved functional status (as assessed by SPPB) in T1D was associated with healthier muscle quality at the medial gastrocnemius, lumbar paraspinal, and tibialis anterior (r=0.29, p=0.02; r=0.26, p=0.05 ; r=0.29, p=0.02) as well as at the medial gastrocnemius (r=0.31, p=0.03) in controls. Greater physical activity (as assessed by MET values) in controls was associated with higher muscle quality at the the tibialis anterior (r=0.27, p=0.05).

In summary, our preliminary data suggest that mScan holds promise for detection of skeletal muscle health

impairments in older adults, including those with T1D, potentially aiding in the management and prevention of related falls and fractures. Further research is required to validate the effectiveness of mScan in T1D and other populations.

Presenter: Chloe Scanlan

Institution: BIDMC **Department:** Orthopedic Surgery

Poster Number: 11

Title: Body Composition and Bone Health in Adults with Type 1 Diabetes

Co-Authors: Kaitlyn Allard, Elaine W. Yu, Mary L. Bouxsein, Fjola Johannesdottir

Abstract:
Fragility fractures are a growing and major health concern in individuals with T1D. T1D have a 2-3-fold higher risk of fracture than non-diabetic controls, which is only partly explained by lower bone mineral density (BMD). In individuals without diabetes, both muscle impairments and increased visceral adipose tissue are associated with increased fracture risk. However, it is unknown how these factors contribute to the high fracture risk seen in T1D. Thus, we conducted a study utilizing previously collected clinical abdominal and pelvis computed tomography (CT) scans from older adults with longstanding T1D and non-diabetic controls to evaluate bone density, muscle and fat deficits.
In this retrospective pilot study, we analyzed CT scans from a cohort of older adults with T1D (n=24) and age-, sex- and race-matched non-diabetic controls (n=50) who underwent abdominal or pelvis CT exams from 2010-2020. Exclusion criteria included history of malignancy, CKD (stage 4 or higher) and history of abnormalities in bone and mineral metabolism other than osteoporosis. Visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) volumes and quality, assessed by CT density, were obtained at L3 level (3D Slicer, TotalSegmentator) http://www.slicer.org). Trunk muscles at L3 level and gluteal muscles were segmented (3D Slicer). Muscle quality and radiomic features were extracted (PyRadiomics v3.1.0). We measured femoral neck areal BMD (FNaBMD) and trabecular volumetric BMD (vBMD) at L3 level using validated phantomless calibration (CliniQCT, Mindways, Austin, TX, USA). We employed t-tests and linear models to estimate the differences in measurements between T1D and controls with adjustment for age, body mass index (BMI) and sex.
The average age of the cohort was 64±10 years, and BMI 27.5±5.9 kg/m2; and were similar between T1D and control (p>0.85). The T1D cohort included 12 men and 12 women with mean diabetes duration of 43±10 years. T1D individuals exhibited a tendency towards higher SAT volume (13%, p=0.11) but lower VAT volume compared to controls (-18%, p=0.05) after adjusting for confounding factors. However, both VAT and SAT density were elevated in T1D compared to controls (13-14%, p<=0.002). Conversely, FNaBMD was observed to be lower in T1D than controls (-8%, p=0.06), whereas L3 trabecular vBMD tended to be higher in T1D individuals compared to controls (12%, p=0.13). Analysis for muscle is ongoing and the finalized findings will be presented at the symposium.
In conclusion, individuals with T1D demonstrate bone deficits specifically at the hip, while the lumbar spine remains unaffected compared to non-diabetic controls. Moreover, T1D group exhibit differences in fat distribution and had higher fat density compared to controls. These findings suggest potential links to metabolic abnormalities and/or dysfunction within adipose tissue. Future studies should delve into whether these alterations in fat composition correlate with skeletal fragility in T1D.

Presenter: Emily R. Moore

Institution: HSDM **Department:** Developmental Biology

Poster Number: 12

Title: The role of BMP2 in appositional bone growth

Co-Authors: Gavin Chen, David Maridas, Ana Garcia Castineiras, Laura Gamer, and Vicki Rosen

Abstract:
The periosteum contains progenitor cells capable of differentiating into osteoblasts and chondrocytes that contribute to bone growth and repair. Bone morphogenetic protein (BMP) signaling is central to the activation and differentiation of periosteal cells, and mice lacking periosteal BMP2 (Prx1Cre;Bmp2fl/fl) have significantly thinner bones and exhibit a complete lack of fracture healing due to decreased periosteal osteogenesis. Here, we investigate the unique periosteal phenotype resulting from removal of BMP2 in periosteal lineage cells.

Periosteal tissue was collected from newborn Prx1Cre;Bmp2fl/fl mutants and Bmp2fl/fl littermate controls for bulk RNAseq analysis. Multiple markers for osteogenesis and BMP signaling were downregulated in mutants, suggesting Prx1Cre;Bmp2fl/fl mice have reduced periosteal activity. We utilized our new periosteum-derived cell line and Smad-binding element analysis to narrow down novel targets that may directly interact with BMP2 in the periosteum.

To further interrogate periosteal activity in appositional growth, we designed an ex vivo growth model. Briefly, femurs from P7 littermates were exposed to oscillatory fluid flow (OFF) for 5 consecutive days. Bmp2fl/fl femurs were slightly longer compared to Prx1Cre;Bmp2fl/fl femurs under static conditions and OFF did not influence length. Femurs from control Bmp2fl/fl mice widened significantly when OFF was applied, but this behavior was lost in Prx1Cre;Bmp2fl/fl explants. Histological analysis revealed the periosteum expanded and bone formation was enhanced in the mid-diaphysis with OFF in control femurs. In contrast, the periosteal surface was comparatively inactive in Prx1Cre;Bmp2fl/fl femurs. To determine whether exogenous BMP2 can recover the thin bone phenotype, culture media was supplemented with 100 ng/mL BMP2. Femurs from control Bmp2fl/fl did not experience significantly more growth and width was partially recovered in Prx1Cre;Bmp2fl/fl femurs. RTqPCR analysis revealed that expression of Bmp2, markers of osteogenesis, BMP signaling targets, and selected novel RNAseq targets are increased in both control and knockout explants when BMP2 is supplemented.

These results raise interesting questions about the source of BMP2 in the periosteal niche. To test this, we isolated primary periosteal cells and resulting bone shafts from P7 Bmp2fl/fl and Prx1Cre;Bmp2fl/fl littermates. Bone shafts were devitalized by repeated freeze/thaw cycles and primary cells with or without BMP2 were seeded on shafts with or without BMP2. We quantified cell survival after 3 days in culture and found that a matrix-derived source of BMP2 resulted in better cell survival. We also seeded our periosteum-derived cells, which express little to no Bmp2, on these shafts and their survival was far superior on the shaft containing BMP2. Collectively, our bulk dataset and novel experimental tools have provided important insights into the role of BMP2 and BMP signaling in periosteal cell activity and function.

Presenter: Camryn Berry BS

Institution: BCH **Department:** Anesthesiology, Critical Care and Pain Medicine

Poster Number: 13

Title: Dissecting the Heterogeneity of Craniofacial Lesions in Patients with Fibrous Dysplasia/McCune-Albright Syndrome

Co-Authors: Nehal Shah MD, Laura A. Drubach MD, Hanne van der Heijden MS, Emma Golden BS, Anthony Westbrook PhD, Stephan Voss MD, Neha Kwatra MD, Laura Romo MD, David Ebb MD, Leonard B. Kaban MD, DMD, Mariesa Cay, Alison M. Boyce MD, Michael Mannstadt MD, Zachary S Peacock MD, DMD, Jaymin Upadhyay PhD

Abstract:
Fibrous dysplasia/McCune Albright Syndrome (FD/MAS) frequently manifests in the craniofacial skeleton. Craniofacial FD lesions exhibit diverse imaging characteristics on radiographs, computed tomography (CT),

Magnetic resonance imaging (MRI), and 18F-sodium fluoride positron emission tomography (18F-NaF PET). A multimodal imaging classification of craniofacial FD lesions may offer clinical insights on progression, intervention strategies, and symptomatology. This prospective, single-site study of 15 FD/MAS patients examined 35 craniofacial lesions utilizing a combination of 18F-NaF PET, MRI, and CT. Symptom presentation (headache frequency, pain severity, neuropathic pain quality, allodynia, photophobia, depression, and anxiety) was also monitored. A principal component analysis and k-means clustering algorithm was used to categorize lesions based on imaging characteristics and to determine if lesion properties were associated with symptom presentation. Clustering analysis revealed three types of lesions based on the magnitude of regional 18F-NaF standardized uptake values (SUV), signal intensities on T1-weighted MRI and fluid-sensitive sequences, and appearance on CT (lucent, sclerotic, and ground glass). Three categories of symptom presentation emerged, with frequent and neuropathic-like pain often coinciding with temporal bone or skull base lesions. This preliminary study provides a foundation for future longitudinal, natural history, or treatment studies, where the prognostic value of baseline craniofacial FD imaging characteristics and clinical symptomatology can be further evaluated.

Presenter: Jason Marvin

Institution: MGH **Department:** Center for Regenerative Medicine

Poster Number: 14

Title: The Role of the Innate Immune Response in Regeneration of the Adult Zebrafish Enthesis

Co-Authors: Sara Silarszka, Marie-Therese Nödl, Stephanie L. Tsai, Jenna L. Galloway

Abstract:
INTRODUCTION: The tendon-bone attachment site (‘enthesis’) transmits dynamic mechanical forces to enable locomotion of the skeleton. Entheses are susceptible to acute ruptures owing to their complex, multi-tissue structure and extracellular matrix (ECM) composition. Clinical interventions such as surgical repair suffer from re-tear rates as high as 94% in the rotator cuff and are unable to attenuate the formation of scar tissue. Recent studies have shown that an inflammatory cascade facilitates repair after rupture, but prolonged inflammation contributes to pathogenesis. Our study investigates the role of the innate immune response in adult enthesis regeneration using wild-type (WT) regenerative and mutant zebrafish deficient in tenascin-C (tnc-/-), a glycoprotein known to regulate wound healing and pro-inflammatory signaling in other organs, that exhibit impaired enthesis healing.

METHODS: The maxillary superficial tendons (MST) of adult transgenic reporter zebrafish (scxa:mCherry – tendon, mpeg1:EGFP – macrophages) in WT and tnc-/- backgrounds were collected for whole-mount multiphoton imaging in this study. Bioinformatics: To determine whether immune dysfunction underlies the tnc-/- phenotype, we performed Ingenuity Pathway Analysis (Qiagen) of existing bulk RNA-sequencing (RNA-seq) data of samples collected at 0 and 7 days post-injury (dpi) following an acute transection of the enthesis. Chemical Screens: To assess the influence of key inflammatory mediators (e.g., prostaglandin E2; PGE2) on enthesis regeneration, we treated zebrafish with 10 µM Diclofenac (non-steroidal anti-inflammatory drug), 10 µM NS398 (selective cyclooxygenase [COX]-2 inhibitor), and 5 µM AH6809 (antagonist against E-type prostanoid [EP] receptors 1-3).

RESULTS & DISCUSSION: tnc-/- samples showed transcriptional signatures associated with a dampened immune response and disrupted ECM and cellular homeostasis, which were corroborated by increased metalloproteinase (MMP-14) protein expression and limited macrophage infiltration at 3 dpi compared to WT (Fig. 1). AH6809- and NS398-treated (data not shown) samples displayed significantly increased gap sizes and reduced collagen/second harmonic generation (SHG) abundance indicative of impaired enthesis healing (Fig. 2A-E). Moreover, Diclofenac and AH6809 treatment blunted macrophage infiltration by 2-fold at 0.5 dpi compared to DMSO controls (Fig. 2F-I). Our data provide evidence that PGE2 signaling is required for adult enthesis regeneration. Ongoing work is uncovering the function of recruited innate immune cells using

genetic ablation lines, in addition to in vivo lineage tracing, cellular (proliferation and apoptosis), and molecular (in situ hybridization chain reaction; HCR) analyses.

Presenter: Sachin Chaugule

Institution: UMass Chan Medical School **Department:** Medicine

Poster Number: 15

Title: Essential Roles of Ubiquitin Specific Protease (USP8) in Postnatal Skeletal Development

Co-Authors: Yeon-Suk Yang, Tadatoshi Sato and Jae-Hyuck Shim

Abstract:
Postnatal bone formation involves the initiation of bone deposition by creating a structural framework through endochondral and intramembranous ossification, in which procedure osteoblasts synthesize new bone matrix, essential for developing and growing long bones. Here, we demonstrated that the deubiquitinating enzyme ubiquitin-specific protease 8 (USP8) is required for osteogenic differentiation and bone formation. Mice lacking USP8 in mature osteoblasts/osteocytes (Usp8DMP1) showed a significant trabecular bone loss in the metaphyseal area of the long bone while cortical bone thickness and porosity were markedly increased. Intriguingly, this cortical bone displayed an increase in osteoblasts numbers, osteoid secretion, and immature woven bone formation, but a decrease in numbers and activity of osteoclasts. This is consistent with the transcriptome analysis of Usp8DMP1 bone RNA showing an increase in osteoblast gene expression and a decrease in osteoclast gene expression. At cellular levels, osteogenic differentiation and extracellular mineralization were substantially increased in Usp8DMP1 bone marrow-derived stromal cells and USP8-deficient osteoblast line, suggesting that USP8 acts as a negative regulator of osteoblast development and function. USP8-deficient cells also showed upregulation of angiogenic, cytokine, and chemokine responsive genes. Overall, our findings suggest a potential role for USP8 in skeletal homeostasis, warranting further investigation into its specific contributions to postnatal bone development.

Presenter: Hannah Houston

Institution: Spaulding Rehabilitation Hospital **Department:** Ambrosio Lab

Poster Number: 16

Title: Can progesterone help to maintain muscle contractility in post-menopausal mice?

Co-Authors: Z Hettinger, G Gilmer, K Wang, N Shahshahan, J Bergmann, E Creed, T Mkandawire, S Sinkar, R Kopchak, F Ambrosio

Abstract:
Postmenopausal women disproportionately experience mobility limitations compared to men, and muscle weakness is correlated with this loss of mobility. Prior research suggests the decline in estrogen during menopause exacerbates muscle weakness, yet the results of estrogen replacement therapies designed to maintain muscle size and strength have been inconsistent. This inconsistency may be due, in part, to the inadequacy of rodent models used to develop therapies. A major contributor to the inadequacy of these models is that aged rodents do not maintain a menopausal phenotype. In this study, we used two different models, chemically-induced menopause in female mice and three-dimensional (3D) muscle organoids, to investigate the impact of menopause on muscle strength. For the mouse model, we injected an ovarian toxin, 4-vinylcyclohexene diepoxide (VCD) into middle-aged (14-16-month old) female mice for 10 consecutive days to simulate menopause. Sesame oil (SO)-injected mice served as controls. After 40 days of injections, tibialis anterior muscle force was measured prior to animal euthanasia in both groups. The VCD-injected mice demonstrated a decrease in muscle contractile force. However, there was no change in

cross-sectional area between VCD-injected and SO-injected miceTo understand the effects of individual sex hormones on muscle, we next measured muscle force production in 3D muscle constructs. Unlike in vivo, muscle constructs allow us to evaluate the effects of individual sex hormones without interference from other systems or cell types. Muscle constructs were cultured in hormone free media and randomized to one of the following groupsPre-menopausal conditions (high 17-β estradiol (E2) and progesterone (P4, post-menopausal conditions (high follicle stimulating hormone (FSH) and luteinizing hormone (LH)), individually with FSH, LH, E2, or P4, or hormone free. Single hormone culture revealed that constructs treated with P4 had an increase in force relative to hormone free. Notably, FSH, LH, or E2 exposure alone showed no effect on force production. To validate this finding in vivo, VCD-injected mice received sham nutella, E2, P4, or E2 + P4 in nutella. Tetanic force measurements were taken after 40 days of treatment. We found that E2+P4, E2 alone, and P4 alone restored muscle force in menopause. Together, these data suggest that the loss of progesterone associated with menopause may lead to muscle force deficits via mechanisms acting directly on the muscle but not involving a change in muscle size. Our data also support the hypothesis that E2 improves muscle contractile force in vivo but not by directly acting on the muscle. Taken together, these findings highlight a previously unappreciated role of progesterone loss, in addition to estrogen loss, in menopause in contributing to skeletal muscle weakness.

Presenter: Ramina Behzad

Institution: UMass Dartmouth **Department:** Bioengineering

Poster Number: 17

Title: Impact of Low Dose Vitamin C on Non-Enzymatic Glycation

Co-Authors: Arune Vickneswaran, Maggie McCafferty, Zackery Joseph Silva, Christian Ray, Lamya Karim

Abstract:
Diabetics have increased susceptibility to bone fractures, possibly linked to diminished bone quality [1]. Non-enzymatic crosslinks known as advanced glycation end-products (AGEs) contribute to the poor quality [2]. Vitamin C [VitC] can inhibit AGE formation in other tissues [3], and we aimed to assess its effects in human bone in vitro. We hypothesized that VitC treatment will reduce AGEs and improve mechanical properties. 60 cortical bone beams from human cadaveric tibiae (young: ~36 years, old: ~76 years) were imaged by microCT, and incubated in vehicle control, Ribose (0.6 M), or Ribose + VitC (0.283 mM). Fluorometric assay (total fAGE assay), cyclic reference point indentation (cRPI) and 3-point bending were conducted. There was no difference in fAGEs levels between young vs old groups. Old bone had lower ultimate stress (-15.07%, $p = 0.03$), ultimate strain (-5.5%, $p = 0.057$), failure stress (-37%, $p = 0.003$), and yield work (-17.64%, $p = 0.026$), and higher failure strain (+41%, $p = 0.056$), versus young bone. Old donors had higher indentation distance increase (IDI, +37.50%, $p = 0.023$), indentation distance (ID, +39.34%, $p = 0.009$), and total indentation distance (TID, +32.10%, $p = 0.010$) versus young. ANCOVA tests indicated there was no influence of age on fAGEs levels ($p = 0.313$), and there was higher fAGEs in Ribose (+228.38%, $p \leq 0.001$) and Ribose + VitC group (+226.58%, $p \leq 0.001$) versus controls. There was no difference in fAGEs in Ribose versus Ribose + VitC groups ($p = 0.998$). Mechanical properties were not different across groups. In conclusion, incubation of ribose successfully induced glycation but VitC did not impact fAGEs or consequent biomechanical properties.

Presenter: Marcela Granados

Institution: Beth Israel Deaconess Medical Center **Department:** Orthopedic Surgery

Poster Number: 18

Title: Femoral Cortical Bone Material Properties in Older Adults with Type 2 Diabetes

Co-Authors: Shannon R. Emerzian, Fjola Johannesdottir, Elaine W. Yu, Mary L. Bouxsein

Abstract:
Patients with type 2 diabetes (T2D) have an increased risk of hip fracture compared to non-diabetics despite normal to high bone mineral density (BMD), suggesting that other factors must account for increased skeletal fragility in this population. One potential underlying mechanism for increased skeletal fragility in T2D beyond BMD is diabetic-induced alterations to bone material properties. Thus, the objective of this study was to investigate the influence of T2D on cortical bone microstructure and material properties.

Whole femora were acquired post-mortem from a commercial tissue bank from individuals with T2D (n = 21) as well as age- and sex-matched controls (n = 21). Cortical beams were extracted from the midshaft of the femur, polished to exact dimensions (2 x 2 x 40 mm), and assessed via imaging and mechanical testing. First, the mid-section of each cortical beam was imaged via micro-computed tomography (µCT, Scanco µCT40, 10 µm isotropic voxel) to quantify beam geometry, tissue mineral density (Ct.TMD), and cortical porosity (Ct.Po). Next, ten cyclic reference point indentation (cRPI) tests were performed on the periosteal surface of the cortical beam approximately 1 mm apart for 20 cycles at 2 Hz with a peak force of 10 N (BioDent, Active Life Scientific, BP2 probe). The cRPI measures during the ten separate tests were averaged for each specimen. Finally, the mechanical properties of the beams were assessed through 4-point bending using a servo-hydraulic testing system (Instron 8511). The upper and lower spans were set to 9 and 27 mm, respectively, to achieve a 1/3 support span ratio per ASTM standards. Tests were performed with the periosteal surface resting on the bottom supports while the actuator moved at a fixed displacement of 3 mm/min. Force, displacement, and geometry data were used to calculate the apparent material properties of the cortical bone, such as bending modulus (GPa), yield stress (MPa), ultimate stress (MPa), toughness to fracture (mJ/mm3), post-yield toughness to fracture (mJ/mm3), work to fracture (mJ), and post-yield work to fracture (mJ). Non-parametric Wilcoxon rank sum tests were used to determine differences between groups.

The T2D specimens included 11 women (52%) and 10 men (48%), with median age at death of 77 years (range: 63-88). Age, sex, and height distribution were not significantly different between groups ($p > 0.33$). However, weight and BMI were significantly greater in T2D compared to non-diabetic controls ($p = 0.023$ and 0.043, respectively).

Data collection for µCT, cRPI, and 4-point bending is ongoing. The finalized findings will be presented at the conference.

Presenter: David E. Maridas

Institution: Harvard School of Dental Medicine **Department:** Developmental Biology

Poster Number: 19

Title: Deletion of Smad1/5/8 in osteoprogenitors and osteoblasts affects postnatal bone acquisition and fracture repair

Co-Authors: David E. Maridas, Jiahui Huang, Vicki Rosen

Abstract:
After birth, the skeleton undergoes a rapid period of bone mass acquisition driven by enhanced osteoblast activity. While sex, genetics, hormones, and environmental factors are known to affect the rate of osteoblast activity, the central mechanism governing postnatal bone gain requires continued investigation. Canonical BMP signaling, via the phosphorylation of Smad1/5/8 proteins, is a pathway crucial for the differentiation of osteoprogenitors into bone-forming osteoblasts. Multiple studies have demonstrated that disruptions of the receptors or ligands involved in the regulation of BMP signaling affect bone mass and fracture healing. However, the skeletal manifestations of direct deletion of Smad1/5/8 in osteoblast-lineage cells have not been examined. Here, we hypothesize that BMP signaling is required by osteoblasts during postnatal bone acquisition and fracture repair. To address our hypothesis, we deleted Smad1/5/8 in 4-week-old mice using

the following inducible strains: Osx-CreERt to target osteoprogenitors (OsxER KO mice) and Col1a1-CreERt to target osteoblasts (Col1ER KO mice). By 9 weeks of age, OsxER KO mice were disheveled, failed to gain weight, and had to be euthanized. DXA analyses showed that BMD increased significantly in both control and Col1ER KO mice but did not change in OsxER KO mice during the study. MicroCT and histological imaging revealed that femurs from both OsxER KO and Col1ER KO had increased trabecular bone volume and porous cortices. Since BMP signaling is important for fracture repair, we induced deletion of Smad1/5/8 in the osteoprogenitors of 12-week-old mice before performing femur fracture. At 10 days post fracture (DPF), cartilage callus was formed in both control and OsxER KO mice. By 21 DPF, most of the cartilage callus had been replaced by bone in control mice but not in OsxER KO mice, where a large cartilage area remained. In summary, our results indicate that canonical BMP signaling is crucial in osteoprogenitors and in osteoblasts to achieve adequate postnatal skeletal acquisition and efficient fracture repair.

Presenter: Tianbai Wang

Institution: Boston University **Department:** Materials Science and Engineering

Poster Number: 20

Title: A Bio-inspired Latent TGF-β Conjugated Scaffold to Improve Cartilage Regeneration

Co-Authors: Tianbai Wang, Celina C. Maldonado, Zhonghao Dai, Prem Nelesh, Andrew Martin, Joanne E. Murphy-Ullrich, Matthew D. Layne, Mark W. Grinstaff, Michael B. Albro

Abstract:
Transforming growth factor beta (TGF-β) is widely used for cartilage tissue engineering (TE). Conventionally, tissue constructs are exposed to supraphysiologic doses of TGF-β (10-100 ng/mL) supplemented in culture medium. While accelerating extracellular matrix (ECM) biosynthesis, these strategies are associated with features detrimental to hyaline cartilage function, including cellular hyperplasia [1] and ECM heterogeneities—resulting from TGF-β penetration limitations [1]. In contrast, TGF-β delivery in native cartilage occurs differently, whereby chondrocytes are surrounded by TGF-β in an inactive complex, termed latent TGF-β (LTGF-β). LTGF-β is highly concentrated in cartilage (~300 ng/mL [2]) but is activated at low rates (0.1-0.7 ng/mL [3]). This regulatory mechanism can maintain ECM biosynthesis but avoid pathological tissue formation from TGF-β excesses. Here, we recapitulate this native regulatory mechanism using a bio-inspired TE scaffold, whereby LTGF-β is conjugated to methacrylate agarose (MeAgr), allowing for a sustained and uniform delivery of physiologic doses of TGF-β, subsequently mitigating detrimental features associated with TGF-β excesses and improving tissue homogeneities. Methods: LTGF-β1 was conjugated to methacrylate MeAgr [4] by incubating at 37C overnight at 0.6, 1.5, and 3 μg/mL (Conj. Low, Medium, High). The scaffold was seeded with bovine chondrocytes (30x10^6/mL), fabricated at Æ5Å~2 mm, and cultured in chondrogenic media for 56 days. LTGF-β constructs were compared to unconjugated constructs exposed to media supplemented (MS) active TGF-β3 at a physiologic dose (0.3 ng/mL), supraphysiologic dose (10 ng/mL), or no TGF-β (TGF-β free). Results and Discussions: LTGF-β conjugation levels increased with incubation dose, reaching native levels (290 Å} 19 ng/mL) for Conj. High, further, ~50% of initial conjugated LTGF-β was retained after 21 days for all doses (not shown), providing a stable LTGF-β reservoir. At day 56, all LTGF-β groups exhibited significant enhancement in Young's modulus (EY) and sGAG content relative to TGF-β free, approaching native cartilage levels (Fig. 1A-B), indicating sufficient TGF-β delivery for tissue growth. Safranin O staining and spatial mechanical testing reflected pronounced spatial heterogeneities in sGAG distribution and tissue mechanical properties of MS constructs, which was greatly mitigated by LTGF-β conjugation (Fig. 2A-C), resulting from uniform delivery of TGF-β. 10 ng/mL active TGF-β caused significant cell cluster formation and upregulated expression of type X collagen (Col10), both are chondrocyte hypertrophic biomarkers. In contrast, LTGF-β conjugation induced isolated cell morphology and mitigated Col10 expression (Fig. 3A-C), suggesting improved cell morphology and cell phenotype by moderate levels of TGF-β through LTGF-β conjugation. Together, the bio-inspired LTGF-β conjugated scaffold regenerates cartilage with native-matched properties, while mitigating undesirable tissue features, which may ultimately serve to improve clinical regeneration outcomes. References: [1] Albro+ 2016

Biomaterials. [2] Morales+ 1991 Arch. Biochem. Biophys. [3] Kim+ 2020 SB3C (No. 409). [4] Paepe+ 2002 Polym. Int.

Presenter: Carolyn Chlebek

Institution: MaineHealth Institute for Research **Department:** Center for Molecular Medicine

Poster Number: 21

Title: In Obese C57Bl6 Mice, Weight Loss due to Caloric Restriction Reduced Bone Morphology but Improved Components of Systemic Metabolism

Co-Authors: Casey McAndrews, Samantha Costa, Clifford J. Rosen

Abstract:
Background: Obesity and calorie restriction individually alter systemic cellular metabolism and affect musculoskeletal tissue health. Obesity reduces trabecular and cortical bone quality. Clinically, obese patients are advised to lose weight, despite the negative effects of calorie restriction on bone in nonobese preclinical models. Little is known about bone quality following weight loss in obese preclinical models. We hypothesized that in obese models, caloric restriction would further worsen bone quality.

Methods: To induce obesity, male and female C57Bl6 mice (8 weeks old) received 60% high fat diet for 12 weeks (high fat feeding phase). Following obesity induction, the HFD-CR groups were acclimated to control low fat diet for 2 wks and then underwent 30% caloric restriction for 8 wks (caloric restriction feeding phase). The HFD groups received 60% kcal high fat diet from ages 8 to 30 wks. LFD mice received control low fat 10% kcal diet for the study duration. Mice were euthanized at 30 wks of age. Body weight, body composition, and bone mineral density were recorded at baseline and at the end of each diet. Glucose and insulin tolerance were recorded at the end of the high fat and caloric restriction feeding phases. At euthanasia, cortical and trabecular bone morphology (μCT) were assessed in the tibia.

Results: In both males and females ,adverse metabolic parameters worsened with high fat feeding but improved by caloric restriction. Compared to sex-matched LFD animals, HFD and HFD-CR mice gained body weight, fat mass, and lean mass during the high fat feeding phase. After the caloric restriction feeding phase, body weight, fat mass, and lean mass were reduced in HFD-CR compared to both HFD and LFD controls. After 12 weeks of high fat feeding, glucose and insulin tolerance were reduced in HFD and HFD-CR mice compared to LFD. Caloric restriction improved both glucose and insulin tolerance in HFD-CR mice compared to both HFD and LFD animals.

Calorie restriction following high fat diet had negative effects on bone in both sexes. In both sexes, HFD-CR mice had reduced cortical area and cortical thickness. In males, both HFD and HFD-CR mice had reduced tibial bone volume fraction compared to LFD. Male HFD-CR animals had smaller trabecular thickness compared to their HFD counterparts. Although female bone volume fraction was not different between diets, HFD-CR female mice had greater trabecular number and smaller trabecular thickness compared to HFD. In male mice, high fat diet reduced areal bone mineral density (aBMD), but HFD-CR and LFD aBMD were not different at the end of the study. In females, aBMD did not change between diet groups.

Conclusions: Despite improvements in components of systemic metabolism, caloric restriction in obese preclinical models reduced bone morphology in both trabecular and cortical compartments. More negative musculoskeletal outcomes were recorded in males compared with females. This work will improve our understanding of the musculoskeletal system following caloric restriction in obese models and will aid in nutritional counseling for obese patients.

Presenter: C R Coveney

Institution: Harvard **Department:** HEB

Poster Number: 22

Title: Joint morphology, osteoarthritis, and synergistic activity of GDF5 regulatory regions

Co-Authors: D E Maridas, P Muthuirulan, Z Liu, T Kahan, B L Proffen, A Kiapour , V Rosen, T D Capellini

Abstract:
Single nucleotide polymorphisms (SNPs) spanning a 130 kb interval containing GDF5 are associated with up to 1.8-fold increases in knee osteoarthritis (OA) risk and 1.6-fold increases in developmental dysplasia of hip (DDH) risk, among other cartilage disease phenotypes. Previous research on the cis-regulatory architecture of the GDF5 locus identified distinct GDF5 regulatory enhancers (R1–R5; GROW1) that control knee and hip cartilage gene expression, and harbor putative risk variants, including regulatory variants downstream of GDF5 (rs4911178 in GROW1; rs6060369 in R4). In vivo, each risk variant leads to morphological changes of the knee and hip joints, respectively, predisposing animals to cartilage degradation and spontaneous osteoarthritis development by one year of age (published). To explore the locus further, we examined other regulatory sequences for their effects on joint biology, including one termed R2 immediately upstream of the GDF5 5'UTR. We first found that deletion of R2 in vivo caused severe down-regulations of Gdf5 expression and significant phenotypic impacts, notably cartilage-driven morphological changes to the knee and hip by eight-weeks post-natal life. These anatomical domains partially overlap those effected by variants in R4 and GROW1. However, in contrast to R4 and GROW1 mutants, morphological changes as a result of the R2 deletion did not lead to increased disease risk (Fig 1a and 1b). Interestingly, in the 5'UTR of GDF5 there is also a commonly cited cis-regulatory variant, rs143384. To understand the impacts that that the "T" risk allele at this variant position has on expression and phenotype, we next engineered humanized single base-pair "T" replacement mice. Using allele-specific expression assays in mice we found that the risk "T" variant has minimal (i.e., statistically insignificant, p=0.12) effects on Gdf5 gene expression in each of the major forelimb and hind limb joints analyzed, yet results in only minor changes to joint morphology localized to the femoral plateau. This slight dysmorphology was not associated with any changes to cartilage thickness, or integrity (Fig 1c and 1d). In theory, this finding points to the importance of previously identified downstream regulatory variants in GROW1 and R4 as primarily causal for hip dysplasia and knee OA. However, as this 5'UTR variant resides next to R2 it could influence R2 activity in joints, and this is currently being assessed through phenotypic studies. Importantly, as the 5'UTR "T" allele at rs143384 resides on the same risk haplotype as R4 rs6060369 "T" and GROW1 rs4911178 "A" alleles it may also impact their activity through epistatic interactions. To assess this possibility, we finally tested in reporter assays in chondrocyte cells different combinations of risk and non-risk variants across the 5'UTR, R4, and GROW1 regulatory regions finding that synergistic interactions occur across the locus. These findings point towards complex genetic underpinnings of risk for different disease phenotypes at GDF5. Overall, our work provides an important developmental context to explain the mechanisms through which human GDF5 genetic variants can lead to joint disease.

Presenter: Gayani Senevirathne

Institution: Harvard University. **Department:** Department of Human Evolutionary Biology

Poster Number: 23

Title: Uncovering the Development of the Pelvic girdle at a single cell level

Co-Authors: Serena Fernandopulle, Stephanie Baumgart, Terence Capellini

Abstract:
Phenotypic changes from an existing ancestral condition highlight the evolution of "key innovations". Of the anatomical domains undergoing considerable evolutionary change, the human pelvic girdle is considered a novelty as it is perhaps the most drastically reorganized

structure of the post-cranium compared to those in chimpanzees and other primates. The human pelvis is composed of several subelements, the ilium, pubis, ischium, and acetabulum each quite different in morphology yet critical for human locomotion and childbirth. Our lab previously performed RNA and ATAC-sequencing on each of these subelements from rare human gestational samples; work that led to the identification of sets of genes and on/off switches that help form each subelement. However, to shed light on the morphogenetic events that have modified the chondrocyte populations underlying human pelvis development and thus contributed to the formation of novel human biology, here we report on our investigation into human pelvic development at single-cell(sc) resolution. Through our use of an integrative sc-multiomics and spatial transcriptomics approach on human pelvic samples, we have gained marked insight into the chondrocyte populations and the soft-tissue environment encapsulating developing human pelvic tissues. We focused on four key developmental windows: E45-51, E52-57, E58-64 and E65-72; embryonic stages when each subelement forms from cartilaginous precursors (E45-51) and subsequently undergoes rapid and extensive growth (E52-E72). Our integrated approach helps to identify a novel chondrocyte growth plate orientation, and a novel ossification pattern, along with specific gene and regulatory element sequences underlying their development. Overall, our work sheds light on the developmental genetic basis of the highly derived nature of human skeletal biology.

Presenter: Nereida Ramirez

Institution: Boston Children's Hospital **Department:** Orthopedics

Poster Number: 24

Title: Therapeutic translation of human pluripotent stem cells-derived cartilage

Co-Authors: Sarah Jachim, Chilan Bou Ghosson Leite, Christian Lattermann, Benedikt Proffen, April Craft

Abstract:
Osteoarthritis is a debilitating and painful musculoskeletal condition that affects over 1 in 5 US adults. Current standards of care involve pain management, or regenerative procedures that fail to restore normal osteochondral architecture. We specialize in generating cartilage tissue from human pluripotent stem cells, and we are evaluating the therapeutic capacity of these tissues by engrafting them into the knees of Yucatan mini-pigs with surgically induced chondral defects. We hypothesize that within 3 months our in-vitro-derived cartilage constructs will integrate with native cartilage and result in lower histology-based OARSI scores than microfracture-treat defects. In our initial 2-week pilot study, we found that the implanted tissues were retained in the defect without any adhesives, maintained important structural features, and continued to produce key extracellular matrix constituents. However, we also observed an inflammatory host response, potentially due to disruption of the subchondral bone during the creation of the chondral defect. From the results of our pilot study, we conclude that pluripotent stem cell-derived tissues engraft within the defect site and maintain their cartilage identity at a tissue and cellular level, making them a promising resource for cartilage repair. Furthermore, we will refine our approach to use a specialized orthopedic drill to increase the precision and depth accuracy with which the chondral defect is made, and we are optimizing the culture conditions of the cartilage tissue implants to increase their thickness.

Presenter: Jingting Yao

Institution: Massachusetts General Hospital **Department:** Radiology

Poster Number: 25

Title: Alveolar Bone Density Assessment in Dental Implantology: Advancing from CT to MRI

Co-Authors: Isabela G.G. Choi, Shidong Xu, Otavio Henrique Pinhata-Baptista, and Jerome L. Ackerman

Abstract:
Background: Accurate evaluation of alveolar bone density is crucial for the successful placement of dental implants, affecting osseointegration and implant stability. Traditional computed tomography (CT)-based methods expose patients to ionizing radiation, raising concerns about long-term safety. Magnetic resonance imaging (MRI), commonly used for soft tissue imaging, is emerging as a promising radiation-free alternative by quantifying bone marrow fat content and, by extension, bone volume fraction. We are exploring the accuracy and clinical applicability of MRI at 15 Tesla (T), 14T, and clinically relevant 1.5T field strengths, for quantifying alveolar bone density. MRI results were compared with cone-beam CT (CBCT)—frequently used in in vivo dental practice—and micro-CT, the gold standard for ex vivo bone mineral density analysis.

Methods: We conducted a series of studies on ex vivo alveolar bone biopsies (15 males, 6 females, mean age 52.9 years), utilizing 15T and 14T MRI to quantify bone marrow fat (BMF) content and correlate it with bone volume fraction (BV/TV), as benchmarked against micro-CT. Subsequently, we conducted another study extending this evaluation to 1.5T MRI, aiming to evaluate its clinical relevance and applicability in dental implantology by comparing its BV/TV estimations with those from micro-CT and 14T MRI.

Results: Initial findings from 15T MRI exhibited a significant inverse correlation between BMF content and BV/TV ($r = -0.68$, $P = 0.045$). Extending these insights, 14T MRI's correlation with micro-CT presented an exceptional congruence ($r = 0.943$, $P < 0.001$), with 1.5T MRI maintaining this trend, demonstrating significant correlations (1.5T vs. Micro-CT: $r = 0.70$, $P < 0.0001$; 1.5T vs. 14T: $r = 0.76$, $P < 0.0001$), thereby affirming the consistency and reliability of MRI across the examined field strengths.

Conclusion: This series of studies validate MRI's capability to offer a safer, non-invasive alternative to traditional CT methods for alveolar bone density assessment. The introduction of 1.5T MRI for clinical application marks a significant leap towards enhancing patient care in dental implantology by eliminating ionizing radiation exposure without compromising diagnostic accuracy, critical for sensitive populations such as children and pregnant women.

Presenter: Ahmed Al Saedi

Institution: HMS **Department:** Endocrinology

Poster Number: 26

Title: CXXC Finger Protein 1 is Required for Osteoblast Differentiation

Co-Authors: Ahmed Al Saedi1,2, Joshua Meyer1, Katelyn Healey1, Molly Persky1, David Maridas3, Lijie Jiang1, Vicki Rosen3,4 and Diana L. Carlone1,2,4

Abstract:
Skeletal development, remodeling, and regeneration are intricately tied to the activity of mesenchymal progenitor cells (MPCs). Recent evidence highlights the role of epigenetics in regulating MPC function, impacting bone formation in both mice and humans. Despite this, the precise mechanisms through which epigenetics, particularly the action of CXXC Finger Protein 1 (CFP1), govern MPC activity and their involvement in bone formation remain unclear. CFP1, identified as an epigenetic regulatory factor facilitating H3-Lys4 trimethylation for transcriptional activation, has shown regulatory roles in progenitor cell differentiation in various systems. However, its specific function in bone formation has yet to be defined. To address this gap, we used Prx1-Cre to delete Cxxc1, the gene encoding CFP1, in MPCs (cKOPrx1). This deletion led to a disruption in chondrocyte differentiation, maturation, and primary ossification, indicating CFP1's critical role in endochondral ossification. Moreover, mutant mice exhibited reduced intramembranous ossification within the calvaria, suggesting CFP1's direct influence on osteoblast differentiation. Further investigations using adenoviral deletion of Cxxc1 in bone marrow stromal cells (BMSCs) and quantitative RT-PCR analysis demonstrated a significant decrease in the expression of osteoblast markers (Runx2, Osx, Col1a1, Ocn), underscoring CFP1's involvement in bone formation. To delve deeper into its role in osteoblast differentiation, CFP1 knockout MC3T3-E1 and W20 cells were generated using CRISPR/Cas9.

Consistent with our earlier findings, mutant cells fail to properly differentiate. Preliminary studies link CFP1 to several signaling pathways including BMP implicating a possible mechanism by which this epigenetic factor controls osteoblast differentiation. In summary, our recent findings establish a connection between CFP1 and both endochondral and intramembranous bone formation. This research sheds light on the crucial regulatory role of CFP1 in MPCs, emphasizing its impact on skeletal development and osteoblast differentiation.

Presenter: Kedkanya Mesil

Institution: Harvard School of Dental Medicine **Department:** Department of Oral Medicine, Infection, and Immunity, Division of Bone and Mineral Research

Poster Number: 27

Title: Bambi, a novel PTH target gene, regulates WNT and TGF-β signaling to affect osteocyte biology

Co-Authors: Sara Monaci, Hiroyuki Okada, Francesca Gori, and Roland Baron

Abstract:
Parathyroid hormone (PTH), plays a crucial role in calcium and phosphorus metabolism, acting on bone and the kidney. Although PTH can induce both bone catabolic and anabolic effects, depending on the duration and periodicity of exposure, daily injections increase bone density in human and animal studies. PTH acts directly on osteoblasts and osteocytes, indirectly influencing osteoclasts. Osteocytes play an essential role in bone homeostasis by secreting various regulators (RANKL, OPG, Sclerostin) that control the activity of both osteoblasts and osteoclasts during the bone modeling and remodeling processes. Additionally, osteocytes release an endocrine factor, FGF23, which targets the kidney and intestine to regulate phosphate metabolism. Our bulk-RNA seq database from osteocyte-enriched cell populations in response to in vivo iPTH treatment in mice showed an increase in the expression of several genes, with Bone Morphogenetic Protein and Activin Membrane-Bound Inhibitor (Bambi) being one of the most significantly upregulated genes by both PTH and Abaloparatide (Liu et al., JCI Insight, 2023). Furthermore, analysis of pooled scRNA-seq public databases of bone cells (Okada et al., Curr Osteoporos Rep, 2023) revealed that Bambi is expressed in a relatively restricted manner in osteocytes. Bambi is a transmembrane protein pseudo-receptor that exhibits structural homology to the transforming growth factor β type I receptor (TGF-βRI) but lacks an intracellular kinase domain. It has been identified as a common transcriptional target and mediator between the Wnt and TGF-β signaling pathways, ultimately enhancing cellular growth by promoting Wnt signaling and inhibiting TGF-β signaling. Although an increase in Bambi expression has been associated with various human pathologies, there is a limited body of literature on Bambi's role in bone-related events, and its impact on osteocyte biology has not been explored. Here we show that, during the OmGFP66 osteocyte cell line differentiation, Bambi is expressed at basal level and continued to increase during differentiation. Importantly, Bambi expression was elevated with PTH treatment, both in a time-dependent manner – most prominently at the 1-hour time point with PTH 50 nM – and during long intermittent treatment with PTH 50 nM for 1 hour per day over 5 days. Following Bambi knockdown (Bambi-KD), we observed a decrease in non-phosphorylated β-catenin and an increase in pSmad2, signaling molecules of the Wnt and TGF-β pathways, respectively. Importantly, Bambi-KD resulted in the upregulation of Sost under steady-state condition, with a significant increase observed in response to PTH treatment, mimicking the inhibition of HDAC4/5 phosphorylation, which allows Mef2c-driven Sost expression. Most interestingly, while reversing the effect of PTH to repress Sost, Bambi-KD had no effect on Rankl (Tnsfs11) expression. Furthermore, Bambi-KD impaired osteocyte mineral deposition, as assessed by Alizarin Red staining, disrupted Fgf23 levels, evidenced by a decline in RNA and protein levels, and impacted osteocyte morphology, with a

reduction in phalloidin-stained osteocytic dendrites and a decrease in Sp7 and Osteocrin expression. In summary, our results unveil the importance of Bambi in osteocyte biology and in their response to PTH, an agent used routinely to increase bone density in osteoporotic patients.

Presenter: Xiaomeng You

Institution: BWH **Department:** Department of Orthopedic Surgery

Poster Number: 28

Title: Cystic fibrosis associated gut microbiome dysbiosis reduces bone growth in early postnatal mice.

Co-Authors: Jisun Paik, Stacey M. Meeker, Kevin S Mears, Sruthi Sridevan, Lucas R. Hoffman, Julia Charles

Abstract:
Cystic fibrosis (CF) is a fatal autosomal recessive disease caused by a mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Linear growth impairment is a common characteristic feature in CF children and is closely associated with long-term health outcomes. Clinical studies and mouse models both demonstrate differential microbiome profiles of CF individuals compared to healthy control cohorts. As the gut microbiome is known to regulate bone development and homeostasis, CF associated gut microbiome dysbiosis may negatively impact bone growth.

This study compared bone phenotypes and linear growth trajectory of C57BL/6 Cftrtm1Unc mutant mice (CF) to their wild-type littermates (WT) under specific pathogen free (SPF) and germ-free (GF) conditions. 4-week-old CF mice showed reduced femur length and diminished serum IGF-1 compared to WT mice under SPF condition, but not in GF condition. However, 4-week dietary intervention of short chain fatty acids failed to show a beneficial effect on bone growth in 4-week-old CF mice. At 14-week-old, CF mice showed reduced femur length under both SPF and GF conditions. Difference-in-differences analysis of body length growth curves showed a significant growth gap between WT and CF mice in SPF versus GF conditions from 1 to 4 weeks but diminishing thereafter. Consistently, 4-week-old CF mice showed more pronounced microbial taxonomic and functional dysbiosis than 14-week-old CF mice. In conclusion, CF-associated gut microbial dysbiosis plays a critical role in bone growth in early postnatal CF mice, while additional factors contribute to impaired growth later in life. Thus, correcting dysbiosis alone may not be sufficient to improve growth retardation in CF children.

Presenter: Audrie L. Langlais

Institution: MaineHealth Institute for Research **Department:** Center for Molecular Medicine

Poster Number: 29

Title: Morphine Decreases Extracellular Vesicles and miRNA Expression: Implications for Opioid-Induced Bone Loss

Co-Authors: Claire Morrow, Peter Caradonna, Kathleen Becker, Katherine J. Motyl

Abstract:
Opioids compromise bone health by reducing bone mineral density and increasing fracture risk. Previously, we identified morphine-induced bone loss in male, but not female, C57BL/6J mice, which was due to reduced bone formation. Bone loss was also associated with reduced circulating micro RNAs (miRNA) expression in serum and bone. However, the source of altered miRNA expression and the effects on bone formation have not been investigated. Based on opioids effects on the nervous system, and low opioid receptor expression in bone, we hypothesized that opioids alter the neural-skeletal axis to cause bone loss. To test this hypothesis, we treated 8-week-old C57BL/6J male mice with morphine (20 mg/kg, s.c., N = 5) or

vehicle (0.9% saline, s.c., N = 5) for 1 hour and isolated serum extracellular vesicles that are released by cells to traffic cargo including miRNA/RNA. Within serum, the concentration of extracellular vesicles tended to be reduced by morphine (p = 0.08), suggesting decreased vesicle secretion contributes to changes in miRNA expression. A second cohort of C57BL/6J mice received an intratibial injection of Fast Blue, a retrograde neuronal tracer, and one week post injection dorsal root ganglion (DRG) which house the cell bodies of sensory neurons were isolated. Using in situ hybridization (RNAScope) we detected co-staining of Fast Blue+ and m-opioid receptor+ (Oprm1) sensory neurons, suggesting opioids may impact sensory nerves directly innervating bone, in addition to systemic effects as seen in serum. Lastly, we have generated an exosome reporter mouse, CD3emGFPl/s/l x Baf53bCre/+, and confirmed expression of CD63emGFP+ vesicles within bone that overlap with the neuronal marker βIII-Tubulin, suggesting their release from nerve terminals to recipient cells. Future studies will be expanded in reporter mice to test how morphine impacts neural-exosomes and miRNA contents in bone during bone loss, and more specifically osteoblast mineralization. This work will critically expand our understanding of how opioids impair bone and may influence future clinical treatment and prevention strategies.

Presenter: Roy B Choi

Institution: MGH **Department:** Endocrine unit

Poster Number: 30

Title: Early postnatal deletion of SIK2/SIK3 in osteoblast lineage promotes anabolic action in the cancellous compartment of mice.

Co-Authors: Nicha Tokavanich, Henry M Kronenberg, Yingzi Yang, Marc N Wein

Abstract:
PTH agonists are one of the FDA-approved anabolic agents to treat osteoporosis. However, these medications require daily injections, a major barrier for patients with a chronic and asymptomatic disease. Therefore, we have studied signaling events downstream of the PTH receptor in order to identify alternative strategies to enhance PTH receptor actions in bone. PTH signaling blocks the cellular actions of salt inducible kinases (SIKs); thus, direct small molecule SIK inhibitors represent a new and promising strategy to enhance bone mass. Despite these advances, major questions remains regarding the cell type(s) responsible for the action of SIKs in PTH signaling in bone. Deletion of SIK2 and SIK3 globally and with Dmp1-Cre shows dramatically increased bone mass and formation, mimicking the skeletal phenotype seen with constitutive PTH1R signaling. However, the role of SIKs early in the osteoblast lineage during skeletal development remains unknown. To test whether SIK2/SIK3 deletion in the osteoblast stage promotes bone anabolic action, we generated control (Osx1-CreERt2; Sik2/Sik3+/+; R26tdTMT) and SIK2/SIK3OSXiDKO (Osx1-CreERt2; Sik2/Sik3f/f; R26tdTMT) mice using tamoxifen inducible Osterix1-CreERt2 mice which contains red florescent marker tdTMT protein. To test whether which time point affect induce more anabolic action, we induce SIK2/SIK3 deletion at P3(postnatal) and P21(pubertal) in both male and female mice. After injection, mice were sacrificed at P28 (P3 gene ablation) and P42 (P21 gene ablation). Interestingly, μCT revealed that SIK2/SIK3 deletion at P3 led to a dramatic expansion of trabecular bone, a phenotype much more severe than what was noted with P21 tamoxifen treatment. Consistent with μCT results, there were dramatically increased tdTMT protein which were observed by red florescent mainly in primary and secondary spongiosa, trabecular region in both P3 injected male and female mice. However, compared to P3, P21 showed slightly increased tdTMT+ cells, suggesting that early postnatal SIK2/SIK3 deletion has a more dramatic effect on the cancellous compartment than deletion of these kinases at P21. Lastly, histology analysis revealed expansion of marrow stromal cells with concomitant reduction in hematopoietic cells in the P3 Osx1-CreERt2 SIK2/SIK3 ablation model. In conclusion, early postnatal deletion of SIK2/SIK3 induces more dramatic anabolic action in the cancellous compartment compared to deletion at P21. These results provide important insights into the cell types responding to SIK2/SIK3 gene deletion during bone development .

Presenter: Courtney Mazur

Institution: MGH **Department:** Endocrine Unit

Poster Number: 31

Title: Identification of RNA localization elements controlling mRNA transcript enrichment in osteocyte dendrites

Co-Authors: Parthena E. Kotsalidis, Christian D. Castro Andrade, J. Matthew Taliaferro, Marc N. Wein

Abstract:
Subcellular compartments like neuronal dendrites and fibroblast protrusions contain local transcriptomes that contribute to morphology and function. Trafficking of mRNA within cells is controlled by RNA binding proteins that recognize localization sequences in mRNA, often found in the 3' untranslated region (3'UTR). Our subcellular transcriptomic analysis identified 420 mRNA transcripts enriched in dendrites of osteocyte-like Ocy454 cells compared to cell bodies ($log2FC^{3}1$, padj<0.01). These transcripts largely encode for ribosomal proteins, electron transport chain, and cytoskeletal proteins. To learn how osteocytes traffic these specific mRNAs to dendrites, we tested the hypothesis that osteocyte dendrite-enriched transcripts contain localization sequences in their 3'UTRs.

We used a heterologous reporter system in which the 3'UTR of one dendrite-enriched mRNA was cloned downstream of the Firefly luciferase (FLuc) coding sequence. Untagged Renilla luciferase (RLuc) served as a control. Ocy454 cells stably expressing one 3'UTR reporter were grown on transwell membranes with 1 mm pores to facilitate fractionation of dendrites from cell bodies. Relative expression of FLuc mRNA to RLuc mRNA was measured by RT-qPCR in dendrites and cell bodies. For 11 of 15 dendrite-enriched transcripts tested, the 3'UTR was sufficient to promote dendrite localization of FLuc, whereas the 3'UTR of cell body-enriched Pth1r mRNA did not cause dendrite localization.

To precisely identify localization sequences within active 3'UTRs, we then conducted a massively parallel reporter assay (MPRA). Eleven 3'UTRs were fragmented into overlapping 260-nucleotide-long sequences such that each nucleotide of 3'UTR was represented in 65 oligos. The pool of 7115 unique oligos was cloned downstream of a GFP reporter and the resulting plasmid library was stably expressed in Ocy454 cells. After fractionation of dendrites from cell bodies, we performed targeted mRNA sequencing to identify which 260-nucleotide sequences caused dendrite localization of the GFP reporter.

Within the 3'UTRs of two mRNA sequences previously investigated in neurons, we found strong peaks of 38 and 49 consecutive dendrite localized oligos, consistent with published data. Since oligos overlap across each 3'UTR, the minimal elements shared across all significantly localized oligos for each 3'UTR are 109 and 67 nucleotides, respectively. Within the 3'UTRs of new osteocyte dendrite-localized mRNAs, we identified peaks of 3-19 consecutive oligos, indicating minimal elements 185-252 nucleotides long. For two 3'UTRs with no significantly localized oligos in the MPRA, minimal localization elements may be longer than 260 nucleotides. We will use the top dendrite-localized 260-nucleotide sequences to identify RNA binding proteins responsible for mRNA transcript enrichment.

Together our data suggests that dendrite-localized mRNA in osteocytes is often trafficked via specific sequences within the 3'UTR. As we uncover the role of this carefully regulated process in osteocytes, this molecular regulatory mechanism may be a future therapeutic target for osteocyte and bone disorders.

Presenter: Divya Venkatasubramanian

Institution: Boston Children's Hospital **Department:** Orthopedic Research

Poster Number: 32

Title: Leveraging single cell analysis to study human cartilage development

Co-Authors: Gayani Senevirathne, Terence Capellini, April Craft

Abstract:
To understand the molecular regulation of articular and growth plate cartilage development in humans, we jointly profiled the transcriptome and open chromatin regions of the distal femur at two different timepoints of human development. These single cell multiomic data linked regulatory elements with gene expression in different chondrocyte and connective tissue subtypes and uncovered transcription factors working in a cell-type specific manner during chondrogenesis. We further built gene regulatory network (GRN) models, with putative targets of TFs, which revealed TFs previously unknown in cartilage biology and suggested new roles for known TFs in specifying distinct chondrocyte lineages. We evaluated one such regulator, NFATC2, using an in vitro human pluripotent stem cell model of chondrogenesis, to demonstrate its role in driving epiphyseal chondrocyte gene programs. The gene regulatory networks we uncovered provide new insights in how cellular fate is determined during human cartilage development and how these networks maintain cartilage health and contribute to cartilage disease.

Presenter: Soha Ben Tahar

Institution: Northeastern University **Department:** Mechanical Engineering

Poster Number: 33

Title: Predicting patterns in limb joint development

Co-Authors: Ester Comellas, Sandra Shefelbine

Abstract:
Embryonic development has intrigued researchers for over a century. Morphogens are the chemical signals facilitating cell-to-cell communication and influencing cell fate and tissue specialization. There are central to this process. The reaction-diffusion model has proven useful in modeling morphogens and pattern formation across diverse biological contexts. However, a gap in our knowledge persists: How do these patterns emerge in the complex context of skeletal limb growth? Addressing this gap underscores the importance of investigating patterning in systems that faithfully replicate relevant biological growth.

This project aims to explore the potential of the reaction-diffusion system as a model for pattern formation in developing axolotl limb skeletal structures. Our approach combines finite element analysis with experimental morphometric measurements. We captured the morphological changes in arm development of an axolotl from Stage 44 to Stage 48 through photographs taken at 24-hour intervals. Modeling includes eight growing steps, extending over eight days. This dynamic growth process is numerically simulated using a finite element model with a moving boundary condition. Our Matlab house-code solves the reaction-diffusion system coupled with the growing process. In this model, one species represents a group of molecules that instructs cells to differentiate into cartilage, while the other species represents a group of molecules that signal cells not to differentiate into cartilage. Therefore, the patterns we are targeting are the ones observed during limb bone development. A sensitivity analysis is conducted to understand the impact of various parameters in the reaction-diffusion system, the temporal and spatial scaling, as well as the addition of a low diffusion epithelium layer. This analysis reveals a limited range of patterns (dots, single stripes, double stripes, and intermediate patterns). Through this exploration, we seek to better understand the potential of the reaction-diffusion system as a model for pattern formation in the context of skeletal limb development.

Presenter: Carolyn Chlebek

Institution: MaineHealth Institute for Research **Department:** Center for Molecular Medicine

Poster Number: 34

Title: Canagliflozin treatment improves musculoskeletal health more in female than male mice

Co-Authors: Casey McAndrews, Samantha Costa, Erin M. MacFarlane, Shoshana Yakar, Clifford J. Rosen

Abstract:
INTRODUCTION: Canagliflozin lowers serum glucose by inhibiting sodium glucose transporter 2 (SGLT2), which is primarily expressed in the kidney. SGLT2 inhibitors are used to treat Type II Diabetes and cardiovascular disease, and have been associated with increased longevity. In preclinical models, short-term treatment with canagliflozin negatively affected trabecular bone whereas long-term treatment reduced cortical bone mineralization in male but not female mice. We aimed to determine the effect of an intermediate period of canagliflozin treatment on bone quality in nondiabetic mice.

METHODS: Under IACUC approval, male and female C57Bl/6J mice aged 3- and 6-months received either control or canagliflozin-containing diet (180 ppm) for six months (n = 8-10/group). Mice were euthanized following six months of treatment, at 9 months or 12 months of age. Body mass, composition, and bone mineral density (BMD) were assessed monthly. Prior to euthanasia, mice received calcein injections (-10 days, -3 days). At euthanasia, fasting blood glucose was recorded and femurs were collected for micro-computed tomography (µCT). One tibia from each animal was paraffin embedded, sectioned, and stained with hematoxylin and eosin. Bone marrow adiposity was assessed within the proximal tibia using ImageJ. Males and females were analyzed separately with a two-way ANOVA using factors of drug and age. One femur from each animal was embedded in plastic, sectioned, and imaged for dynamic histomorphometry. Mineral apposition rate and bone formation rate were calculated in Osteomeasure. Dynamic histomorphometry in the trabecular bone was assessed in all animals, and males and females were analyzed separately with a two-way ANOVA using factors of drug and age. Dynamic histomorphometry in the cortical bone was measured in 12 mo animals, and a t-test was used for each sex to determine differences between canagliflozin and control mice. Calcium and phosphate levels in the urine were normalized to creatinine, and evaluated at baseline and during the last week of the study. Urine levels were analyzed within each age- and sex cohort, using a two-way ANOVA with factors of drug and timepoint.

RESULTS: Canagliflozin (CANA) did not alter body weight, but significantly lowered blood glucose levels. In females, CANA increased the change in areal bone mineral density over the course of the study. Trabecular bone volume fraction and number were increased with CANA in both sexes. CANA increased trabecular thickness in females but not males. CANA reduced trabecular mineralizing surface in males but did not change bone formation rate in either sex. All CANA-treated animals had increased femoral cortical thickness and decreased marrow area compared with controls. In females, CANA increased cortical bone area and moment of inertia. Cortical bone formation rates were calculated in 12 mo animals; canagliflozin reduced endocortical mineral apposition rate and bone formation rate in both sexes. Periosteal bone mineral apposition rate and bone formation rate were not different with CANA treatment. SLGT2 inhibition reduced bone marrow adiposity in females, but not males. Bone marrow adipocytes in CANA-treated females were smaller compared to age-matched controls. Canagliflozin increased calcium urinary output in all cohorts but did not alter phosphate levels in the urine.

DISCUSSION: Despite the lack of SGLT2 expression in bone, in this study both cortical and trabecular bone morphology were improved with 6 months of canagliflozin treatment. In females, canagliflozin attenuated age-related bone marrow adiposity accumulation. More benefits to musculoskeletal health were measured in females than males. Although the CANVAS clinical trial reported increased risk of bone fracture with canagliflozin treatment, non-CANVAS studies did not record increased fractures with CANA. Variability in canagliflozin-associated fracture risk may be due to variation within patient populations or differences in fracture risk at the time of treatment initiation. CANVAS patients had Type II Diabetes, likely increasing their baseline fracture risk. Use of canagliflozin has expanded to non-diabetic populations to treat cardiovascular disease. This study demonstrated that in nondiabetic C57Bl/6J mice, canagliflozin treatment improves bone morphology in both sexes and reduces marrow adiposity in females. The increased use of canagliflozin in

nondiabetic patients warrants further investigation into the sex-specific effects of this treatment on musculoskeletal health.

Presenter: Shannon R. Emerzian

Institution: BIDMC **Department:** Orthopedic Surgery

Poster Number: 35

Title: Older Women with Longstanding Type 1 Diabetes Have Lower Femoral Strength and Region-Specific Deficits in Trabecular Bone Mineral Density of the Femoral Neck

Co-Authors: David C. Lee, Fjola Johannesdottir, I-Hsien Wu, John Gauthier, Surya Vishva Teja Jangolla, Marc Gregory Yu, Hetal S. Shah, George L. King, Tony M. Keaveny, Klaus Engelke, Elaine W. Yu, Mary L. Bouxsein

Abstract:
Type 1 diabetes (T1D) is associated with an increased risk of hip fracture, but the factors underlying skeletal fragility in older adults with T1D are not well understood. This study assessed regional differences in bone mineral density (BMD) and strength of cadaveric femora from postmenopausal women with longstanding T1D and non-diabetic controls.

Whole femora were acquired post-mortem from female Joslin Medalists with T1D ≥50yrs (n=11); age and sex-matched non-diabetic control femora were obtained from a tissue bank (n=10). Femora were scanned via axial computed tomography (CT, Siemens). CT scans were analyzed using Medical Image Analysis Framework (MIAF)-Femur, with cortical thickness (Ct.Th) and volumetric BMD (total=Tt; trabecular=Tb) assessed at the total hip (TH) and femoral neck (FN). FN volumes were further divided into quadrants: superior anterior (SA) and posterior (SP) as well as inferior anterior (IA) and posterior (IP). Femoral strength and DXA-equivalent TH and FN areal BMD (aBMD) and T-score were calculated using Biomechanical CT analysis (BCT, O.N. Diagnostics). Individuals were considered high fracture risk if they had either low aBMD (T-score≤-2.5) or fragile bone strength (≤3000N). The ratio of fall force to femoral strength was computed using a soft-tissue attenuated fall force and femoral strength from BCT. Wilcoxon rank sum tests assessed group differences; percent differences are between group medians.

The T1D group had an average (mean±SD) BMI=25.1±3.4kg/m2, HbA1c=8.3±0.9%, T1D duration=65±5yrs, age at onset=13±8yrs, and age at death=78±10yrs; age and BMI were not different between groups. 73% of T1D and 40% of controls were considered high risk (p=0.2). TH Tt.BMD and aBMD did not differ between groups. FN aBMD (p=0.07) and Tb.BMD (p=0.06) were lower in T1D (Table). Within the FN, women with T1D had Tb.BMD deficits that were largest in the SP (-35%, p=0.04) and SA (-35%, p=0.10) quadrants. Ct.Th did not differ between groups at any site. Women with T1D also had lower femoral strength (-26%, p=0.03), but similar fall force (p=0.25), resulting in a greater load-to-strength ratio (+28%, p=0.04).

These findings reveal deficits in femoral BMD and strength and greater load-to-strength ratio in older women with T1D, indicating increased susceptibility to hip fracture. As hip fractures may initiate in the superior FN, trabecular bone deficits in this region may contribute to the high risk of hip fracture in older adults with T1D.

Presenter: Stephanie L. Tsai

Institution: Massachusetts General Hospital **Department:** Center for Regenerative Medicine

Poster Number: 36

Title: Elucidating injury-site specific regenerative programs to rebuild the tendon

Co-Authors: Marie Noedl, Mor Grinstein, and Jenna L. Galloway

Abstract:
Tendons are essential connective tissues that transmit forces from muscle to bone. Their unique highly ordered, matrix-rich architecture is critical for proper function. Tendon injuries are common and frequently occur within the tendon or at the tendon-bone attachment. Current treatments are limited, costly, and variable in efficacy, leaving patients with impacted quality of life and altogether contributing to a growing economic healthcare burden. To date, the development of new therapeutic strategies has been hindered by the lack of experimental tendon regenerative models which may be leveraged to elucidate cellular and molecular mechanisms required for proper regeneration. While adult mammalian tendons can heal, tendon cells, or tenocytes, fail to respond and disorganized scar tissue with impaired function forms instead. Using lineage tracing and multiphoton imaging, we demonstrate that unlike their mammalian counterparts, adult zebrafish tenocytes can proliferate, migrate, and regenerate the tendon and tendon-bone attachment following full tear injuries. To investigate the molecular basis for zebrafish tenocyte plasticity, we performed single cell transcriptomics during homeostasis and regeneration in both injury models. We identify and characterize shared early transient injury-responsive cell-states which diverge during later stages into site-specific regenerative programs seemingly functionally driven by TGF-beta signaling in the tendon and Wnt signaling in the tendon-bone attachment. Finally, we present a spatial zebrafish tendon cell atlas and elucidate evolutionarily conserved cell populations. Our work debuts the adult zebrafish tendon as an invaluable regenerative model and opens avenues to generate genetic tools for performing cross-species comparative studies to uncover mechanisms driving regeneration versus fibrosis.

Presenter: Jingshu Liu

Institution: Tufts University **Department:** Immunology

Poster Number: 37

Title: THE ROLE OF MAST CELLS IN OSTEOARTHRITIS

Co-Authors: Jingshu Liu, Sihan Liu, Anjali Rajesh Mamidwar, Matthew Gordon, Daniel Sun, Zerong You, Jianren Mao, Irene Tsilioni, Li Zeng

Abstract:
INTRODUCTION: Osteoarthritis (OA) is characterized by joint degeneration and chronic inflammation. Mast cell (MC), one of the major immune cells increased in OA synovium, orchestrates inflammation by degranulation to releasing preformed mediators as well as de novo synthesis of cytokines. Recent study found MC-deficient mice had decreased DMM-induced OA, suggesting MCs are required for OA progression. However, the regulation of MCs in OA remains unknown. In this study, we characterized synovial MCs in OA and investigated their function and regulation.

METHODS: Human OA synovium and cartilage were obtained from Tufts Medical Center (IRB exempt). Mouse experiments were approved by IACUC. 10-week male C57BL/6 mice were subjected to ACLT surgery, following by intraarticular injection of compound 48/80 (C48/80) or lentiviral Wnt7a and GFP, as well as von Frey analysis. Isolated mouse joints were cultured for 7 days with MC injection on day 2 and day 5. Joints were evaluated by IHC/IF using CD117, FcεRI, tryptase, chymase, PGP9.5 antibodies, or by histology with avidin, safranin O/fast green, toluidine blue. Human LADR MCs were stimulated with IgE/anti-IgE (for chondrocytes (CC) and synoviocytes (FLS) treatment), LPS, or substance P (SP, MC activator). Statistical analysis was done using GraphPad Prism 9, $p < 0.05$ will be considered significant.

RESULTS: 1. MCs in human OA synovium have distinct markers. MCs are widely spread in human OA synovium and can be identified by toluidine blue, as well as surface markers CD117 and FcεRI (Fig. 1A, B). Human synovial MCs can be divided into two subtypes, MCT and MCTC, by the expression of MC-specific proteases tryptase and chymase, suggesting heterogeneity of MCs (Fig. 1C). 2. MCs in mouse joints correlate with OA severity and are located near nerve endings. MC numbers increased when OA progressed in ACLT OA knees (Fig. 2A). Mouse synovial MCs also have heterogeneity with MCT and MCTC subtypes

(Fig. 2B). MCs and nerve endings reside closely in the synovium, prompting the investigation of OA pain (Fig. 2C). 3. MC activation induces catabolic gene expression and lead to joint destruction and pain in OA. MMP levels were increased in MC-CM treated CC and FLS, suggesting MCs promotes catabolic gene expression in joint cells (Fig. 3A). C48/80, a chemical that causes MC degranulation, resulted in increased articular cartilage damage and nociceptive pain in ACLT OA joints (Fig. 3B). Injection of activated MCs led to increased cartilage damage in ex vivo cultured mouse joints (Fig. 3C, D), suggesting MC is sufficient to contribute to OA progression. 4. Wnt7a inhibits MC activation and OA progression. Wnt7a was found to be expressed in MCs and was inversely correlated with OA severity (Fig 4A). ELISA showed that pre-treatment with Wnt7a inhibited MC degranulation and de novo synthesis of inflammatory cytokines (Fig. 4B). IA Injection of lenti-Wnt7a reduced joint damage, MC infiltration and degranulation in ACLT OA joints, suggesting that Wnt7a is sufficient to regulate MC activity in OA.

CONCLUSIONS: MCs play a key role in OA progression by promoting catabolic gene expression and contributing to OA pain. Wnt7a inhibits MC activation, OA pain and progression.

Presenter: Quentin A. Meslier

Institution: Northeastern University **Department:** Bioengineering

Poster Number: 38

Title: 3D labeling and imaging of osteocyte mRNAs and proteins in adult mouse bone.

Co-Authors: Timothy J. Duerr, James R. Monaghan, Sandra J. Shefelbine

Abstract:
"Introduction: Osteocytes sense external forces and initiate the bone mechanoadaptation response by regulating their molecular expression. Current methods to investigate changes in osteocyte genes and proteins expression in response to loading do not preserve the 3D spatial information. There is a need for 3D tools to investigate molecular expression in osteocytes in their spatially preserved location. Tissue clearing has been used for visualization of bone blood vessels, nerves, and endogenous fluorescence. However, labeling of the osteocyte mRNA and proteins in whole-mount bone remains challenging due to osteocytes' location within the dense bone matrix. In this work, we present a method for 3D immunofluorescence and mRNA staining in whole-mount mouse bone. We also share protocols, highlight opportunities, and identify the challenges of this novel 3D labeling method.
Methods: All experiments were approved by IACUC. The right tibia of 23 weeks old C57BL/6 mice were loaded using uniaxial compression model. Left legs were kept as control. Following loading protocol, tibia were collected, fixed with paraformaldehyde, and decalcified using EDTA. mRNA labeling: we used HCRFISH to label Sost mRNA transcripts. Protein labeling: once decalcified, samples were preserved using SHIELD (LifeCanvas Technologies), an epoxy-based solution that has been shown to protect tissue structure and molecular components. Then, an enzymatic matrix permeabilization step was performed to enable antibody penetration and immunolabeling. Primary antibodies specific to sclerostin were incubated with the samples for 2 days. As an alternative approach, we report the use of customized fluorescent nanobodies to target and label sclerostin. Nanobodies do not require matrix permeabilization, but their fluorescent signal is dimer. Cell detection: A custom neural network was used to detect cells. Lightsheet images were segmented to only include cortical bone. The number of positive cells detected was normalized by the total number of nuclei. Imaging: All samples were mounted in agarose gel and their refractive index were matched to 1.52. Images were acquired using lightsheet microscopy.
Results: mRNA labeling: Sost and nuclei signals were acquired throughout the mouse tibiae and presented as a 3D reconstruction (Fig.1). On average, 350,000 cells were detected in the cortical bone based on nuclear staining (Fig.2.B). Preliminary results show a

decrease in the percentage of Sost-positive cells at about 25% and 55% of the midshaft length of loaded bones (n=3) compared to control legs (n=5) (Fig. 2.C). Protein labeling: We successfully labeled sclerostin protein in whole-mount mouse tibia using traditional antibodies (Fig.3). Matrix permeabilization has been found to be critical for optimal antibodies penetration. For an adult mouse tibia, we recommend 6 h of enzymatic permeabilization. SHIELD was found to preserve samples integrity during permeabilization. Discussion: Various factors can affect the enzymatic permeabilization such as the size of the sample, enzyme lots and concentration, permeabilization time, epoxy concentration,, and presence of the bone marrow. Significance: To our knowledge, it is the first whole-mount immunolabeling protocol for skeletally mature mouse bones. This method will allow 3D investigation of bone cells molecular signal and enable correlation with their 3D environment. Current work focuses on finalizing cell detection analysis for both mRNA and protein labeling"

Presenter: Abdulrahman Idrees

Institution: Boston University **Department:** Translational Dental Medicine

Poster Number: 39

Title: Sema3A: a Potential Regulator of Myeloid cells

Co-Authors: Emaad Saad, Gokce Kiryaman, Myra Banville, Paola Divieti Pajevic

Abstract:
Introduction: Semaphorin 3A (Sema3A) is an osteocyte-derived protein that promotes osteogenesis and suppresses osteoclastogenesis by binding to neuropilin1 (Nrp1) on osteocytes/osteoblasts and osteoclasts. Studies show that semaphorins are key regulators of cell migration, cell death and synapse formation during nervous system development. Moreover, they are involved in a variety of cellular processes, such as angiogenesis and immune function. In this study, we investigated the effects of osteocyte-derived Sema3A on myeloid cell proliferation and differentiation in vitro. We hypothesized that osteocytes regulate myeloid cell proliferation and differentiation through the action of Sema3A.

Methods: CRISPR/Cas9 technique was used to target Exon9 of Sema3A in Ocy454-12H cell line which was then used to generate conditioned medium. Bone marrow mononuclear cells (BMNCs) were isolated from femurs of wild type C57BL/6N female mice 8-10 weeks old and then differentiated into myeloid cells and osteoclasts. To validate the deletion of Sema3A we performed real time PCR. Osteocytes migration was investigated by scratch assay. Methocult was used to investigate myeloid colony formation. Migration assay was performed to examine the effect of CM on the migration rate of BMM. TRAP staining and pit resorption assay were used to analyze osteoclasts formation and function.

Results: Our data indicates that the absence of Sema3A in osteocytes leads to significant increase in RANKL expression. Ocy454-12H Sema3A KO cells were found to be significantly more abundant in the area of a scratch wound compared to the control group, demonstrating that Sema3A controls osteocytes migration. CM from Osteocytes lacking Sema3A significantly increased the number of CFU-GM and promoted the migration of BMMs. Ocy454-12H CM significantly increased the proliferation of BMM independently of Sema3A expression. Lastly, CM from Ocy454-12H lacking Sema3A significantly increased the number of osteoclasts.

Conclusion: Our data suggests that Sema3A from osteocytes may play a role in controlling osteocytes migration, differentiation and proliferation of granulocytes and monocytes, BMM migration and osteoclast proliferation.

Presenter: Myra Banville

Institution: BOSTON UNIVERSITY **Department:** TRANSLATIONAL DENTAL MEDICINE

Poster Number: 40

Title: Establishment of A Novel Murine Alveolar Osteoblastic Cell Line

Co-Authors: Gokce Kiryaman, Abdulrahman Idrees, Irobosa Enabulele, Paola Divieti Pajevic

Abstract:
Introduction: Cell lines play an essential role in scientific research as they provide a reproducible platform for experiments. Several osteoblastic cell lines have been generated to date including MC3T3-E1, Saos-2, MG-63, hFOB 1.19, and ROS 17/2.8. However, none of them originated from alveolar bones. Alveolar bone is different than the other bones of the skeleton regarding structure and function and studies are needed to better understand the physiology and functions of alveolar bone cells. Aim of this study is to generate a novel alveolar osteoblast cell line in order to examine characteristics of these cells thoroughly.

Methods:, 8-10 week old female C57BL/6 wild-type mice were housed in a controlled, pathogen-free environment maintaining a 12-hour light–dark cycle on a standard chow diet. Mice were euthanized following approved IACUC protocols and mandibles were aseptically isolated and freed of soft tissues. Incisor teeth were extracted and the remaining bone was minced and digested twice for 30 mins at 37oC using a solution containing 3 mg/ml of Collagenase I and 4mg/ml of Dispase II. Cells were collected at the end of the digestions and both cells and bone fragments were cultured in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum and 1% antibiotic-antimycotic. Lentiviral particles expressing a temperature sensitive SV40 (Simian virus 40) large T Antigen and puromycin resistance gene were used to infect cells after they reached 80% confluence.

Results: We performed 5 independent experiments for a total of 8 mice. Cells were successfully isolated from both cells and bone fragments from all animals. Nine cell isolations, consisting of 5 preparations from alveolar fragments (AB) and 4 from alveolar cell digestion (AC), were subsequently infected with an SV40 T Antigen, puromycin-resistant Lentivirus. To date we have isolated 14 single cell colonies, 5 of which were further characterized. To demonstrate the osteoblastogenic phenotype of these cells we cultured them in DMEM containing ascorbic acid (50 µg/ml)and Beta-Glycerolphosphate (10mM). Alkaline phosphatase (ALP) and Von Kossa staining demonstrated the presence of numerous ALP+ cells and bone nodules. Additional characterization of these clones is currently underway.

Conclusion: We have successfully isolated and immortalized several alveolar cells and initial analysis of 4 single cell clones revealed a high expression of ALP and extensive matrix mineralization, demonstrating the osteogenic phenotype of these cells.

Presenter: ALI HADIAN AMREI

Institution: University of Boston GSDM **Department: P**eriodontology

Poster Number: 41

Title: Synergistic Effect of High Glucose and LPS on Osteoclast Differentiation

Co-Authors: Weiyuan Ma

Abstract:
The association between diabetes and periodontitis-related bone loss has been established, yet the mechanisms underlying how high glucose exacerbates inflammation-induced bone loss remains elusive. This study explores the interplay of high glucose and lipopolysaccharides (LPS) on osteoclast differentiation

and periodontitis-related bone loss.

RAW264.7 cells were cultured with the Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL), M-CSF (Macrophage colony stimulating factor) or both, then exposed to varying glucose concentrations and LPS. Osteoclast formation was assessed on days 4 and 6, Moreover, the effect of LPS on osteoclast differentiation was assessed one day and 4days after adding RANKL, M-CSF, or both to RAW264.7 cells.

Result: Elevating glucose concentrations from 25mM to 50mM on both days (day 4 and day 6), both with and without LPS, leads to a decline in TRAP activity. Additionally, at all glucose levels, LPS enhances TRAP activity reduction. Moreover, introducing LPS one day after RANKL resulted in significantly less differentiation of osteoclast cells compared to a 4-day delay.

In conclusion, glucose concentrations exerted a significant impact on TRAP activity, and the timing of LPS introduction played a crucial role in osteoclast differentiation.

This study contributes valuable insights into the dynamic interplay of osteoclasts in the context of diabetes and periodontitis, paving the way for future research and potential therapeutic strategies.

Presenter: Lipi A. Marion

Institution: MGH **Department:** Endocrinology

Poster Number: 42

Title: Contributions of Skeletal Microarchitecture and Fall Risk to Fractures in Adults with Long-standing Type 1 Diabetes Mellitus

Co-Authors: Andria I. Fremaint, Fjola Johannesdottir, Enrico Cagliero, Vanita R. Aroda, Mary L. Bouxsein, Elaine W. Yu

Abstract:
INTRODUCTION:
Type 1 diabetes mellitus (T1D) is associated with a marked increase in fracture risk, a phenomenon not entirely explained by lower DXA-measured BMD. Recent studies suggest that T1D may lead to deficits in bone microarchitecture, but results are inconsistent. Whether the heightened fracture risk in T1D is driven by mechanisms primarily intrinsic to bone or extrinsic remains unknown. Thus, our objective was to assess fracture history, falls and bone microarchitecture in adults with long-standing T1D.

METHODS:
We recruited 97 individuals with T1D and 53 non-diabetic controls aged >50 years as part of the T1D Bone Health Connection (BEACON) Study, an ongoing prospective observational cohort study enrolling from the greater Boston area. We measured DXA-BMD of the spine, femoral neck, and total hip, as well as volumetric BMD (vBMD), bone microarchitecture and estimated bone strength by high-resolution peripheral quantitative computed tomography (HRpQCT) at the 4% distal radius (R4), 7% distal tibia (T7) and 30% diaphyseal tibia (T30). We also assessed self-reported prevalent fractures and recent falls. We used linear regression models with adjustments for age, sex, height and weight to analyze group differences in bone parameters as well as multivariate logistic regression to assess independent associations between variables of interest and binary outcomes.

RESULTS:
Mean age of the full cohort (n=150) was 64±7 years; 51% were women. T1D and control groups were similar in age (p=0.20) and sex distribution (p=0.46), though T1D subjects had a higher mean BMI (27.9+5.0 vs 26.0+4.9 kg/m2, p=0.03). T1D subjects (mean disease duration 46±10 years, HbA1c 7.1±1.0%) reported a higher prevalence of fractures compared to controls (39% vs 6%, p<0.001). DXA-BMD did not differ between the two groups at any site, though T1D participants had lower cortical vBMD at T7 (-5.5%, p <0.001) and

lower cortical area (-5.4%, p=0.008) and thickness (-4.9%, p=0.02) at T30. Unexpectedly, T1D subjects had higher trabecular thickness at T7 (+3.5%, p=0.008). Failure loads at T7 and R4 were similar between T1D and controls. While there were no intergroup differences in the proportion of individuals who had >1 fall in the past year (19% vs 13%, p=0.40), among those who fell, T1D subjects reported a higher fall frequency than controls (median 2 vs 1 fall/person, p=0.045). A fall in the past year was associated with higher odds of prevalent fracture [OR 4.9 (95% CI 1.8-13.8)].

CONCLUSION:
This study highlights the increased fracture risk in adults >50 years with T1D. Differences in fall risk and deficits in cortical microarchitecture may contribute to fractures in T1D. Although we observed minor differences in bone microarchitecture in our cohort, these did not impact peripheral bone strength. Further studies are needed to elucidate the precise mechanisms for the increased fracture susceptibility in this population.

Presenter: Jae hyuck Shim

Institution: UMass Chan Medical School **Department:** Horae Gene Therapy Center

Poster Number: 43

Title: AAV-mediate Gene Therapy for Fibrodysplasia Ossificans Progressiva

Co-Authors: Yeon-Suk Yang, Jung-Min Kim, Jun Xie, Guangping Gao

Abstract:
Heterotopic ossification is the most disabling feature of fibrodysplasia ossificans progressiva, an ultra-rare genetic disorder for which there is currently no prevention or treatment. Most patients with this disease harbor a heterozygous activating mutation (c.617G>A;p.R206H) in ACVR1.

This study identify recombinant adeno-associated virus 9 (rAAV9) as the most effective serotype for transduction of the major cells-of-origin of heterotopic ossification. We use AAV9 delivery for gene addition by expression of codon-optimized human ACVR1 (ACVR1opt), ACVR1R206H allele-specific silencing by AAV-compatible artificial miRNA and a combination of gene addition and silencing.

In mouse skeletal cells harboring a conditional knock-in allele of human mutant ACVR1 and in patient-derived induced pluripotent stem cells, AAV gene therapy ablated aberrant Activin A signaling and chondrogenic and osteogenic differentiation. In ACVR1R206H mice treated locally in early adulthood or systemically at birth, trauma-induced endochondral bone formation was markedly reduced, while inflammation and fibroproliferative responses remained largely intact in the injured muscle. Remarkably, spontaneous heterotopic ossification also substantially decreased in in ACVR1R206H mice treated systemically at birth or in early adulthood.

To further improve therapeutic efficacy and safety of AAV gene therapy, we developed AAV carrying the combination of ACVR1opt and artificial miRNAs targeting Activin A and its receptor ACVR1R206H while limiting the expression in non-skeletal organs such as brain, heart, lung, liver, and kidney. The advanced AAV ablated aberrant activation of BMP-Smad1/5 signaling and osteogenic differentiation of ACVR1R206H skeletal progenitors. Both local and systemic delivery of AAV gene therapy resulted in a significant decrease in endochondral bone formation in ACVR1R206H mice.

Collectively, we develop promising gene therapeutics that can prevent disabling heterotopic ossification in mice, supporting clinical translation to patients with fibrodysplasia ossificans progressiva.

Presenter: Kai Wang

Institution: Spaulding Rehabilitation Hospital **Department:** Physcial Medicine and Rehabilitation

Poster Number: 44

Title: Combining mitochondrial transplantation and magnetic field stimulation to improve aged skeletal muscle regeneration

Co-Authors: Nafiseh Shahshahan, Fabrisia Ambrosio, Kai Wang

Abstract:
Mitochondrial dysfunction is one of the hallmarks of aging. In skeletal muscle, age-related mitochondrial dysfunction causes functional declines in muscle stem/progenitor cells (MPCs), which ultimately impairs aged muscle regeneration. Studies have shown that transplantation of healthy mitochondria can enhance skeletal muscle regeneration. However, the functional improvements of injured skeletal muscle after mitochondrial transplantation remain limited. On the other hand, magnetic field stimulation has been shown to improve mitochondrial function by inducing mitophagy of damaged mitochondria, increasing mitochondria biogenesis, and enhancing mitochondrial respiration. Therefore, we hypothesize that combining mitochondrial transplantation with magnetic stimulation represents a promising regenerative rehabilitation strategy to improve aged MPC function and aged muscle regeneration. To test our hypothesis, we collected mitochondria of young MPCs that were isolated from 3-5-month-old mice, and we used those mitochondria to treat aged MPCs isolated from 24-26-month-old mice. A Helmholtz coils-based magnetic field stimulation chamber was used to generate static magnetic fields. We set up four experimental groups (i.e., no treatment control, mitochondrial transplantation alone, magnetic field stimulation alone, mitochondria transplantation combined with magnetic field stimulation) to systemically evaluate the effects of mitochondrial transplantation and magnetic field stimulation on MPC proliferation and myogenic differentiation. As shown in Figure 1, compared to no treatment control, magnetic field stimulation did not influence aged MPC proliferation but promoted cell myogenic differentiation and myotube formation. By contrast, treatment with young mitochondria promoted both cell proliferation and differentiation, and such promotion effects were further enhanced by magnetic field stimulation. These results indicate that mitochondrial transplantation and magnetic field stimulation synergistically enhance aged MPC proliferation and myogenic differentiation. Given the critical role of MPCs in skeletal muscle regeneration, our study suggests that the combination of mitochondrial transplantation and magnetic field stimulation can be a promising regenerative rehabilitation modality to improve aged muscle repair.

Presenter: Yu Jin

Institution: Harvard School of Dental Medicine **Department:** Developmental Biology

Poster Number: 45

Title: Shh+ stem cells regulated by mechanotransduction maintain nucleus pulposus homeostasis and promote its regeneration

Co-Authors: Guoyan Liang,Qian Cong, Yizhong Hu, Yuchen Liu, Yingzi Yang

Abstract:
The intervertebral disc (IVD) links vertebral bodies in the spine, allows movement and resistance to tension and compression forces between each vertebra, and maintains a constant intervertebral space that prevents compression of the spinal nerves. The physiological function of the IVD depends on the cellular and molecular composition of the nucleus pulposus (NP), which plays a key role in managing the mechanical loading of the IVD. IVD degeneration primarily results from the disrupted homeostasis and diminished regenerative capacity within the nucleus pulposus (NP). However, the existence and functional roles of NP stem cells in adult NP remained elusive.

Here we identified the rare Sonic hedgehog (Shh)-positive cells in the adult NP as unipotent stem cells with robust proliferative and clonogenic capacities. The Shh+ cells located in the outer NP layer at the NP-

annulus fibrosus (AF) interface, which are activated to proliferate in response to mechanical stimuli or injury in adult Shh-CreER; Rosa26tdTomato mice during long-term lineage tracing. We show that the Shh+ NP cells are required to maintain NP homeostasis as their depletion triggers rapid NP degeneration and spine discomfort using the Shh-CreER; Rosa26tdTomato/DTA mice. Additionally, the Shh+ NP cells are essential for NP regeneration in an IVD injury model and their numbers decline with age, aligning with the reduced regenerative capacity of aged NP. We also found younger human IVD samples contain more SHH-positive NP cells compared to older ones.

Notably, combining with previous research and our single cell sequencing results, we found Yap-mediated mechanotransduction is essential in maintaining the Shh+ stem cells. Genetic Yap loss markedly reduces the Shh+ cell number and abolishes post-injury NP regeneration in Shh-CreER; Rosa26tdTomato; YAPf/f;TAZf/f mice. Conversely, Yap activation in the Shh+ cells increases their numbers and promotes NP regeneration uncovered by Shh-CreER; Rosa26tdTomato/rtTA; TetOYAPS127A (TetOYAP) mice. Our studies identify NP stem cells, and their regulation by Yap in IVD maintenance, aging and regeneration.

Presenter: Louis C. Gerstenfeld

Institution: Boston University **Department:** Orthopedic Surgery

Poster Number: 46

Title: Use Of The Human Serum Proteome To Assess The Progression Of Fracture Healing

Co-Authors: Serkalem Demissie; Ryan Kim; Robert Azario; Jason Lowe; Robert O'Toole; David B. Weiss; Paul Matusewski; George Hanson; Stephen Warner; Joshua L. Gary; Roman Natoli; Renan Castillo; Paul Tornetta III; METRC Major Extremity Trauma Research Consortium

Abstract:
Serum protein markers may potentially be used to predict fracture healing. However, only a limited number of studies in either animals or humans have been performed (Reviewed in 1). The overall goal of this preliminary prospective observational study was to define the potential efficacy of a serum protein-based diagnostic for predicting the progression or failure of fracture healing in closed humeral diaphyseal fractures through observation of the serum proteome over a six-month healing period.

Methods:. Patient Group: Patients were enrolled under an approved sIRB overseen by the Major Extremity Trauma Research Consortium . We assessed nine patients (seven that healed and two non-unions). Primary Inclusion criteria: 1) Skeletally mature, ages 18-70 (inclusive), 2) Diagnosis of extra-articular fractures of the proximal humeral metaphysis (AO/OTA 11 type A2.1, A2.2 and/or A2.3) with diaphysis involvement or diagnosis of isolated closed extra-articular fractures of the humeral shaft (AO/OTA 12), 3) Treated by closed non-operative immobilization in a functional brace, and 4) Approached for consent within three weeks of injury. Primary Exclusion criteria: 1) Humeral shaft fractures that extend into the articular surface (i.e., AO/OTA 13), 2) Pathologic fractures, 3) Additional bone injuries or injury that involved a trauma activation with entry of more than two AIS codes, and 4) Pregnant or lactating women. Time Course of Study: The first study visit was at the first follow up visit at 1-3 weeks after injury and thereafter at 4-6, 7-9, 12-16 and 26-32 weeks. At the regularly scheduled visit at 7-9 weeks, progression to union was assessed. Nonunion was defined as gross motion at the fracture site and mRUST score of less than six at this visit. If assessed as a nonunion the treating physician discussed the option of waiting until next examination to further assess healing or consider surgical treatment to try to improve healing. If the decision is to have surgical treatment, the last blood draw was at the time of surgery. Proteomic Assay: Total plasma was prepared using a standardized protocol across all participating sites. Samples were stored at -80oC until they were shipped on dry ice to the primary study site where they were organized and freeze thawed one time to generate 200 microliter aliquots. Samples were then transferred to Somalogic (Boulder, Colorado) who provided an aptamer based proteomic profiling as a commercial service using the SomaScan Discovery Assay that screened ~7000 proteins per sample. Statistical and Bioinformatics Approaches: In this analysis, the first time point (1-3 weeks post injury) was used as the reference for statistical comparisons, to which each

subsequent time was compared. A paired t-test was used to compare the first visit against each subsequent visit (temporal change), and 2-sample t-test to compare the two outcome groups (union vs. nonunion). Only proteins showing a fold change (FC) at 1.25 or greater and a $p<0.01$ were considered to be differentially expressed (DE). The protein groups showing differences by time and between the union and nonunion groups were then used for ontology analysis using either DAVID (2) or Metascape (3).

Results 739 proteins were identified that met our identification criteria that were different by time. 576 showed differences between the nonunion and union groups. 100 proteins overlapped between the two groups. A total,121 of the 739 proteins were associated with matrisome (extracellular matrix production). Some notable proteins included Col10A1, periostin, and Col9A1. The second most prominent group seen in normal healing were associated with blood vessel development of which 61 proteins were observed. The proteins associated with the matrisome also were the most prevalent type of protein that passed screening for DE between the nonunion and union groups. Other interesting groups that are of note seen only in the non-healing protein group included immune cytokines and proteins associated with the adaptive immunity. Analysis of specific differences between the union and nonunion groups. Assessments of individual proteins such as COMP-1 (Cartilage oligomeric protein) VEGF 121 (Vascular Endothelia Growth Factor A121) and BMP5 (Bone Morphogenetic Protein 5) were used as examples of proteins with different patterns of serum profiles between the healer and non-union groups as well as were informative mechanistically to the underlying biology of healing and non-union

Discussion In this first analysis, our goal was to show both the feasibility and efficacy of using a serum proteomic approach to observe the biological progression of fracture healing to predict nonunion. While none of the protein groups had significance with an FDR< 0.05, which is the basis for acceptance for this type of large data analysis, we did observe biological relevancy of the protein groups that were identified to the known biology of fracture healing. In order to assess how many patients we would need to reach acceptable levels of statistical significance we carried out a simulation study for power assessment of our current data using varying sample sizes, proportions of DE proteins, FC differences and varying FDR values. As an example using 10% DE proteins (~760), while controlling FDR at 5%, we found that FDRs will be controlled on average at 0.054, 0.043, 0.035 for N=25, 30, and 35, respectively. These assessments suggest that our analysis is easily scalable to identify individual proteins having both prognostic and diagnostic value.

Clinical Relevance Even though the current study was less statistically robust, the ontology and pathway analysis of the DE proteins was useful to both successfully identify biological processes associated with the progression of healing and identify biological mechanism that are associated with failed healing. As an example, the current data, for the first time, shows the actual temporal progression of the biological processes of human fracture healing. It places the initiation of a seeable endochondral process between 4-6 week after injury while placing peak of cartilage formation during endochondral bone formation in the humerus in a window between 7 and 12 weeks. From a mechanistic point of view, it will provide further clarity on why healing fails, identify what adjunct therapies may have useful efficacies, and when adjunct therapies might show greatest efficacy in their clinical application Acknowledgement: Funded through DOD Contract number W81XWH-19-1-0796 (UNION) 1. Chitwood et al. Biomarkers 2021, 26(8) 703. 2. Huang da, W. et al. Nucleic Acids Res.35 (Web Server issue). 3. Zhou, Y. et al. Nat Commun. 2019, 3;10(1):1523

Presenter: Qian Cong

Institution: Harvard School of Dental Medicine **Department:** Developmental Biology

Poster Number: 47

Title: Identification of progenitor cells that contribute to heterotopic ossification in Progressive Osseous Heteroplasia

Co-Authors: Yingzi Yang

Abstract:

Heterotopic ossification (HO) is a debilitating condition characterized by the pathologic formation of ectopic bone that occurs as a common complication after injury or as a manifestation of particular genetic disorders. The identity of progenitor cells that contribute to GNAS mutation induced heterotopic ossification relevant to progressive osseous heteroplasia (POH) is unknown, which limits the effective treatment or prevention of ectopic bone formation and expansion. Utilizing an inducible lineage-tracing mouse (Gnasf/f; Pdgfra-CreERT2; Rosa26LSL-TdTomato), we found that Pdgfra+ progenitor cells were induced to become osteoblasts 6 weeks post systemic Tamoxifen injection. The ectopic bone was much more severe 3 months and 6 months post Tamoxifen injection, suggesting that Pdgfra+ cells is the major cell population in HO formation. Interestingly, we found that Platelet-derived growth factor (PDGF) signaling were much upregulated in Gnas mutant cells. Pharmacological inhibition of PDGF signaling with Imatinib, effectively abolished halted HO progression without affecting normal bone homeostasis, offering a previously unrecognized therapeutic rationale for HO prevention. Furthermore, our previous data revealed the progression of ectopic bone into deep muscular regions in POH mouse models, suggesting a potential role for committed muscle cells in ectopic bone formation. However, it is noteworthy that Gnas mutant mature myotube cells lack the ability to undergo reprogramming towards ectopic bone formation. Moreover, skeletal muscle precursors and muscle satellite cells in vivo contributed minimally to heterotopic ossification, even after injury. Thus, Pdgfra-expressing progenitor cells differentiate into osteoblasts, contribute to heterotopic bone formation and expansion in POH, shedding light on potential cell-specific therapeutic interventions.

Presenter: Parthena Kotsalidis

Institution: MGH **Department:** Endocrine Unit

Poster Number: 48

Title: Genome-wide CRISPR screen to identify novel regulators of osteocyte maturation and dendrite formation

Co-Authors: Courtney Mazur, Marc Wein

Abstract:
Osteocytes are bone-embedded cells with many important functions, accomplished in part due to specialized actin-based dendritic projections that develop during differentiation from pre-osteoblasts to osteocytes. These projections enable cell-cell communication, coordination of osteoblasts and osteoclasts, transport of nutrients, mechano-sensing, and maintenance of the surrounding bone. To identify novel genes that regulate osteocyte differentiation, we conducted a FACS-based genome-wide CRISPR-interference (CRISPRi) screen in the Ocy454 osteocyte-like cell line.

To identify an appropriate marker for our flow cytometry-based pooled CRISPRi screen, we first used RNA-sequencing data from Ocy454 cells to select genes encoding cell surface proteins that are associated with osteocyte maturity. From this analysis, integrin beta-3 subunit (CD61) was selected based on its strong, reproducible upregulation at the cell surface over differentiation time as detected by flow cytometry. Furthermore, osteocytes in vivo express integrin beta-3 primarily along their dendrites.

To conduct the screen, we infected Ocy454 cells stably expressing dCas9-KRAB with a lentiviral library consisting of over 60,000 sgRNAs targeting mouse gene promoters (3 guides/gene target). After 10 days of differentiation, cells were labeled with CD61-PE antibody and SYTOX Green dead cell stain and sorted into CD61-high and CD61-low populations. The top and bottom 10% of CD61-PE conjugated cells were recovered, genomic DNA was isolated, and the sgRNAs in each population were sequenced to determine genes that affected CD61 expression.

In each of two replicates, 4.6-13.3 million cells per group were collected for sequencing, and sgRNAs targeting Itgb3 (the gene encoding CD61) were most enriched in the cd61-low group, which served as a robust positive control. Additional hits enriched in the cd61-low group (genes required for cd61) and cd61-high group (genes that inhibit cd61) were chosen for further validation in a non-screening format. One was

eukaryotic translation initiation factor 3 subunit B (Eif3b), which we further investigated using sgRNAs targeting distinct areas of the promoter, full gene knockout, and shRNA-mediated knockdown. We subsequently ran flow cytometry and validated that inhibition of Eif3b led to decreased expression of CD61. Using the results of the screen we are working towards identifying additional genes that will help us better understand the regulation of osteocyte maturity and dendrite formation.

Presenter: Xinchen Wu

Institution: Harvard School of Dental Medicine **Department:** Developmental Biology

Poster Number: 49

Title: Gαs R201H acts through Sik2/Sik3 to affect Yap/Taz in Fibrous Dysplasia

Co-Authors: Qian Cong

Abstract:
FD is a crippling genetic skeletal disorder caused by mosaic activating mutations (R201H or R201C) of the α subunit of stimulatory G protein (Gαs), encoded by GNAS. In this illness, normal bone cells reside next to cells that express the mutant Gαs. The mutant cells fail to form normal bone and instead form weak, disorganized, and poorly mineralized bone that leads to deformity, fracture, and severe pain, leading to functional impairment and wheelchair confinement. Our FD models are established based the Gnasf(R201H) mouse line in which the human FD mutation (R201H) has been conditionally knocked into the corresponding mouse Gnas locus. Lats1 and Lats 2 are activated by phosphorylation at different sites by Hippo Kinases Mst1/2 in the Hippo pathway. We observed increased LATS1/2 phosphorylation and Lats-mediated Yap and Taz inactivating phosphorylation in GαsR201H BMSCs. Furthermore, bulk RNA seq analysis of the GαsR201H BMSCs and WT BMSCs revealed upregulation of hippo signaling. We observed severely reduced bone formation in developing long bones of the Osx1-Cre; Gnasf(R201H)/+ mice was associated with downregulated Yap protein levels.

In previous study, Sik2/Sik3 deletion phenocopies FD. At present, very little is known about how SIKs might control Yap/Taz activity, with no studies addressing connections between SIKs and Yap in mammalian cells. Sik2/Sik3 may phosphorylate other proteins in the Hippo pathway to control gene expression in a cell specific manner. SK-124, a new SIK2/3 inhibitor, inhibited Sik2/Sik3 as phosphorylation of HDAC4/5/7, the known substrates of Sik2/3, was reduced. The LATS1/2 and Yap/Taz phosphorylation were increased in SK-124 treated cells as we also observed in GαsR201H BMSCs, indicating Sik2/Sik3 may play critical roles regulating Yap/Taz activities downstream of Gαs. We expressed Lats1 and Lats2 in LATS1/2 DKO HEK293A cells by plasmid transfection. 48 hours later, we treated the cells with SK-124 and found increased LATS1/2 activity measured by increased Yap phosphorylation at S127 in Lats1 or Lats2 expressing cells, whereas no Yap phosphorylation was detected in LATS1/2 DKO cells with or without SK-124 treatment. Our finding suggests that Sik2/Sik3 may regulate Yap/Taz activities by direct phosphorylation on LATS1/2 and LATS1/2 DKO abolished effects of Sik2/Sik3 on Yap/Taz.

Presenter: Fuhua Wang

Institution: Harvard School of Dental Medicine **Department:** Developmental Biology

Poster Number: 50

Title: Sexual Dimorphic Requirement of Piezo1 in Suppressing Osteoclast Differentiation from Monocytes

Co-Authors: Yizhong Hu, Yu Jin, Yingzi Yang

Abstract:

Mechanotransduction plays a critical role in maintaining bone homeostasis. The absence of mechanical loading results in bone loss by inhibiting osteoblastic bone formation and promoting osteoclastic bone resorption. While mechanotransduction in skeletal cells have mainly been attributed to osteocytes, whether osteoclasts contribute to this process remains unclear. While deletion of the mechanosensitive ion channel Piezo1 from Ctsk+ mature osteoclasts did not affect skeletal mass, we found that Piezo1 deletion from Csf1r+ monocyte precursors of osteoclast resulted in significant trabecular bone loss due to increased osteoclast number with no change in bone formation, indicating that mechanosensing by Piezo1 is required to regulate osteoclast differentiation. Importantly, these changes were found in female mice but not in young male mice, suggesting the sexual dimorphic nature of this regulation.

Mechanistically, mechanical stimulation by fluid shear stress inhibited osteoclast differentiation from primary bone marrow monocytes (BMM) measured by TRAP staining and RT-qPCR analysis of mature osteoclast genes (Ctsk, Acp5). Pharmacological activation of Piezo1 mimicked the effects of mechanical stimulation and inhibited osteoclast differentiation, while Piezo1-deficient BMMs isolated from Csf1r-iCre; Piezo1f/f mice exhibited increased osteoclast differentiation. Interestingly, male and female BMMs exhibited similar responses to Piezo1 deficiency in vitro when cultured in standard complete media, suggesting that the sexual dimorphic response observed in vivo may be due to circulating factors such as gender-specific hormones. Indeed, when cultured in charcoal-stripped FBS devoid of growth factors such as estrogen, increased osteoclast differentiation due to Piezo1 deficiency was blunted in male and female BMMs. Bulk RNA-sequencing analyses identified downregulation of Hippo/Yap signaling in female Piezo1-/- BMMs. Indeed, pharmacological Yap activation by TDI-011536 inhibited osteoclast formation while Yap inhibition by verteporfin promoted osteoclast formation. Genetic activation of Yap in vivo in Csf1r-iCre; TetO-Yap* mice resulted in increased bone mass due to reduced osteoclast number in female mice but not male mice. Importantly, in Csf1r-iCre; Piezo1f/f; TetO-Yap* mice, osteoclast number was restored to normal levels and trabecular bone loss in female mice due to Piezo1 deficiency in osteoclast precursors was completely rescued. Taken together, these data demonstrate a sexual dimorphic role of mechanosensing by Piezo1 via Yap activation in suppressing osteoclast differentiation in bone homeostasis.

Presenter: Mustafa Unal

Institution: BIDMC/Harvard Medical School **Department:** Department of Orthopedic Surgery

Poster Number: 51

Title: Enhancing Fracture Toughness Prediction in Human Cortical Bone through Machine Learning Integration with Raman Spectroscopy Data and Clinical Parameters

Co-Authors: Ramazan Unlu, Ibrahim Tuluce, Jeffry S Nyman

Abstract:
Accurately predicting bone mechanical properties is crucial for personalized fracture risk assessment. This requires a comprehensive approach that considers diverse facets of bone quality. Although artificial intelligence (AI) and machine learning (ML) are increasingly prevalent in scientific and engineering domains, their utilization in bone research is still limited. However, AI/ML algorithms show potential in analyzing diverse datasets of bone quality measurements obtained through various techniques.

The purpose of this study was to investigate the ability of several AI/ML algorithms to predict fracture toughness properties of human cortical bone. This study represents the first attempt to use AI/ML to predict fracture toughness properties using a dataset that includes Raman spectroscopy (RS) measurements and clinical parameters such as age, sex, porosity, and volumetric bone mineral density (vBMD).

The dataset consisted of data from 58 human cortical bone specimens, including crack initiation toughness (Kinit) and J-integral, as well as age, sex, porosity, vBMD, and RS-derived metrics. To identify the most effective model for minimizing the error in predicting actual fracture toughness properties, we assessed five commonly utilized AI/ML algorithms: eXtreme gradient boosting (XG Boost), gradient boosting (GB), Support

Vector Regression (SVR), k-nearest neighbors (KNN), and extra trees regressor (ETR).

Despite the limited sample size, the AI/ML algorithms demonstrated commendable performance in predicting true values of Kinit and J-integral, with an error range of approximately 10-15% relative to the true values.

In summary, our findings highlight the potential of AI/ML algorithms to predict actual bone fracture toughness properties in human cortical bone by utilizing a combination of diverse bone quality measurements. The main challenge now is obtaining extensive datasets that include a variety of measurements to further support the use of AI/ML algorithms in bone research.

Presenter: Zachary R. Hettinger ***No poster***

Institution: Harvard Medical School **Department:** Physical Medicine and Rehabilitation

Poster Number: 52

Title: Follicle stimulating hormone impairs muscle strength in aged female mice through adipogenesis of fibro-adipogenic progenitor cells

Co-Authors: Gabrielle Gilmer, Rylee Kopchak, Hannah Houston, Kai Wang, Juliana Bergmann, Ekaterina Creed, Nafiseh Shahshahan, Michael Hermanto, Tabo Mkandawire, Suyash Sinkar, Amanda Miller, Fabrisia Ambrosio

Abstract:
Muscle strength in women is highly correlated with circulating sex hormone levels, yet little is known how menopause influences the loss of strength with age in females. Contributing to this knowledge gap is the lack of preclinical models that adequately mimic human menopause, thereby preventing the development of effective treatments. Here, we chemically induced menopause in middle-aged female mice and compared muscle strength between aged menopausal and non-menopausal female mice. We found that menopause significantly reduced muscle strength compared to non-menopausal controls, but there were no effects on myofiber size. Instead, we found that menopause compromised muscle quality, as evidenced by intercellular fibrosis and fatty deposition. We then turned our attention to evaluating cells principally responsible for the maintenance of muscle extracellular matrix (ECM), fibro-adipogenic progenitor cells (FAPs). In addition to increasing muscle fibrosis and fat accumulation, menopause also increased the abundance of FAPs. To better understand the responses of FAPs to the menopausal transition, we next isolated FAPs from control mice and exposed cells in vitro to either menopause or non-menopausal serum. We found that post-menopause serum induced FAP adipogenesis and increased the release of pro-degradatory ECM remodeling factors. In a separate cohort, we exposed cells to individual sex hormones at either pre-menopause concentrations (i.e., high estradiol (E2) or high progesterone (P4)), or post-menopause concentrations (i.e., high follicle stimulating hormone (FSH) or high luteinizing hormone (LH)). Exposure of FAPs to individual sex hormones revealed that the influence of menopause on FAP transformation towards a pro-fibrotic and pro-adipogenic phenotype was primarily mediated by FSH. Intriguingly, when we related changes in FAP abundance to changes in circulating sex hormone levels throughout the menopause time course in vivo, we observed that FAP accumulation in muscle closely tracked with the rise of FSH at the onset of menopause. Last, to determine whether FSH alone recapitulates the deleterious effects of menopause on aged female muscle function, we administered either FSH, E2, or FSH + E2, to a cohort of ovariectomized (OVX) aged female mice. By removing the influence of endogenous feedback control from the hypothalamic-pituitary-gonad axis, the relative impact of administered single sex hormones on muscle function can be determined. We found that FSH administration impaired muscle function compared to OVX controls, and the effect was not rescued with E2 treatment. Our results provide mechanistic insights into how a menopause-induced increase in FSH dysregulates FAPs and impairs muscle strength. These findings provide new avenues for the development of targeted treatment options for postmenopausal females.

Presenter: Chilan B. G. Leite

Institution: Brigham and Women's Hospital **Department:** Orthopedics

Poster Number: 53

Title: Attenuation of Inflammation and Posttraumatic Osteoarthritis Following Joint Injury via the Maresin 1-LGR6 Axis

Co-Authors: Hannah Fricke, Luciana P. Tavares, Julie Mekhail, Gergo Merkely, Janey Whalen, Jessica Lehoczky, Julia F. Charles, Christian Lattermann

Abstract:
Introduction:
Joint injuries, particularly anterior cruciate ligament (ACL) tears, often lead to chronic inflammation and subsequent posttraumatic osteoarthritis (PTOA), causing significant morbidity. Current therapeutic approaches inadequately address the persistent inflammatory response post-injury. Specialized pro-resolving mediators (SPMs), such as Maresin 1 (MaR1), have emerged as potential candidates for resolving inflammation and promoting tissue repair. However, their role in PTOA remains underexplored. This study aims to elucidate the temporal dynamics of inflammatory markers and SPM expression, specifically MaR1, post-ACL injury, and evaluate the therapeutic potential of MaR1 in a mouse model of ACL transection (ACLT).

Methods:
Eight-week-old C57BL6/J male mice underwent ACLT, and synovial fluid, periarticular tissue, and tibiofemoral joints were collected at various time points post-surgery for analysis. LGR6-deficient mice were utilized to investigate the role of MaR1 signaling in inflammation resolution. Additionally, the effect of intraarticular MaR1 administration on PTOA progression was assessed in C57BL6/J mice.

Results:
ACLT induced acute joint inflammation characterized by leukocyte infiltration and elevated pro-inflammatory cytokines. MaR1 levels peaked early post-injury and were associated with increased expression of MaR1 biosynthetic enzymes and receptors. LGR6 deficiency exacerbated inflammation and PTOA severity, underscoring the importance of MaR1-LGR6 signaling in resolution of inflammation. Intraarticular MaR1 treatment attenuated inflammation, reduced leukocyte recruitment, suppressed pro-inflammatory gene expression, and ameliorated PTOA development.

Conclusion:
This study demonstrates the critical role of MaR1 in inflammation resolution post-ACL injury and highlights its therapeutic potential in mitigating PTOA progression. Targeting SPM pathways, particularly MaR1, may represent a novel therapeutic approach for preventing chronic joint inflammation and associated degenerative changes following ACL injury.

Presenter: Agustina Rodriguez

Institution: Umass Chan Medical School **Department:** Medicine

Poster Number: 54

Title: In vitro proteomics analysis of human immunodeficiency virus type 1 enhancer-binding protein 3 (Hivep3) in osteocytes

Co-Authors: Tadatoshi Sato

Abstract:
Background: Human Immunodeficiency Virus Type 1 Enhancer-binding Protein 3 (Hivep3) serves as a transcription factor pivotal in modulating the equilibrium between bone formation and resorption, thereby

contributing to the maintenance of physiological bone mass levels. Apart from its role in adult bone formation regulation, Hivep3 exerts a positive influence on the expression of interleukin-2 (IL2) within T-cells. Moreover, Hivep3 governs the expression patterns of numerous critical developmental genes. While Hivep3 demonstrates inhibitory effects on osteoblasts, its function within osteocytes remains incompletely elucidated, presenting an area warranting further investigation. Methods/results: This study utilized lentiviral eGFP overexpression and flag-tagged mouse Hivep3 expression vectors to establish stable Ocy454-eGFP (Ocy-eGFP) and stable Ocy454-Flag-Hivep3 (Ocy-fHivep3) osteocytic cell lines via puromycin selection. After the osteocyte differentiation culture at 37c for 2 weeks, Flag-Hivep3 protein expression was confirmed by western blotting and confocal microscopy (Figure 1A). Then, Ocy-eGFP and Ocy-fHivep3 cells were harvested with TNT buffer with proteinase inhibitor, NaF, vanadate, and DTT and isolated to the proteins. Those protein lysates were incubated with Flag-beads at 4c overnight and performed electrophoresis with 4-20% gradient and CBB staining (Figure 1B). Post-flag-IP samples were analyzed by mass spectrometry (MS). As a result, MS analyses detected 1529 peptides corresponding to 42 unique proteins in the eGFP sample and 4160 peptides corresponding to 498 unique proteins in the fHivep3 sample. Using 498 unique Hivep3 binding protein data sets, we performed the enrichment pathway analyses and found 273 KEGG pathways significantly enriched as Hivep3-related signaling/metabolism pathways such as proteasome, TCA cycle, glycolysis/gluconeogenesis. Conclusion: These findings suggested that Hivep3 in osteocytes might be involved in protein degradation/recycling and energy metabolism, contributing to bone formation through the osteocytes.

Presenter: Hirotaka Iijima

Institution: Harvard Medical School **Department:** Physical Medicine & Rehabilitation

Poster Number: 55

Title: Network medicine-based mechanistic dissection of chondroprotective effects of rehabilitation program via extracellular vesicles

Co-Authors: Kai Wang, Ella D'Amico, Wan-Yee Tang, Renee J. Rogers, John M Jakicic, Fabrisia Ambrosio

Abstract:
INTRODUCTION: Extracellular vesicles (EVs) have emerged as a potent mechanism through which the beneficial effects of rehabilitative exercise are transmitted throughout the body. Yet, the mechanisms by which beneficial information is transmitted from EVs to recipient cells are poorly understood, precluding a holistic understanding of how exercise promotes cellular and tissue health. As a first step to address this critical knowledge gap, using articular cartilage as a model, this study introduced a network medicine paradigm to simulate how exercise facilitates communication between circulating EVs and chondrocytes, the cells resident in articular cartilage.

METHODS: First, through a systematic review and subsequent bioinformatics, we identified 16 EV miRNAs significantly changed after an acute bout of aerobic exercise in elderly participants (GSE144627). Next, we sought to assess the biological impacts of the 16 EV miRNAs on articular cartilage. To simulate these effects, we implemented a network propagation approach to the miRNA regulatory cartilage-specific functional gene network. Finally, follow up experimental studies were designed to interrogate the direct influence of exercise-primed circulating EV on chondrocyte function. Circulating EVs were isolated from older adults ages 65-85 years old (n=10) before and after a 12 week aerobic exercise. The exercise protocol was performed for 5 days per week and consisted of a combination of 1 supervised and 4 home-based, non-supervised sessions per week, which progressed from 70 minutes per week to 150 minutes per week of moderate-intensity activity. Pre- and post-exercise EVs were then co-cultured with aged human chondrocytes seeded onto a substrate that mimics the matrix stiffness of aged human cartilage (100kPa).

RESULTS: Network medicine-based inference revealed that circulating EVs activated by aerobic exercise perturb chondrocyte-matrix interactions and downstream cellular aging processes, including “PI3K/Akt

signaling" and "TGF-beta signaling". Follow up experimental studies supported the findings from the computational analyses and found that pathogenic matrix signaling in chondrocytes was abrogated in the presence of exercise-primed EVs, restoring a more youthful cellular phenotype, as determined by chondrocyte morphological profiling and evaluation of chondrogenicity (i.e., increased Sox9 and type II collagen expression). Epigenetic reprogramming of the gene encoding the longevity protein, α-Klotho, mediated these effects, as evidenced by decreased Klotho promoter methylation level.

DISCUSSION/CONCLUSION: This study introduced a network paradigm approach to infer functional targets of exercise-primed EVs in recipient aged chondrocytes. The findings of this study provide mechanistic evidence that exercise transduces rejuvenation signals to circulating EVs, endowing EVs with the capacity to ameliorate cellular health even in the presence of unfavorable microenvironmental signals. While this study focused on articular cartilage, a major conceptual innovation of the proposed method is the demonstration of the mechanistic link between exercise-primed EVs and cellular aging using an integrated approach of network medicine and experimental verification. We anticipate that the network medicine-based paradigm introduced in this study may have broader implications in the field of rehabilitation medicine and aging research, extending beyond cartilage.

Presenter: Juliana Bergmann

Institution: Spaulding Rehabilitation Hospital **Department:** Physical Medicine & Rehabilitation

Poster Number: 56

Title: Light Exposure Induces Muscle Progenitor Cell Migration Post-Injury in vitro

Co-Authors: Gabrielle Gilmer, Kai Wang, Zachary Hettinger, Antonio Woollard, Fabrisia Ambrosio

Abstract:
Phototherapy has been utilized as a therapeutic to enhance regeneration and promote healing of acute injuries, but the underlying mechanisms are still poorly understood. One possible explanation for the beneficial effect of phototherapy is that it leverages tissue regenerative cascades that are mediated by an endogenously present photon emission mechanism at the site of injury. For example, it has been suggested that the photosensitive protein, Cryptochrome, in the retina of birds helps them geolocate through a quantum mechanism that alters the oscillations of a flavin-bound radical pair based on the Earth's magnetic fields. While there is evidence to support this quantum-based migration pattern at the level of an entire organism, to our knowledge, there have been no studies as to whether similar mechanisms direct cellular migration patterns of biological units (i.e., cells) within an organism. Here, we used muscle progenitor cells (MPC) as a model system to explore the potential positive effects of photon emission and application.

Young male and female MPCs were isolated for in vitro experiments, and a scratch wound assay was utilized to model injury. Photon emission was measured to test the hypothesis that ultralow photon emission occurs at the site of injury. After applying the scratch, there was a significant increase in photons emitted in all visible spectra both immediately after and 24 hours after injury. To determine the spectra of the emitted photons, band pass filters were used to limit the photons to a single color (red (710-730 nm), blue (437-457 nm), or green (520-540 nm)). Photon emission within the blue spectrum significantly increased immediately after injury, while green photons significantly increased 24 hours post-injury when compared to baseline.

Next, exogenous light of variable timing, intensity, and spectra was applied to MPCs post-scratch injury via light emitting diodes that were controlled by encoded Arduino computers. Scratch width (as a measure of wound closure) was analyzed via staining for F-actin (a cytoskeletal element), imaging, and using ImageJ analysis software to determine the distance between cells on opposite sides of the scratch. Low duration (1 second exposure/hour for 6 hours), low intensity (21.44 mW/m2), blue light exposure significantly decreased scratch width, while neither red nor green light had an effect.

Bulk RNA-sequencing (RNA seq) and mass spectrometry proteomics analyses were then performed to

explore fundamental molecular mechanisms driving MPCs response to light. In the context of injury, the RNA seq data showed that light-induced changes to gene expression involved the regulation of ions, especially calcium within the mitochondria and cytoplasm. When analyzing changes in protein expression through proteomics, light exposure alone upregulated proteins found in the RhoA/ROCK pathway. This suggests post-transcriptional modification of proteins involved in the RhoA/ROCK pathway, which is known for mediating stem cell migration.

Taken together, the data suggests that an unknown photoactive molecule within MPCs alters mitochondrial calcium channels. This change in calcium flux may lead to modifications of proteins associated with cell motility, including those involved in the RhoA/ROCK pathway. Next steps include validating the potential relationships derived here and identifying candidate molecules that could underlie a quantum-based model of cellular migration.

Presenter: Ramina Behzad

Institution: University of Massachusetts Dartmouth **Department:** Bioengineering

Poster Number: 57

Title: Impact of Irisin on Glycation in Bone Tissue and Cells

Co-Authors: Lamya Karim

Abstract:
Patients with T2D have normal to high bone mineral density—yet, paradoxically, they have increased risk for fracture. Thus, mechanisms underlying skeletal fragility are poorly understood, making it difficult to develop suitable tools for diagnosing, preventing, and treating fractures in these patients. T2D is associated with the accumulation of advanced glycation end-products (AGEs) by non-enzymatic glycation, which is a spontaneous reaction between amino acid residues on collagen and extracellular sugars that can be simulated in vitro. Increased AGEs contribute to numerous diabetic complications including poor eye health, cardiovascular issues, and kidney disease. AGE buildup can lead to poor bone quality, inhibit osteogenesis, and deteriorate bone mechanical integrity. Despite the harmful effects of AGEs, there is little information about AGE inhibition in human bone. Many inhibitors that went through pre-clinical and initial clinical testing resulted in harmful side-effects. Therefore, identifying natural compounds to use as an inhibitor is critical. One such inhibitor is the myokine irisin. It has been reported that irisin reduces apoptosis and alters gene expression in immature MLO-Y4 osteocytes [1], promotes osteogenesis in adipose-derived cells in presence of AGEs [2], and is present in low concentrations in patients with T2D [3]. Thus, we plan to test irisin to understand how it impacts bone tissue and mature osteocyte cell behavior in hyperglycemic environments. We hypothesize that the addition of irisin to a hyperglycemic environment will result in decreased gene and protein expression of dysfunctional turnover progress in osteocyte bone cells, and will improve mechanical properties of bone tissue. Results from this work will illuminate molecular- and cellular-level mechanisms of skeletal fragility in diabetes and help design corresponding studies in an animal model.

Presenter: Maria Sukhoplyasova

Institution: Massachusetts General Hospital **Department:** Endocrine Unit

Poster Number: 58

Title: The Relationship of Dietary Advanced Glycation End-products and Glycemic Control in Type 1 Diabetic Adults

Co-Authors: Ananya Murthy, BS, Grace Jung, BS, Brianna Gray, LDN, Enrico Cagliero, MD, Vanita Aroda, MD, Elaine Yu, MD

Abstract:
"Introduction:
Accumulation of advanced glycation end products (AGEs) are thought to play a crucial role in the pathophysiology of diabetic complications, yet the contributions of dietary AGE (dAGE) intake to systemic AGE levels and glycemic control have not been established. Utilizing a newly developed methodology for evaluating dAGEs, our objective was to test whether dAGE impact systemic AGEs and glycemic outcomes in adults with longstanding Type 1 Diabetes (T1D).
Methods:
We examined adults aged 50+ years participating in the T1D BEACON cohort (n=79 T1D, n=45 non-diabetic controls). We developed a novel algorithm to quantify dietary AGE intake by associating items from the 2014 Block Food Frequency Questionnaire with their AGE content (as determined by ELISA from a previously published dataset; Urribarri et al., 2010). Daily dAGE intake was normalized using the nutrient density method (Willett et al., 1997). Additional measurements included autofluorescent skin AGEs (AGE Reader, DiagnOptics Technologies), diabetic history, hemoglobin A1C and 14-day continuous glucose monitoring to assess percentage time in glucose target range (70-180 mg/dL). We compared dAGEs between T1D and controls and evaluated associations of dAGEs with outcomes using independent t-tests, Pearson's correlations, and multivariate linear regression with adjustments for age, sex, and body mass index (BMI).
Results:
Mean age and sex distribution were similar in both the T1D and control groups (63.48 ± 6.7 years, 52.4% female). However, BMI was higher in the T1D group compared to the control group (27.4 ± 5.0 vs 25.8 ± 5.0 vs. kg/m^2, p = 0.08). In the T1D cohort, the mean ± SD duration of diabetes was 46.2 ± 10.2 years, with an A1c of 7.0 ± 1.0%. Utilizing our novel algorithm, we found that the mean daily normalized dAGE intake was higher in the T1D group compared to the control group (8.8 ± 2.3 kU/kcal vs. 7.5 ± 2.5 kU/kcal, p = 0.002). However, this difference did not remain significant after multivariate adjustment (adj p = 0.224). Skin AGEs were also higher in the T1D group compared to the control group (3.0 vs 2.6 AU, adj p < 0.001). Within the full cohort, we observed a significant correlation between dAGE intake and skin AGEs (r = 0.32, adj p < 0.001), driven primarily by the association within the T1D group (r = 0.30, adj p = 0.007). Moreover, dAGE intake was associated with higher A1C values within the full cohort (r = 0.32, adj p = 0.001). There was also a trend indicating that higher dAGE intake correlated with decreased time in the target glucose range (r = -0.25, adj p = 0.07). Furthermore, we found a significant increase in skin AGEs among those with a history of at least one fracture, excluding face, fingers, and toes, compared to those without such a history (3.1 AU vs 2.8, adj p = 0.033) within the full cohort.
Conclusion:
Our analyses have revealed novel associations between dietary advanced glycation end product (dAGE) intake, skin AGEs, and glycemic outcomes in adults with type 1 diabetes. This highlights the significance of dietary patterns in influencing systemic AGEs and physiological outcomes within diabetic populations. Future studies should validate this novel dAGE assessment in external cohorts and explore the potential impact of dietary AGE intake on diabetic complications. Moreover, further research is necessary to fully understand the role of AGEs in bone health and fracture risk."

Presenter: Crystabella Nevarez

Institution: Massachusetts General Hospital **Department:** Endocrine Unit

Poster Number: 59

Title: The Relationship of Dietary Advanced Glycation End-products and Glycemic Control in Type 1 Diabetic Adults

Co-Authors: Ananya Murthy, BS, Grace Jung, BS, Brianna Gray, LDN, Enrico Cagliero, MD, Vanita Aroda, MD, Elaine Yu, MD

Abstract:
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Methods:
We examined adults aged 50+ years participating in the T1D BEACON cohort (n=79 T1D, n=45 non-diabetic controls). We developed a novel algorithm to quantify dietary AGE intake by associating items from the 2014 Block Food Frequency Questionnaire with their AGE content (as determined by ELISA from a previously published dataset; Urribarri et al., 2010). Daily dAGE intake was normalized using the nutrient density method (Willett et al., 1997). Additional measurements included autofluorescent skin AGEs (AGE Reader, DiagnOptics Technologies), diabetic history, hemoglobin A1C and 14-day continuous glucose monitoring to assess percentage time in glucose target range (70-180 mg/dL). We compared dAGEs between T1D and controls and evaluated associations of dAGEs with outcomes using independent t-tests, Pearson's correlations, and multivariate linear regression with adjustments for age, sex, and body mass index (BMI).
Results:
Mean age and sex distribution were similar in both the T1D and control groups (63.48 ± 6.7 years, 52.4% female). However, BMI was higher in the T1D group compared to the control group (27.4 ± 5.0 vs 25.8 ± 5.0 vs. kg/m^2, $p = 0.08$). In the T1D cohort, the mean ± SD duration of diabetes was 46.2 ± 10.2 years, with an A1c of 7.0 ± 1.0%. Utilizing our novel algorithm, we found that the mean daily normalized dAGE intake was higher in the T1D group compared to the control group (8.8 ± 2.3 kU/kcal vs. 7.5 ± 2.5 kU/kcal, $p = 0.002$). However, this difference did not remain significant after multivariate adjustment (adj $p = 0.224$). Skin AGEs were also higher in the T1D group compared to the control group (3.0 vs 2.6 AU, adj $p < 0.001$). Within the full cohort, we observed a significant correlation between dAGE intake and skin AGEs ($r = 0.32$, adj $p < 0.001$), driven primarily by the association within the T1D group ($r = 0.30$, adj $p = 0.007$). Moreover, dAGE intake was associated with higher A1C values within the full cohort ($r = 0.32$, adj $p = 0.001$). There was also a trend indicating that higher dAGE intake correlated with decreased time in the target glucose range ($r = -0.25$, adj $p = 0.07$). Furthermore, we found a significant increase in skin AGEs among those with a history of at least one fracture, excluding face, fingers, and toes, compared to those without such a history (3.1 AU vs 2.8, adj $p = 0.033$) within the full cohort.
Conclusion:
Our analyses have revealed novel associations between dietary advanced glycation end product (dAGE) intake, skin AGEs, and glycemic outcomes in adults with type 1 diabetes. This highlights the significance of dietary patterns in influencing systemic AGEs and physiological outcomes within diabetic populations. Future studies should validate this novel dAGE assessment in external cohorts and explore the potential impact of dietary AGE intake on diabetic complications. Moreover, further research is necessary to fully understand the role of AGEs in bone health and fracture risk."