PITTSBURGH CENTER FOR INTERDISCIPLINARY BONE AND MINERAL RESEARCH SYMPOSIUM

CROSS-TALK IN BONE BIOLOGY: BASIC SCIENCE AND CLINICAL IMPLICATIONS

May 3, 2023
University of Pittsburgh
University Club

University of Pittsburgh

Agenda

8:00am
Registration Opens/Poster Set Up
Breakfast available 8:00 - 10:00am

9:00 - 9:15am
Welcome and Opening Remarks
Shilpa Sant, PhD

9:15 - 10:30am
Session 1: Bone and Metabolic Disorders
Moderators: Pouneh K. Fazeli, MD, MPH and Kristen J. Koltun, PhD

9:15am
Deficiency of the Trps1 transcription factor impairs development of bone and dental mineralized tissues
Dobrawa Napierala, PhD

9:35am
Bone metabolism and the paradox of bone marrow fat in anorexia nervosa
Pouneh K. Fazeli, MD, MPH

9:55am
Human Performance Optimization and Musculoskeletal Injury Prevention: NMRL Research Portfolio on Bone Health
Brad Nindl, PhD

Short 5 minute Talks from Submitted Abstracts:

10:15 am
Effect of acute and chronic high load intensity resistance exercise on bone-related biomarkers in men and women
Kristen J. Koltun, PhD; Postdoctoral Associate

10: 21 am
The Association Between 24-Hour Activity with Incident Fracture Risk in the Osteoporotic Fractures in Men Study (MrOS)
Lauren Roe, PhD; ACSM-CEP Aging T32 Pre-Doctoral Fellow

10:30am - 10:45am
Coffee Break

10:45am - Noon
Session 2: Bone and Cancer
Moderators: Giuseppe Intini, DDS, PhD, and Deborah L. Galson, PhD

10:45am
Genomics and epigenomics of bone and soft tissue sarcomas
Ben Nacev, MD, PhD

11:05am
DNA damage and immunobiology in Ewing sarcoma
Kelly Bailey, MD, PhD

11:25am
Bone Targeted Agents and Breast Cancer Recurrence
Adam Brufsky, MD, PhD

Short 5 minute Talks from Submitted Abstracts

11:45am
Targeted Studies of Temporomandibular Joint Disorder (TMD):
A Study of Immune System Surveillance Evasion Mechanism(s) in Humans
Courtney Lucas, MS; Graduate Student

11:51am
Generation and characterization of a multifluorescent osteosarcoma mouse model
Taiana Leite, DDS, MS; Graduate Student

Noon - 1:15pm
Lunch

1:15pm - 2:30pm
Session 3: Regeneration and Functional Integration of Mineralized Tissues
Moderators: Fatima N. Syed-Picard, MSE, PhD, and Hang Lin, PhD

1:15pm
Mechanobiology of bone development and regeneration
Invited Speaker: Joel D. Boerckel, PhD

1:35pm
Synthetic biology and programmable organoids
Mo Ebrahimkhani, MD

1:55pm
Stem Cell versus TGFB-1 Mediated Repair of Segmental Boney Defects
Juan Taboas, PhD

Short 5 minute Talks from Submitted Abstracts

2:15pm
Elucidating the role of keratin 75 in enamel using Krt75tm1Der knock in mouse model
Brent Vasquez, MS; Graduate student

2:21pm
Integration of tooth root organoids with rodent mandibular bone.
Tia Calabrese, Graduate student

2:30pm - 2:45pm
Coffee Break

2:45pm - 3:45pm
Keynote Presentation: Translating Genetics of Skeletal Dysplasias into Therapy
Brendan Lee, MD, PhD

3:45pm - 4:30pm
Mentoring Sessions

Work/Life Balance
Moderated by Pouneh K. Fazeli, MD, MPH, and Alejandro Jose Almarza, PhD

Mentoring Malpractice
Moderated by Deborah Galson, PhD, and Shilpa Sant, PhD

Working with NASA
Moderated by Giuseppe Intini, DDS, PhD

Working at the Interface between Engineering and Biology
Moderated by Hang Lin, PhD, and Fatima Syed-Picard, MSE, PhD

4:30pm - 6:00pm
Poster Session and Reception

Symposium Co-Chairs

Giuseppe Intini, DDS, PhD
Associate Professor
Department of Periodontics and Preventive Dentistry
University of Pittsburgh School of Dental Medicine

Dr. Intini's research focuses on skeletal stem cells and of bone cancer stem cells. He uses genetic strategies and in vivo imaging to study the location and function of these cells and the molecular mechanisms that control their "stemness" in health and disease.

Shilpa Sant, PhD
Associate Professor
Department of Pharmaceutical Sciences and Bioengineering
University of Pittsburgh School of Pharmacy

Dr. Sant's Research group utilizes biomaterials and micro-/nanotechnology-based approaches to develop physiologically relevant three-dimensional microenvironments with the goal to elucidate how microenvironmental factors drive cellular behaviors in disease progression as well as in repair/regeneration.

Speakers

Kelly Bailey, MD, PhD
Assistant Professor
Department of Pediatrics
University of Pittsburgh School of Medicine

Dr. Kelly Bailey is a physician scientist in the Division of Pediatric Hematology and Oncology at the UPMC Children's hospital of Pittsburgh and an Assistant Professor of Pediatrics at the University of Pittsburgh School of Medicine. Dr. Bailey is the Co-Director of the Mario Lemieux Institute for Pediatric Cancer Research at UPMC Children's. Dr. Bailey received her MD and PhD (Cancer Cell Biology) from West Virginia University. She completed a pediatric residency integrated research program at The University of Michigan in 2013 followed by a fellowship in Pediatric Hematology and Oncology.

Dr. Bailey is a pediatric oncologist specializing in the clinical treatment and biology of bone sarcomas, with a focus on the adolescent primary bone tumor Ewing sarcoma. She directs a laboratory-based research program that focuses on understanding the intersection of DNA damage and immunobiology with the translational goal of discovering better treatment options for patients with advanced Ewing sarcoma. Dr. Bailey was awarded the UPMC Children's Resident Teaching award in both 2018 and 2022. Nationally, Dr. Bailey is a member of the Society for Pediatric Research. She holds leadership roles within the Children's Oncology Group (COG), currently heading the Localized Ewing sarcoma Task Force, a group of 30+ national bone tumor experts tasked with developing the next national clinical trial for localized Ewing sarcoma. Dr. Bailey is also national Vice Chair for the COG clinical trial AOST2121, examining a maintenance immunotherapeutic for the treatment of relapsed osteosarcoma.

Joel Boerckel, PhD
Assistant Professor
Departments of Orthopaedic Surgery and Bioengineering
University of Pennsylvania Perelman School of Medicine

Joel received all his degrees in Mechanical Engineering, getting his Ph.D. from the Georgia Institute of Technology in 2011. His graduate work in tissue engineering led to an interest in angiogenesis and postdoctoral training in vascular biology at the Cleveland Clinic. A serendipitous failure led him to developmental biology. He started

his lab at the University of Notre Dame in 2014, and was recruited to the University of Pennsylvania in 2017, where he is jointly appointed as Assistant Professor in the departments of Orthopaedic Surgery and Bioengineering. The Boerckel lab studies how mechanotransduction influences embryo development and postnatal regeneration in the skeleton and the vasculature. Joel is passionate about accelerating science dissemination and impact through preprints (e.g., bioRxiv) and building supportive scientific communities. Find him on twitter @jboerckel

Adam Brufsky, MD, PhD
Professor
Department of Medicine
University of Pittsburgh School of Medicine

Adam M. Brufsky, MD, PhD, is Professor of Medicine at the University of Pittsburgh School of Medicine. He serves as Co-Director, Comprehensive Breast Cancer Center and Medical Director, Women's Cancer Center at the UPMC Magee Women's Hospital, Pittsburgh, PA. He also serves as the Associate Director for Strategic Initiatives for the UPMC Hillman Cancer Center in Pittsburgh, Pa.

Dr. Brufsky received an in Chemistry (Cum Laude) from Dartmouth College in Hanover, New Hampshire. He earned his MD and his PhD in Developmental Biology at the University of Connecticut School of Medicine in Farmington, CT. He was an Intern and Resident in Internal Medicine at Brigham and Women's Hospital, Harvard Medical School, Boston, MA. He then completed a Fellowship in Medical Oncology and Bone Marrow

Transplantation and the Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, where his appointments included Associate Physician and Instructor in Medicine at Dana Farber Cancer Institute and Harvard Medical School in Boston, Mass.

Dr. Brufsky is board certified in Internal Medicine and Medical Oncology by the American Board of Internal Medicine. He is an active member of the American Society of Clinical Oncology and the American Association for Cancer Research. He has authored or coauthored more than 300 abstracts and research articles in leading journals, including the New England Journal of Medicine, Journal of Clinical Investigation, Journal of Clinical

Oncology, and Lancet Oncology. Dr. Brufsky is a Principal Investigator on a number of research grants funded by the National Institutes of Health, Susan G. Komen Foundation, and US Army Breast Cancer Research Program.

Mo Ebrahimkhani, MD
Associate Professor
Department of Pathology
University of Pittsburgh School of Medicine

Dr. Mo Ebrahimkhani is an Associate Professor in the Department of Pathology, School of Medicine, University of Pittsburgh. He is also a member of the Division of Experimental Pathology and the Pittsburgh Liver Research Center. Prior to his current position he was an assistant professor in the School of Biological and Health Systems Engineering at Arizona State University and adjunct faculty of medicine at Mayo Clinic. He performed his Postdoctoral training at the Department of Biological Engineering in Massachusetts Institute of Technology (MIT).

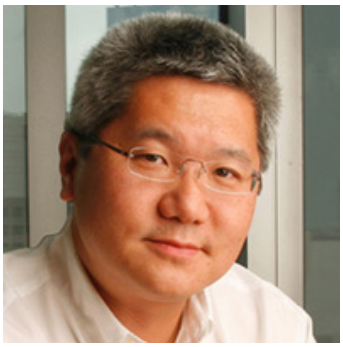
Dr. Ebrahimkhani has an MD degree from Tehran University of Medical Sciences and was awarded a European Association for Study of Liver Sheila Sherlock Fellowship to investigate regenerative processes at University College London. His lab combines human stem cells, synthetic biology and in vivo mouse models to understand tissue development and regeneration and develop technologies to modulate these processes in a personalized fashion. Dr. Ebrahimkhani is the recipient of several research awards including RO1s from NIH, Mayo Clinic accelerated regenerative medicine award and New Investigator Award from Arizona Biomedical Research Council. He is also a member of PLOS ONE Editorial Board (2018- present).

His lab research combines systems and synthetic biology-based approaches to program development of induced pluripotent stem cells across the developmental trajectories and towards human designer liver organoids and hematopoietic niches. This approach will open novel opportunities for next generation genomically engineered human tissues, personalized disease modeling and more effective regenerative therapies. His vision is to advance regenerative medicine through integrating systems and synthetic biology.

Pouneh Fazeli, MD, MPH
Associate Professor
Department of Medicine
University of Pittsburgh School of Medicine

Dr. Pouneh Fazeli is Director of the Neuroendocrinology Unit at the University of Pittsburgh School of Medicine and an Associate Professor of Medicine. She attended the Perelman School of Medicine at the University of Pennsylvania before completing a residency in internal medicine at Columbia and a fellowship in endocrinology at the Massachusetts General Hospital. She is a clinical/translational researcher whose research

program is focused on understanding neuroendocrine adaptations to undernutrition and fasting. She has performed clinical studies investigating therapies for the treatment of bone loss in women with anorexia nervosa, a psychiatric disease characterized by self-induced starvation, and is currently investigating the effects of transdermal estrogen on bone parameters in this population through an NIH-funded protocol. Clinically, Dr. Fazeli sees patients with pituitary disease in the Neuroendocrinology Clinic at the University of Pittsburgh Medical Center (UPMC) and is Medical Director of the Pituitary Center of Excellence at UPMC.



Brendan Lee, MD, PhD
Robert and Janice McNair Endowed Chair and Professor
Molecular and Human Genetics
Baylor College of Medicine

Dr. Brendan Lee is the Robert and Janice McNair Endowed Chair in Molecular and Human Genetics, Professor and Chairman of the Department of Molecular and Human Genetics at Baylor College of Medicine (BCM). Dr. Lee co-directs the joint MD Anderson Cancer Center, University of Texas Health, and BCM Lawrence Family Bone Disease Program of Texas, and the BCM Center for Skeletal Medicine and Biology. He is Founder and Director of the Skeletal Dysplasia Clinic at Texas Children's Hospital, and of the Medical Student Research Pathway at Baylor. As a pediatrician and geneticist, Dr. Lee studies structural birth defects and inborn errors of metabolism. He has published over 320 peer reviewed papers and over 80 invited reviews, chapters, and books. His work has garnered over $82M in continuous NIH funding over his 25 years as an independent investigator. He currently leads the NIH BCM Undiagnosed Diseases Network Clinical Site, NIH BCM RE-JOIN Consortium site, NIH Brittle Bone Disorders Consortium, and the NIH All of US Evenings with Genetics Education Program. He holds multiple patents in drug discovery and gene therapy and several licensed technologies are in industry-sponsored clinical trials (for osteoarthritis, Osteogenesis Imperfecta, and Maple Syrup Urine Disease).

Dr. Lee has received local, national, and international recognition including election to the National Academy of Medicine, Fellow of the American Association for the Advancement of Science, the Texas Academy of Medicine, Engineering, Science, and Technology (TAMEST), the Association of American Physicians, the American Society for Clinical Investigation, and the Society of Pediatric Research. He has also been awarded the American Society of Human Genetic Curt Stern Award for Outstanding Scientific Achievement, the American Society for Bone and Mineral Research William F. Neuman Award for outstanding contributions to science, teaching, research, and administration, the TAMEST Peter and Edith O'Donnell Award in Medicine, the Society for Pediatrics

Research E. Meade Johnson Award for Pediatrics Research, the Michael E. DeBakey Excellence in Research Award, the American Philosophical Society's Judson Darland Prize for Patient-Oriented Clinical Investigation, and Foreign Member, National Academy of Medical and Surgical Sciences in Napoli, Italy. He has served on multiple nonprofit boards and advisory panels including the Advisory Committee to the Director (ACD) of the NIH.

Dr. Lee was previously an Investigator of the Howard Hughes Medical Institute prior to becoming Chairman of the Department of Molecular and Human Genetics at Baylor College of Medicine in 2014. The Department is the leading genetics and genomics program in the world integrating basic/translational/clinical research, prenatal/pediatric/adult clinical service, and molecular pathology activities. His global engagement activities include the establishment of the joint Baylor College of Medicine-Chinese University of Hong Kong Center for Medical Genetics in Hong Kong.

Ben Nacev, MD, PhD
Assistant Professor
Department of Medicine
University of Pittsburgh School of Medicine

Dr. Nacev is a laboratory-based physician scientist with a focus on sarcoma medical oncology and chromatin dysregulation in cancer. He is an Assistant Professor in the Division of Hematology and Oncology in the Department of Medicine at the University of Pittsburgh. He is a Member of the UPMC Hillman Cancer Center where he directs a research lab focused on studying sarcoma-associated alterations in genes that regulate the epigenome.

Dr. Nacev received his MD and PhD degrees from the Johns Hopkins University School of Medicine where he studied under PhD mentor, Prof. Jun O. Liu in the Department of Pharmacology and Molecular Sciences. Dr. Nacev's doctoral thesis focused on drugs with repurposing potential as angiogenesis inhibitors, including itraconazole and cyclosporine. Dr. Nacev's work explored the mechanisms of this antiangiogenic activity, including work towards target identification.

Following doctoral training, Dr. Nacev joined the Osler Medical Residency where he received internal medicine training at the Johns Hopkins Hospital. He then went on to Medical Oncology fellowship at the Memorial Sloan Kettering Cancer Center where he served as co-Chief Fellow and later joined the clinical faculty. In parallel, Dr. Nacev completed postdoctoral research training at The Rockefeller University, studying under Prof. C. David Allis. During this time, Dr. Nacev's research focused on identifying and

understanding the mechanisms of a new class of cancer-associated histone mutations known as oncohistones. In 2022, Dr. Nacev joined the faculty at the University of Pittsburgh.

Dr. Nacev cares for adult patients with sarcomas in the medical oncology clinic, an experience which motivates and inspires his laboratory research program.

Dobrawa Napierala, PhD
Associate Professor
Department of Oral and Craniofacial Sciences
University of Pittsburgh School of Dental Medicine

Dr. Dobrawa Napierala is an Associate Professor, School of Dental Medicine, Center for Craniofacial Regeneration, University of Pittsburgh. Prior to her appointment to Pitt in 2016, Dr. Napierala held positions at the University of Alabama at Birmingham, Birmingham, AL (2011-2016), and Baylor College of Medicine, Houston, TX (2008-2011).

Dr. Napierala received an MSc, Biotechnology in 1995 from the A. Mickiewicz University, Poznan, Poland. In 1999 she received a PhD in Chemical Sciences/Biochemistry from the Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland. She completed her post-doctoral training in molecular biology and genetics from Baylor College of Medicine (1999-2008).

Dr. Napierala served as the president of the Mineralized Tissue Group of the International Association for Dental Research (2017-18). She was also a co-organizer of the 13th International Conference on the Chemistry and Biology of Mineralized Tissues (2019)

A list of her current research interests includes:

Molecular and cellular biology of the mineralization process.
Genetic diseases and animal models of skeletal and dental development and homeostasis.
Phosphate signaling in mineralizing tissues.
Regeneration and repair of mineralizing tissues.
Biogenesis of mineralization-competent extracellular vesicles.

Bradley Nindl, PhD
Professor
Department of Sports Medicine and Nutrition
University of Pittsburgh School of Health and Rehabilitation Sciences

Bradley C. Nindl, PhD, FACSM is the Director of the Neuromuscular Research Laboratory/Warrior Human Performance Research Center and tenured professor and Vice Chair for Research in the Department of Sports Medicine and Nutrition in the School of Health and Rehabilitation Sciences at the University of Pittsburgh. He also has dual appointments as the Senior Military and Scientific Advisor for the University's Center for Military Medicine Research and at the McGowan Institute for Regenerative Medicine and an adjunct professor in the Department of Military and Emergency Medicine at the Uniformed Services University of Health Sciences in Bethesda, MD.

Prior to coming to the University of Pittsburgh, Nindl worked for over 20 years in successive scientific leadership roles as an Army Medical Department government scientist working for the US Army Research Institute of Environmental Medicine (USARIEM) within the US Army Medical Research and Materiel Command (MRMC) from 1999-2011 and the Army Institute of Public Health (AIPH) within the US Army Public Health Command (USAPHC) from 2012-2015. He was the MRDC Research Task Area Manager for Physiological Mechanisms of Musculoskeletal Injury from 2008-2011. Nindl graduated from Philips Exeter Academy in 1985, received a Bachelor of Science in biology from Clarkson University in 1989, a Master of Science in physiology of exercise from Springfield College in 1993, a Doctor of Philosophy in physiology from The Pennsylvania State University in 1999 and a Master of Strategic Studies from the US Army War College in 2012.

His research interests span human performance optimization/injury prevention and biomarker domains with a focus on adaptations of the neuromuscular and endocrine systems (growth hormone/insulin-like growth factor-I axis) to both exercise and military operational stress. He is internationally recognized for his work in these areas and was Co-Chair of the 3rd International Congress on Soldiers' Physical Performance (ICSPP) in 2014 and has performed research sabbaticals at the University of Jyvaskyla in Finland (2009) and the University of Wollongong in Australia (2014) with the Finnish and Australian Defence forces, respectively. He is currently a principal investigator or co-invenstigator on funded studies addressing resiliency, biomarkers, physical and musculoskeletal readiness and training adaptations from the DoD (MRDC and ONR), NIH, NASA and the British Ministry of Defence.

Nindl's previous awards include the American College of Sports Medicine (ACSM) Young Investigator Award in 2002, NIH Biological Remodeling and Plasticity Young Investigator Travel Award in 2002, ACSM Exchange Lecturer for the American Orthopedic Society for Sports Medicine Annual meeting in 2006, Distinguished Visiting Professorship in Exercise Physiology at the University of Jyvaskyla, Finland in 2009, Australian DSTO Black Box Lecturer in 2014, US Army's Surgeon General "9A" Proficiency Designator (the Army Medical Department's (AMEDD) highest award for professional excellence, bestowed on less than 2% of AMEDD military officers) in 2013, Springfield College Peter V. Karpovich lecturer in 2018, Order of Military Medical Merit (O2M3) in 2019 and Texas American Sports Medicine Fall Lecturer in 2019. He is an associate editor for Journal of Science and Medicine in Sport and the Journal of Strength and Conditioning Research, editorial board member for Medicine and Science in Sports and Exercise and Growth Hormone and IGF-I Research and a Fellow in the American College of Sports Medicine.

He has over 175 peer-reviewed publications indexed on PubMed that have been cited over 5,000 times with an h-index of 41. He is also an Army Reserve Officer (COL) having continuous military service since 1991 when he enlisted and has served in staff and command positions at the platoon, company, battalion and brigade level. He is currently a Senior Research Fellow (IMA) at the Joint Special Operations University, Special Operations Command in Tampa, FL and served as the Brigade Commander of the Southeast Medical Area Readiness Group (SE-MARSG) in Nashville, TN under the Army Reserve Medical Command (AR-MEDCOM) from 2017-2019. He was deployed in 2004-2005 in Mosul, Iraq as an executive officer (XO) for a military transition team (MiTT) with the 98th DIV(IT) embedded with the 25th ID as an Iraqi Army advisor and was awarded a Bronze Star and the Combat Action Badge. He and his wife Jeanne have 5 children: Ashley, Lyndsey, Zachary, Joshua and Cooper.

Juan Taboas, PhD
Associate Professor
Department of Oral and Craniofacial Sciences
University of Pittsburgh School of Dental Medicine

Dr. Juan Taboas is an Associate Professor with the Department of Oral and Craniofacial Sciences in the School of Dental Medicine and the Department of Biomedical Engineering in the Swanson School of Engineering at the University of Pittsburgh. He is also a Professor in the Clinical and Translational Science Institute.

Dr. Taboas earned his undergraduate degree in Mechanical Engineering at the University of Florida and earned his master's in Engineering Science (Biomechanical Engineering) from Stanford University. He then entered the MS/PhD program at the University of

Michigan, where he earned a doctorate in Biomedical Engineering. He performed his postgraduate training at the National Institute of Standards and Technology (Polymers Division, NIST) and at the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS, NIH) before he came to Pittsburgh.

Dr. Taboas is a member of Tau Beta Pi, the Society for Biomaterials, the Orthopaedic Research Society (ORS), the Tissue Engineering International and Regenerative Medicine Society (TERMIS), and the American Association for Dental Research (AADR). He is a grant reviewer for the National Institutes of Health (NIH) and the Department of Defense (DoD), and he reviews manuscripts for several journals, including Acta Biomaterialia, Biomedical Materials, PLOS One, and The Journal of Dental Research.

Dr. Taboas' research seeks to regenerate craniofacial and skeletal tissues. His laboratory focuses on regenerating bone and tissues that interface with bone, such as the pulp of teeth and the cartilages of the growth plate and mandibular condyle. They have developed scaffold biomaterials, drug delivery systems, and live-cell imaging methods to study the biology and regeneration of these tissues. With these tools, they can control stem cell differentiation, tissue formation and endochondral ossification. The work is funded by the NIH and DoD.

Moderators

Pouneh Fazeli, MD, MPH
Associate Professor
Department of Medicine
University of Pittsburgh School of Medicine

Dr. Pouneh Fazeli is Director of the Neuroendocrinology Unit at the University of Pittsburgh School of Medicine and an Associate Professor of Medicine. She attended the Perelman School of Medicine at the University of Pennsylvania before completing a residency in internal medicine at Columbia and a fellowship in endocrinology at the Massachusetts General Hospital. She is a clinical/translational researcher whose research program is focused on understanding neuroendocrine adaptations to undernutrition and fasting. She has performed clinical studies investigating therapies for the treatment of bone loss in women with anorexia nervosa, a psychiatric disease characterized by self-induced starvation, and is currently investigating the effects of transdermal estrogen on bone parameters in this population through an NIH-funded protocol. Clinically, Dr. Fazeli sees patients with pituitary disease in the Neuroendocrinology Clinic at the University of Pittsburgh Medical Center (UPMC) and is Medical Director of the Pituitary Center of Excellence at UPMC.

Deborah L. Galson, PhD
Associate Professor
Department of Medicine
University of Pittsburgh School of Medicine

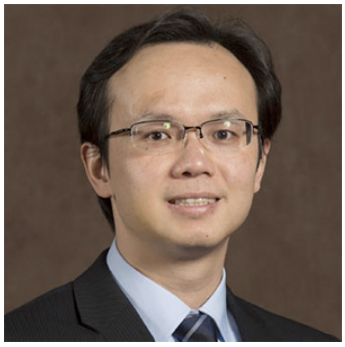
The Galson lab research has two main themes: (1) The molecular mechanisms driving aberrant osteoclasts in Paget's disease of bone. (2) Cancer-Bone interactions with a focus on Myeloma bone disease – communication between cancer cells and both osteoclasts and osteoblasts.

Giuseppe Intini, DDS, PhD
Associate Professor
Department of Periodontics and Preventive Dentistry
University of Pittsburgh School of Dental Medicine

Dr. Intini's research focuses on skeletal stem cells and of bone cancer stem cells. He uses genetic strategies and in vivo imaging to study the location and function of these cells and the molecular mechanisms that control their "stemness" in health and disease.

Kristen J. Koltun, PhD
Postdoctoral Associate
Neuromuscular Research Lab
Department of Sports Medicine and Nutrition
University of Pittsburgh School of Health and Rehabilitation Sciences

Dr. Koltun's current research focuses on investigating sex-differences in the physiological mechanisms underlying bone adaptation to physical activity in exercising and military populations.



Hang Lin, PhD
Associate Professor
Department of Orthopaedic Surgery
University of Pittsburgh School of Medicine

Fatima Syed Picard, MSE, PhD
Assistant Professor
Department of Oral Biology
University of Pittsburgh School of Dental Medicine

POSTER #1

Phosphate (P_i) changes protein-protein interactions between transcription factors Osterix and Trps1 in mineralization-competent cells

Socorro, Mairobys[1]; Keskinidis, Paulina[1]; Hoskere, Priyanka[1]; Fujikawa, Kaoru[1]; Roberts, Catherine[1]; Lukashova, Lyudmila[1]; Verdelis, Kostas[1]; Napierala, Dobrawa[1]

[1] Department of Oral and Craniofacial Sciences, University of Pittsburgh School of Dental Medicine, Pittsburgh, PA

Objectives: Transcription factors (TFs) function in complexes with other components of molecular machinery controlling gene expression. These complexes are dynamic structures, which composition, hence the functional outcomes, change depending on the cell developmental stage and in response to extracellular stimuli. Trps1 and Osterix (Osx/Sp7) are TFs co-expressed in the same cells during skeletal and dental development, regulate formation of mineralized extracellular matrix (ECM), and share at least one common target gene (*Bglap*). Hence, we hypothesized that Trps1 and Osx/Sp7 interact at the protein-protein level, and that this interaction is regulated by availability of P_i—a signaling molecule that stimulates formation of mineralized ECM. Additionally, we analyzed consequences of osteoblast-specific *Trps1* deficiency on the expression of Osx/Sp7.

Methods: Trps1-Osx/Sp7 interaction were analyzed in bones and teeth of newborn (P0) mice using the proximity ligation assay (PLA), and in 17llA11 osteogenic cells using co-immunoprecipitation (co-IP). Effect of P_i on the formation of the Trps1-Osx/Sp7 complex was analyzed in 17IIA11 cells under physiological (1mM P_i) and hyperphosphatemic (5mM P_i) conditions. We analyzed long bones of 4wk old wildtype and *2.3kbCol1a1-Cre^{ERT2};Trps1$^{fl/fl}$* (aka *Trps1^{Col1a1}*cKO mice) by microCT, histology and IHC. Expression of Sp7/Osx in 17IIA11 *Trps1*-KD cells was analyzed by qPCR.

Results: Analyses of *Trps1^{Col1a1}* cKO long bones revealed the importance of osteoblast-expressed Trps1 for bone mass accrual and expression of Osx/Sp7. Furthermore, our analyses uncovered that Trps1 and Osx/Sp7 form a molecular complex in osteoblasts lining the alveolar bone, and in developing odontoblasts. Interestingly, the co-IP assay confirmed our in situ results and revealed that Trps1-Osx/Sp7 interaction was diminished within 1h of exposing cells to the hyperphosphatemic conditions.

Conclusion: We identified that Trps1 and Osx/Sp7 TFs directly interact at the protein level in osteoblasts and odontoblasts, which suggest that their target genes are differently regulated when only one of these TFs is present. Our data suggests that availability of P_i regulates the activity of osteogenic TFs, which provides some mechanistic insights into mechanisms of abnormal mineralization in phosphate homeostasis disorders. Moreover, results of this study provide insight on interaction between extracellular stimuli and transcriptional machinery in skeletal and dental tissues development, homeostasis, and disease.

POSTER #2

Effect of acute and chronic high load intensity resistance exercise on bone-related biomarkers in men and women

Kristen J. Koltun[1], Adam J. Sterczala[1], Nicole M. Sekel[1], Kellen T. Krajewski[1], Brian J. Martin[1], Shawn Flanagan[1], Chris Connaboy[1], Sophie L. Wardle[2], Thomas J. O'Leary[2], Julie P. Greeves[2], Bradley C. Nindl[2]

[1]Neuromuscular Research Laboratory/Warrior Human Performance Research Center, Department of Sports Medicine and Nutrition, University of Pittsburgh, Pittsburgh, PA
[2]Army Health and Performance Research, Army Headquarters, UK

Weight-bearing physical activity can stimulate bone adaptation directly via mechanotransduction by bone cells and indirectly via changes in the hormonal milieu. This investigation explored the effect of an acute bout of resistance exercise, and the potential influence of sex and training status, on biomarkers of bone metabolism and muscle-bone crosstalk. Healthy young men (n=21, 29±1y, 26.6±1.0 kg/m^2) and women (n=18, 27±1y, 24.1±0.08 kg/m^2) performed a 6 setx10 repetition squat test (75% 1RM) before and after a 12-week, laboratory controlled, military-specific, resistance and high-intensity interval training program (3 day/week, 4 mesocycles, linearly progressive intensity/mesocycle to improve peak force and power). Before and after completion of the 12-week training program, blood samples collected at rest, immediately following exercise, and 2 hours post-exercise were measured for βCTX, P1NP, sclerostin, osteocalcin, IGF-1, and irisin. Generalized linear mixed effects models tested the effects of sex (male, female), training status (Baseline, 12wk), and exercise (rest, post, recovery), and their interactions, on biomarker concentrations (α=0.05). Main effects of acute exercise (p≤0.002) were observed for IGF-1 (+12%), irisin (+21%), osteocalcin (+27%), and P1NP (+8%); concentrations increased immediately following exercise and returned to resting concentrations within 2 hours of recovery. Sex*exercise interactions were observed for βCTX and sclerostin (p≤0.020); men had a greater decline in βCTX concentration from rest to recovery (-37 vs. -26%) and a greater increase in sclerostin concentration from rest to post-exercise (+53 vs. +38%) than women. Main effects of sex were observed for irisin and P1NP (p≤0.002); men had lower irisin (7.38 vs. 10.41 ug/mL) but greater P1NP (66.9 vs. 54.279 pg/mL) concentrations than women. Osteocalcin was greater after completion of the 12-week program than before (main effect: p=0.021; 22657.5 vs. 20872.1 pg/mL). A significant training*time interaction was observed for sclerostin (p=0.026); concentrations increased following acute exercise at both timepoints (37-56%), but only remained elevated into recovery pre-training and retuned to resting values in recovery following training. Changes in concentrations of biomarkers of bone metabolism and muscle-bone crosstalk, which may promote adaptive bone formation, were observed in men and women after an acute bout of resistance exercise and following 12 weeks of resistance training.

POSTER #3

Assessing the impact of melatonin alone or combined with micronutrients on the relationship between markers of inflammation and bone in peri- and postmenopausal women with or without osteopenia

Afsana Jahna[1], Michelle Han[1], Lauren O'Donnell[1], Holly Lassila[2], Paula Witt-Enderby[1]

[1] Division of Pharmaceutical, Administrative and Social Sciences, [2] Division of Pharmacy Practice Duquesne University School of Pharmacy

Background: In the Melatonin Osteoporosis Prevention Study (MOPS; NCT01152580), melatonin (3mg, po, 6 mos, nightly) restored bone marker (NTx:Osteocalcin) ratios back to equilibrium in perimenopausal women. In the Melatonin-micronutrients Osteopenia Treatment Study (MOTS; NCT01870115) RCT, melatonin (5mg) added in combination with strontium citrate (450mg), vitamin D3 (2000IU), and vitamin K2 (60mcg; MSDK) improved BMD, P1NP levels; reduced bone turnover; and decreased CRP levels in postmenopausal women with osteopenia. As inflammation is a critical driver for osteoclast activation, melatonin, through its anti-inflammatory actions, may be attenuating osteoclast-mediated bone resorption protecting against bone loss.

Objective: To determine the impact of melatonin alone or in combination with micronutrients on the relationship between inflammatory markers, CRP, TNFα, IL-6 and bone markers (OC, NTx, CTx, P1NP) and BMD.

Methods: Serum levels of CRP, TNFα, and IL-6 collected from the MOPS and MOTS were assessed by ELISA and then correlated with bone markers and BMD. Pearson's correlation coefficient, *R,* determined the degree of correlation.

Results: In the MOTS, CRP inversely correlated with BMD for femoral neck (R=-0.73), hip (R= -0.54) and vertebra (R= -0.16) in women taking placebo, which was reversed or lost in the MSDK group [CRP vs femoral neck (R=0.47); vertebra (R=0.47); hip (R=0.006)]. These correlations between CRP and BMD were paralleled by similar and expected correlations between the bone markers, CTx, OC and P1NP (i.e., positive correlations occurred between CRP and CTx or OC and negative correlations to P1NP in placebo, which were lost or lessened in MSDK groups). For TNFα, positive correlations were observed for CTx regardless of treatment [TNFa vs CTx: placebo (R=0.43) and MSDK (R=0.50)]. In the MOPS, positive correlations between CRP and NTx (R=0.72) or (OC 0.81) were observed for placebo, which were lost in the melatonin group [CRP and NTx (R=-0.01) or OC (-0.14)]. For both RCTs, CRP and TNFα demonstrated positive correlations in placebo groups, which remained in women taking MSDK but reversed in women taking melatonin. Melatonin and MSDK affect CRP and TNFa uniquely suggesting distinct mechanisms. CRP, a liver-derived inflammatory marker, may be targeted by melatonin to lower CRP levels attenuating NTx- or CTx-mediated bone loss.

POSTER #4

Precision Druggability of the PTH type 1 Receptor

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G protein-coupled receptors (GPCRs) for peptide hormones are notoriously difficult to target by small molecules because their large orthosteric peptide-binding pocket embedded deep within the transmembrane domain limits the identification and development of non-peptide small molecule ligands. Using the parathyroid hormone type 1 receptor (PTHR) as a prototypic class B GPCR target, and a combination of molecular dynamics simulations and elastic network model-based methods, we demonstrate that PTHR druggability can be effectively addressed. Here we found a key mechanical site that modulates the collective dynamics of the receptor and used this ensemble of PTHR conformers to identify selective small molecules with strong negative allosteric and biased properties for PTHR signaling in cell and PTH actions in vivo. This study provides a computational pipeline to detect precise druggable sites and identify allosteric modulators in PTHR signaling that could be extended broadly to GPCRs to expedite discoveries of small molecules toward the treatment of diverse hormonal and metabolic diseases.

POSTER #5

Tissue chip for assessing the influences of potential disease-modifying osteoarthritis drugs (DMOADs) on subchondral bone

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Introduction: Osteoarthritis (OA) is a painful and disabling joint disease affecting millions worldwide. The lack of clinically relevant models limits our ability to predict therapeutic outcomes prior to clinical trials where most drugs fail. Therefore, there is a need for a model that accurately recapitulates the whole joint disease nature of OA in humans. The emerging microphysiological systems provide a new opportunity. The Lin Lab recently established a miniature knee joint system, known as the miniJoint, in which human bone marrow-derived mesenchymal stem cells (hBMSCs) were used to create an osteochondral complex, synovial-like fibrous tissue, and adipose tissue analogs. The utility of the miniJoint system is especially necessary for assessing the efficacy of DMOADs for both bone and cartilage regeneration during OA.

Methods: HBMSCs were suspended in 15% methacrylated gelatin (GelMA) and placed in an insert for the differentiation into an osteochondral tissue. Following 4 weeks of individual culture the osteochondral tissue was co-cultured with a synovial-like fibrous and adipose tissue analogs for an additional 4 weeks. A "synovitis"-relevant OA model in the miniJoint was procured by treating synovial-like tissues with interleukin-1β (IL-1β), and then a combined treatment of oligodeoxynucleotides (ODNs) suppressing the nuclear factor kappa beta (NF-κB) genetic pathway and bone morphogenic protein- 7 (BMP-7) was introduced.

Results: Here we employ a combination of therapeutics to assess alterations in the osteochondral tissue after osteoarthritis induction. We specifically focus on bone and cartilage regeneration and a reduction in inflammatory markers associated with OA. The combined treatment with BMP-7 and ODN reduced inflammation in the bone, cartilage, and synovial-like fibrous tissue and the bone portion of the tissue showed an increase in bone markers such as alkaline phosphatase, osteopontin, and osteocalcin. Furthermore, cartilage matrix proteins were upregulated such as aggrecan and collagen type II.

Conclusion: For the first time, this study demonstrated the potential of the miniJoint in developing disease-modifying OA drugs. The therapeutic efficacy of co-treatment of NF-κB ODNs and BMP-7 can be further validated in future clinical studies.

POSTER #6

One carbon metabolite as candidate regulators of bone loss during fasting.

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Aim: Fasting may have beneficial effects on longevity, as has been demonstrated in non-human models. However, a known detrimental effect associated with fasting is loss of bone mass. Our aim was to identify novel metabolic regulators of fasting-associated bone loss.

Methods: Bone microarchitecture measured by high resolution peripheral quantitative CT (HR-pQCT), bone turnover markers (ELISA), and metabolites (mass spectrometry) were measured longitudinally during a 10-day, 0-calorie fast in healthy humans [N=12, median age: 29.3 years (21.8-48.3), 8 premenopausal females]. Metabolic candidates of bone loss were further investigated using in vivo and in vitro models.

Results: After a 10-day fast, trabecular number (Tb.N) and bone volume (BV/TV), as measured by HR-pQCT, significantly decreased (Tb.N:-9.2%+7.9 (mean+SD), p=0.02; BV/TV:-0.7%+0.7, p<0.05), whereas trabecular thickness (Tb.Th) and separation (Tb.Sp) significantly increased (Tb.Th:10.3%+8.7, p=0.02; Tb.Sp:11.0%+9.4, p=0.02) at the radius. After 5 days of fasting, P1NP significantly decreased compared to baseline (-55%, p<0.05). We then conducted a pathway analysis to identify metabolite signaling pathways regulating bone loss. The strongest correlates included one carbon metabolism. Indeed, multiple one carbon metabolites, including methionine, demonstrated a similar temporal pattern of dynamic increase with fasting and provided rationale for a new hypothesis - one or more one carbon metabolites impair bone formation. To test this, we conducted an in vivo experiment with 3 groups: control; methionine excess (60mg/day); and fasting (24h). After 5 days of intervention, transcription of genes related to bone metabolism, including Igf-1 and Ocn, significantly decreased in femurs of mice treated with excess methionine compared to controls (p<0.05); fasted mice followed the same pattern as excess methionine mice. To test whether excess methionine may induce negative effects on osteoblasts, we investigated effects of excess methionine on osteoblast activity. There was reduced gene expression of genes related to bone formation (P1NP, ALPL) and osteoblast function (OCN, OPG, ATF4, RUNX2) following treatment with excess methionine (100 and 500 µmol). Consistent with gene expression changes, excess methionine attenuated RUNX2 and ATF4 protein levels.

Conclusions: Excess methionine induces transcriptional changes associated with negative effects on osteoblast function. As methionine levels increase with fasting, methionine may have a role in mediating fasting-associated bone loss.

POSTER #7

The Association Between 24-Hour Activity with Incident Fracture Risk in the Osteoporotic Fractures in Men Study (MrOS)

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Lower physical activity (PA), sleep, and higher sedentary behavior (SB) are individually associated with fractures. Time spent in these behaviors is inherently linked by the confined 24-hr day, but previous analyses examining one activity often have omitted information about all other activities. We evaluated the combined role of PA, SB, and sleep on incident fracture risk using compositional data analysis. Men (N=2,910) attending the Year 7 (2007-2009) MrOS visit (mean age 79.0±5.1 yrs) with accelerometry data (SenseWear® Pro3 Armband) were included in this analysis. PA (>1.5 Metabolic Equivalents of the Task; METs), SB (<1.5 METS) and sleep at night were measured over an average of 5.1±0.3 days. The 24-hr activity compositions were represented as isometric log ratios (ILR). ILRs were predictors in Cox Proportional Hazards regression models to estimate the risk of incident clinical fracture by the proportion of time in one activity relative to all other activities simultaneously. Self-reported fractures were confirmed with radiographic reports. During an average follow-up of 8.7±4.4 yrs, 669 men had fractures. In minimally adjusted models (age, race, clinic, season of accelerometer wear), more SB at the expense of sleep and PA was associated with greater fracture risk (HR: 1.53, 95% CI: 1.12, 2.07), and more sleep at the expense of PA and SB was associated with lower fracture risk (HR: 0.71, 95% CI: 0.52, 0.97). Further adjustment for height, weight, diabetes, health status, smoking, comorbidities, and impaired instrumental activities of daily living, did not alter associations (sedentary vs. PA/sleep - HR: 1.54, 95% CI: 1.14, 2.08; sleep vs. PA/SB - HR: 0.68, 95% CI: 0.50, 0.92). In contrast, no association between PA vs. sleep/SB existed with fractures in minimally or fully adjusted models. In conclusion, when accounting for the inherent co-dependence of daily activities, men with higher proportions of sleep relative to the proportion of PA and SB had a lower risk of incident fractures, while men with higher proportions of SB relative to the proportion of PA and sleep had an increased risk of fractures. Future studies could evaluate if fracture risk is lowered by increasing PA or sleep at the expense of SB.

POSTER #8

Withdrawn

POSTER #9

Force plate leg power and velocity were associated with the risk of Major Osteoporotic Fracture (MOF): the Osteoporotic Fracture in Men Study (MrOS)

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Previous studies have shown that muscle function is associated with risk of falls and fractures, however, most studies have examined strength(force), but not power (force*velocity). A novel jump test on a force plate measures weight-bearing leg power and allows the dissection of peak power into its components, force and velocity. We investigated the association between jump test leg power measures and MOF risk in men from the MrOS study. The study included 1841 older men (median age 84 years, range 77-101 years) who completed the Year 14 visit. Peak jump power (Watt/kg body weight), force (Newton/kg body weight) at peak power, and velocity (m/s) at peak power were measured by jump tests on a force plate at Year 14 visit. Participants were followed for up to 5 years. Fractures were identified by triannual questionnaires, with all fractures confirmed by medical record. Cox proportional hazards models were used to estimate hazard ratios (HR) and 95% Confidence Intervals (CIs) of MOFs. 599/1841 (33%) men were not able to complete the jump test mainly due to low function. 265/1841 (14%) men had an incident clinical fracture within 5 years, of which 136 (51%) were MOFs. In the fully adjusted Cox regression model, one SD increment of peak power/kg body weight was associated with 29% reduced hazard of MOF (HR 0.71, 95% CI 0.54-0.92). Similarly, one SD increment of velocity at peak power was associated with 24% reduced hazard of MOF (HR 0.76, 95%CI:0.59-0.98). Further adjustment of femoral neck (FN) bone mineral density (BMD) and fall history did not attenuate the association. Compared to the referent group, the fourth (highest) quartile of peak power and velocity were also significantly associated with decreased risk of MOF, P=0.003 and P=0.025 for trend, respectively. Only highest quartile of force at peak power was significantly associated with risk of MOF (P for trend not significant). Peak leg power and velocity, but not force, during a force plate jump test predicted MOF risk independent of FN BMD. Power, which incorporates velocity, may play a more important role than force in reducing the risk of a fracture.

POSTER #10

Reduced CD73 activity and FOXO1 methylation in medial arterial calcification.

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Background: Arterial calcification due to deficiency of CD73 (ACDC) is a rare genetic disease causing medial arterial calcification (MAC) in lower-extremity arteries. ACDC is caused by inactivating mutations in the gene encoding the CD73 enzyme, which converts extracellular AMP to adenosine. We previously reported that reduced CD73 levels are found in non-genetic common forms of MAC, suggesting that CD73 is critical for peripheral vascular health. Adenosine can signal via binding to adenosine receptors or can enter into the cell via transporter proteins. Intracellular adenosine levels regulate the methionine cycle which is essential for methyltransferase protein activity. Methyltransferases metabolize the methyl-donor, S-adenosyl-L-methionine (SAM) to S-adenohomocystine (SAH), and elevated levels of SAH feed back to inhibit methyltransferase activity. Arginine methylation is a common post-translational modification where a methyl group is added onto arginine residues of a protein. Arginine methylation is catalyzed by protein arginine methyltransferases (PRMT) which transfer methyl group from SAM to the arginine residues. Reduced adenosine may enhance PRMT activity and the transfer of methyl group to an acceptor molecules. Methylation of FOXO1 transcription at specific arginine residues can promote its nuclear localization. We found that in CD73-deficient cells, FOXO1 drives the expression of the osteogenic gene ALPL.

Hypothesis: The reduction of adenosine production increases PRMT activity to induce FOXO1 nuclear localization via arginine methylation.

Methods: Western blot was used to quantify the total levels of arginine methylation in control and CD73-deficient smooth muscle cells (SMCs) at baseline and after exogenous adenosine and AMP treatment. To investigate how reduced CD73 promotes FOXO1 localization in ACDC patient fibroblasts, we assessed by immunofluorescence whether blocking a protein arginine methyltransferase activity altered FOXO1 nuclear localization.

Results: Our preliminary data shows that global arginine methylation levels are elevated in CD73-deficient SMCs compared to wild-type cells, suggesting elevated PRMT activity. In the absence of CD73, exogenous AMP promoted the nuclear localization of FOXO1, and inhibition of PRMT5 activity inhibits FOXO1 nuclear localization.

Conclusion: Our data suggest the lack of CD73-mediated adenosine production regulates FOXO1 via promoting arginine methylation.

POSTER #11

Bone Mineral Density, Microarchitecture, and Biomarker Responses to 12-weeks of Concurrent Exercise Training in Young Men and Women

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Understanding sex-specific bone adaptations to arduous training may yield insight into stress fracture susceptibility and ultimately reduce musculoskeletal injuries in the military.

PURPOSE: To examine sex-specific adaptations in bone microarchitecture, body composition and turnover markers in recreationally active and military-aged men and women undergoing a 12-week, militarily relevant strength and high-intensity interval training program.

METHODS: Recreationally active men (n=21, 29 ± 1 y, 1.78 ± 0.08 m, 84.3 ± 3.0 kg, 24.77 ± 7.87 % BF) and women (n=18, 27 ± 1 y, 1.64 ± 0.06 m, 65.0 ± 2.0 kg, 30.58 ± 6.83 % BF) completed the training program. Total body areal BMD and composition (Lunar iDXA, GE Healthcare), volumetric bone density (vBMD) and bone strength at the tibial metaphysis (4% site) and tibial diaphysis (30% site) (high-resolution peripheral quantitative computed tomography (HRpQCT), XtremeCTII, Scanco Medical AG) and biochemical markers of bone metabolism (βCTX and P1NP) from fasted venous blood samples were measured before and after training via immunoassays. All outcomes were assessed with 2 x 2 (sex [male vs female] x time [pre vs post training]) mixed-measures ANCOVAs and reported as main effects. Bone analyses were controlled for change in total mass as alterations in body mass are known to illicit adaptive skeletal responses.[1,2]

RESULTS: Training increased total body ($p = .008$) and trunk aBMD ($p < .001$), total body lean mass ($p = .001$), leg lean mass ($p < .001$), arm lean mass ($p < .001$), and trunk lean mass ($p = .001$) and decreased regional fat percentage for the total body ($p = .027$), arms ($p = .017$), and legs ($p = .007$) in men and women. No training-induced changes were observed in vBMD, bone strength or bone biomarkers. Basal βCTX and P1NP exhibited main effects of sex ($p < .025$) such that men exhibited greater markers of bone turnover than women.

CONCLUSION: The bone and body composition findings seem to indicate that men and women respond similarly to military relevant training. Although the training program was insufficient to induce significant tibial adaption, these findings demonstrate that it is possible to improve bone mineral density and body composition without significantly stressing the tibia.

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POSTER #12

Myocardin-related transcription factor promotes osteoclast differentiation and bone colonization of breast cancer cells

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Bone is a frequent site for breast cancer metastasis. Conditioning of local tumor microenvironment through crosstalk between tumor cells and stromal cells in the metastatic niche is a major driving force for bone colonization of cancer cells. The objective of the present study was to determine the role of Myocardin-related transcription factor (MRTF - a major cofactor for the transcription factor serum-response factor, SRF) in bone colonization of breast cancer cells. We found that stable suppression of MRTF isoforms (MRTF-A and MRTF-B) dramatically impairs bone colonization of breast cancer cells in experimental metastasis assay, a finding that is recapitulated in the setting of pharmacological inhibition of MRTF. Our in vivo studies further demonstrated that MRTF's interaction with SRF is absolutely critical for metastatic colonization of breast cancer cells. To gain mechanistic understanding, we next performed RNA-sequencing analyses of 3D cultures of breast cancer cells under genetic and pharmacological perturbations of MRTF, and compared differentially expressed genes in these settings against a 21-gene signature specifically associated with bone metastasis of breast cancer cells. These studies followed by confirmatory qRT-PCR and immunoblot analyses identified connective tissue growth factor (CTGF), a cell-secreted factor known for promoting osteoclastic differentiation/activity and osteolytic lesions by tumor cells, to be transcriptionally upregulated by MRTF in an SRF-dependent manner. These findings were further supported by bioinformatic analyses-based confirmation for MRTF's positive association with CTGF expression in human breast cancer samples, and differential expression of CTGF between isogenic pairs of bone-colonization-competent vs incompetent breast cancer cell lines. Indirect co-culture studies demonstrated that loss of MRTF expression inhibits osteoclast differentiation of primary bone-marrow derived monocytes in a paracrine signaling manner. Based on these findings, we conclude that MRTF inhibition could be a novel strategy to suppress osteoclastic activity and skeletal involvement in metastatic breast cancer.

POSTER #13

Generation and characterization of a multifluorescent osteosarcoma mouse model

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BACKGROUND: Despite marked evolution in treatment, the 5-year survival rate for metastatic osteosarcoma (OS) remains as low as 20%. To study OS, various mouse models have been introduced, most commonly xenograft models. However, their use is limited by several factors, including the need to use immunosuppressed mice and cell lines that have suffered mutations throughout the years. Recently developed genetically-engineered mice can overcome these disadvantages, by means of Cre-mediated conditional inactivation of tumor suppressor genes in cells of the osteoblastic lineage. However, these new mouse models exhibit two significant limitations: first, since they constitutively inactivate tumor suppressor genes during embryogenesis, they simultaneously develop OS and other forms of more undifferentiated sarcomas; second, they cannot be utilized to trace the tumor cells during tumor development.

MATERIALS AND METHODS: To overcome these barriers, we have developed a novel mouse model of OS, utilizing a tamoxifen-inducible Prx1-CreER-EGFP transgene to inactivate tumor suppressors p53 and Rb1 post-natally, in osteoprogenitor cells that express EGFP. In addition, to track cells of the osteoblastic lineage during tumor development, we included transgenes expressing the fluorescent mCherry and cyan proteins under the Osx and Col1 promoters, respectively. Tumor and metastasis development were radiographically monitored and cell expression of the various fluorochromes was evaluated by traditional and intravital fluorescent microscopy, as well as by in vivo flow cytometry.

RESULTS: Similar to what is observed in humans, 89% of the mice developed OS lesions, the majority of which were in the hindlimbs (76%). The latency of cancer development was 30 weeks, and 46% of the mice developed metastases. As observed in humans, metastases consistently develop in lungs. Tracing of the fluorochrome expression indicates that Osx-expressing cells (mCherry-expressing cells) are the most abundant cells in tumors, metastases, and in circulation.

CONCLUSION: This novel post-natally inducible mouse model of OS resembles the human disease and allows to track fluorescent cells during cancer development in vivo. Osx-expressing cells are the most abundant circulating cells and may represent a potential target for development of novel OS therapies.

POSTER #14

PRICKLE1 and FOCAD are expressed in skull base chondrocytes

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Background: In normal human populations, single nucleotide polymorphisms (SNPs) near both PRICKLE1 and FOCAD are associated with much wider midfaces. FOCAD is a tumor suppressor gene, that functions to connect the cell to the extracellular matrix. FOCAD is associated with early onset colorectal, breast and glioblastoma tumors and loss of FOCAD expression increases cellular motility. The skull base undergoes endochondral ossification and it does not fully mature until the 2nd decade of life. In fact, abnormalities in the skull base can be present at birth or later with the development of skull base chondrosarcomas. Both disorders are difficult to treat because of the proximity of the brain to the skull base. Robinow syndrome (RS), is a rare genetic syndrome where patients develop a wider face and patients with a subtype of RS also experience ectopic bone growth in the skull base foramina. We model RS using the Prickle1Beetlejuice/Beetlejuice (Prickle1Bj/Bj) mice. We have observed that the Prickle1Bj/Bj skull base chondrocytes are disorganized resulting in a wider, shorter cranial base. At the molecular level, we observed that the localization of Prickle1 protein is abnormal in chondrocytes, and the Prickle1Bj protein has decreased co-localization with FOCAD.

Methods & Results: Using immunofluorescence staining we tested the localization of FOCAD and Prickle1 in a subset of UPMC cranial base chondrosarcoma cases. In these tumors we observed FOCAD and Prickle1 expression and a subset of cells had an overlap of FOCAD and Prickle1 expression. Intriguingly, in one case, we observed Prickle1 localization that is reminiscent of the disorganized chondrocytes in the Prickle1Bj/Bj cranial base chondrocytes.

Conclusions: Our data support the conclusion that skull base chondrocytes may require FOCAD and Prickle1 for normal development and this may be dysregulated during neoplastic growth.

POSTER #15

Targeted Studies of Temporomandibular Joint Disorder (TMD): A Study of Immune System Surveillance Evasion Mechanism(s) in Humans

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The incorporation of new techniques in addition to foundational approaches in human genetics to evaluate mechanisms of disease onset, modes of inheritance, associations, and development, are evolving applications in research. This study aims to evaluate genetic variants for chronic, inflammatory conditions which participate in immune system surveillance evasion to propagate disease progression, by assessing single nucleotide polymorphisms (SNPs) associated with metastatic cancer for oral health variables temporomandibular joint disease (TMD) and periodontitis. Identification and evaluation of the SNPs to determine genetic associations will provide a clearer depiction of the relationship between the chronic, inflammatory condition of TMD and periodontitis, which affect the bone quality and health of oral and craniofacial microstructures, and disease progression/severity. Discovered genetic associations may serve as biomarkers to aid efforts for preventive measures and interventional support. In addition to an evaluation of TMD and periodontitis, confounding inflammatory conditions categorized as sub-cohorts, will also be evaluated, as data has shown that individuals with one chronic inflammatory condition commonly present with additional confounding inflammatory conditions. As a result, it is important to understand the impact of having confounding conditions in comparison to individuals without disease and those with only one affliction. Approximately 7,000 human profiles of the University of Pittsburgh Dental Registry and DNA Repository (DRDR) will allow genomic characterization of 17 SNPs for the following sub-cohort profiles: periodontitis, obesity, asthma, type II diabetes, periapical lesions, rheumatoid arthritis/autoimmune diseases, and dry socket to identify genetic alteration associations for use as biomarkers for oral disease predisposition and severity. Preliminary data involving catechol-O-methyltransferase (COMT) (SNP rs4818) for asthma, rheumatoid arthritis/autoimmune disease, and periapical lesions have revealed the initial comparison of the frequency of distribution between each of the 3 sub-cohorts (asthma vs. rheumatoid arthritis/autoimmune disease ($p=0.91$), rheumatoid arthritis/autoimmune disease vs. periapical lesions ($p=0.56$), asthma vs. periapical lesions ($p=0.62$)) to reveal there are no statistical differences or effect between disease states. Distribution of the genotypes are in Hardy Weinberg equilibrium ($p>0.05$) and have a chi-square value with 2 degrees of freedom between 0.01 and 0.9. The study will continue to evaluate the remaining sub-cohorts which display a strong bone phenotype.

POSTER #16

Deficiency of Trps1 in osteoblasts and cementoblasts impairs formation of alveolar bone and cementum, compromising periodontal structure

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Objectives: Trichorhinophalangeal syndrome (TRPS) is caused by a heterozygous mutation in the TRPS1 gene. This gene encodes a transcription factor that regulates the expression of multiple genes involved in the formation of mineralized tissues. TRPS patients present with skeletal dysplasia, including defective development of endochondral bones, and abnormal dental and craniofacial development. Recently, GWAS studies have revealed TRPS1 as a bone mineral density-associated locus and periodontal disease risk locus. Periodontitis is a chronic inflammatory disease that leads to the destruction of the alveolar bone. These suggest a link between Trps1 and homeostasis of mineralized tissue in the periodontium, namely alveolar bone and cementum anchoring of periodontal ligaments; however, the role of Trps1 in the development and homeostasis of periodontium is unknown. Thus, we hypothesized that Trps1 is involved in osteogenesis and cementogenesis through regulation of gene expression in osteoblasts and cementoblasts.

Methods: We compared alveolar bone and tooth roots of 1st mandibular molars of 4wk old WT and $Trps1^{Col1a1}$ cKO mice (N=5/genotype/sex) by micro-Computed Tomography (μCT), histology, and immunohistochemistry (IHC).

Results: μCT analyses revealed significantly decreased formation of alveolar bone, and shorter and thinner tooth roots in $Trps1^{Col1a1}$cKO compared with WT mice. There were no significant differences in the mineral density of alveolar bone and tooth roots. Semi-quantitative histological analyses showed reduced cementum area in $Trps1^{Col1a1}$cKO mice compared to WT mice. IHC revealed fewer Osterix-positive cells on the surface of alveolar bone and cementum in $Trps1^{Col1a1}$cKO mice than in WT mice. Furthermore, we detected decreased levels of tissue-nonspecific alkaline phosphatase (TNAP) in alveolar bone, cementum, and periodontal ligament in $Trps1^{Col1a1}$cKO mice in comparison with WT mice. Picrosirius staining revealed impaired organization of collagen fibers in periodontal ligaments in $Trps1^{Col1a1}$cKO mice.

Conclusions: Our in vivo analyses demonstrated that Trps1 supports expression of major proteins involved in bone and cementum formation. Furthermore, the impaired alveolar bone and cementum formation in $Trps1^{Col1a1}$cKO mice highlights the importance of this transcription factor for the function of osteoblasts and cementoblasts, as well as formation and sound periodontium.

POSTER #17

Comparison of a Novel Photo-Crosslinkable Hydrogel with Revascularization for Regenerative Endodontic Therapy in the Presence of Intra Bony Defects Associated with Endo-Perio Lesions

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Introduction: Periodontal disease of immature permanent teeth is challenging to treat, such as the destruction of periodontal ligaments and the formation of intra-bony defects. Regenerative endodontic therapies have emerged as a novel approach to remove underlying infection and restore tooth vitality. Here we induce Endo-Perio lesions in a canine model and evaluate the safety and efficacy of a novel hydrogel compared to revascularization.

Methods: The photo-crosslinkable Gelatin methacrylate/ Heparin methacrylate (GelMA/HepMA) hydrogel was fabricated in-house from porcine derived gelatin (45 kDa) and heparin (15 kDa). To induce Endo-Perio lesions, the pulp chamber of mandibular premolars and first molars of 8-month-old beagles were exposed to saliva via endodontic access through the crown. A day later, pulpectomy was performed and teeth were treated with either revascularization or the hydrogel. After two weeks, the periapical disease and bone tissue response to different treatments was evaluated with histopathology (H&E and Brown and Brenn Staining) and micro-CT.

Results: The initial results indicated that there are no differences between the two studied groups in terms of inflammatory cell infiltration. Furthermore, bone density and periapical and radicular bone volume did not show any differences between the revascularization and the hydrogel groups.

Conclusion: The GelMA/HepMA hydrogel can be considered a promising alternative for revascularization in immature infected teeth with endo-perio lesions.

Keywords: Endo-Perio Lesions; Photo-crosslinkable hydrogel; Regenerative Endodontic Therapies

POSTER #18

Structural Differences in the Tibial Metaphysis Between Female NCAA Division I Cross-Country Runners and Gymnasts

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Bone geometry and microarchitecture vary between athletes with different habitual loading patterns because bone adapts to withstand loading demands. As gymnastics involves infrequent high impact loading and running involves repetitive medium impact loading, differences in the tibial structure are expected between these athletes.

PURPOSE: Investigate differences in geometry and microarchitecture of the distal tibial metaphysis between collegiate female athletes competing in gymnastics and cross-country.

METHODS: High resolution peripheral quantitative computed tomography was used to assess the distal tibia of NCAA Division I female cross-country runners (n = 17, age = 19.0 ± 0.9yrs, BMI = 20.6 ± 1.4kg/m2) and gymnasts (n = 16, age = 19.5 ± 1.4yrs, BMI = 23.3 ± 1.8kg/m2). Scans were taken at 4% of tibial length and evaluation software measured bone parameters. Finite element analysis estimated stiffness and failure load. Unadjusted group comparisons were conducted using independent samples t tests, followed by analysis of covariance adjusting for baseline BMI. Data are presented as mean±SD, α=0.05, two-sided.

RESULTS: Unadjusted group comparisons showed that gymnasts exhibited greater total area (1067.4±100.3mm2, 950.8±65.9mm2, p<.001), trabecular area (995.5±103.2mm2, 880.7±64.5mm2, p<.001), and trabecular number (2.1±0.2mm-1, 1.9±0.2mm-1, p=.015) than runners. Stiffness (228.7±47.5Nmm-1, 190.5±46.9Nmm-1, p=.027) and failure load (12.2±2.4N, 10.3±2.4N, p =.026) were also greater in gymnasts than runners. Group differences analyzed after adjusting for BMI remained significant for total area (p=.030), trabecular area (p=.020), and trabecular number (p=.008); whole bone stiffness and failure load were no longer significant (p≥.381). Cortical volumetric bone mineral density, area, and thickness were not significantly different between groups in either analysis (p>.05).

CONCLUSION: Gymnasts presented with more favorable bone structure than runners, possibly due to higher forces experienced during training and competition. Differences in tibial metaphysis bone structure between gymnasts and runners, which persist after controlling for BMI, indicate that the adaptive bone formation response to sport training is specific to demands of the sport.

POSTER #19

Phosphate Signaling Is Mediated via Parathyroid Hormone Receptor 1-PLC-PKC Pathway.

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Inorganic phosphate (Pi) is a structural element in mineralized tissues and a signaling molecule, which regulates mineralization of an extracellular matrix (ECM). Studies demonstrated that Pi signaling involves FGFR1 and Erk1/2 kinase. However, the molecular mechanisms of Pi sensing and cellular mediators of Erk1/2 activation in response to Pi remain unknown. Our goal is to elucidate Pi signaling in cells producing bone (osteoblasts) and dentin (odontoblasts). Therefore, for in vitro studies, we selected 17IIA11 odontoblast and MLO-A5 osteoblast/pre-osteocytes cell lines, which have molecular characteristics of committed osteogenic cells, and rapidly produce mineralized ECM upon stimulation with standard osteogenic medium. Two independent lines of experiments with 17IIA11 cells demonstrated that the loss of their ability to respond to Pi coincides with downregulated expression of parathyroid hormone receptor 1 (Pth1r). Hence, we hypothesized that Pth1r is required for Pi signaling in committed osteogenic cells. To test this hypothesis, we generated Pth1r-deficient 17IIA11 and MLOA5 cell lines using shRNA approach. Pth1r-deficient cells, unlike shScr controls, did not activate Erk1/2 upon stimulation with Pi . They also failed to mineralize under osteogenic conditions. Because Pth1r can initiate various signaling cascades, including protein kinase A (PKA)-cAMP and phospholipase C (PLC)-protein kinase C (PKC), we investigated which signaling axis downstream of the Pth1r transmits the response to Pi . We compared activation of pErk1/2 in response to Pi in the presence and absence of specific pharmacological inhibitors. Erk1/2 phosphorylation was attenuated only when phospholipase C (PLC) was inhibited with D609 but not by PKA inhibition with H-89. Furthermore, inhibition of PLC downstream signals with pan-PKC inhibitor Bisindolylmaleimide I (GF109203X) resulted in a dose-dependent decrease of pErk1/2 levels in comparison to untreated controls. To identify the PKC isoform involved in Pi signaling, we utilized selective PKC inhibitors Sotrastaurin (inhibits classical PKCs - PKCα and PKCβ, but not PKCγ) and Staurosporine (inhibits PKCα and PKCγ, but not PKCβ). We found a dose-dependent decrease in Erk1/2 activity only with Staurosporin but no effect with Sotrastaurin. Altogether, our in vitro identified Pth1r as a candidate receptor/sensor of Pi, that mediates Pi signaling through PLC-PKCγ axis in committed osteogenic cells.

POSTER #20

Influence of Medical Marijuana on Osteogenesis during Bone Healing – an *in vitro* study

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Objective: Recently, many states legalized the use of medical marijuana in clinical use with the notion of potential health benefits of the certain properties and usage in analgesic, immunomodulatory and anti-inflammation. The active component, Δ^9-tetrahydrocannabinol, activates the CB1 and CB2 cannabinoid receptors, thus mimicking the action of endogenous cannabinoids. Given the recent finding that both CB1 and CB2 affect bone metabolism, further endocannabinoid signaling has also been shown to regulate proliferation and differentiation of mesenchymal stem cells (MSCs), Traditionally, fracture management involves prolonged immobilization during the recovery with and without surgery and thus prolonged discomfort for patients. It is of great interest to explore the influence of medical marijuana with potential impact in this common prevalent disorder. In this preliminary study, we conducted an in vitro study to examine the influence of a synthetic cannabinoid agonist, Win-55,212-2(Win) on human mesenchymal stem cells-derived osteoblast. The cell viability and phenotypes were assessed by MTS assay, real time polymerase chain reaction (PCR), and alizarin red staining.

Methods: With the approval from IRB, MSCs were isolated from healthy human bone marrow. At P5, cells were subjected to 21 days osteogenesis culture and then treated with/without different concentration of Win-55 (0.01, 0.1, or 1 μM) for another 48 hours. MTS assay were employed to test the half-maximal (50%) inhibitory concentration (IC50) at different time point. The cell phenotype was assessed by real-time PCR and alizarin red staining.

Results: We tested 7 doses, from 0.001μM up to 5 μM, determined that the IC 50 of Win-55 on human osteoblast for 1-7 days was ~ 1.5 μM (Figure 1), which was slightly lower than the doses we found in previous study on chondrocyte. As shown in Figure 2, gene expression of inflammatory cytokines IL-1βand TNF-αshowed significant reduction. There was increased osteoblastic phenotype in RUNX2 and OPN at relative high dose of Win-55. Calcium deposition and mineralization was mostly observed and enhanced by treatment with 1μM Win (Figure 3).

Conclusion: High dose of Win may directly cause the upregulation of osteoblastic henotype and reduction of inflammation. There was also increased calcium deposition and mineralization in MSCs derived osteoblast, suggesting that cannabidiol might lead to improvement in fracture healing and provide a novel therapeutic option for the bone regeneration.

POSTER #21

Erosive Tooth Wear, Alcohol Intake, and Genetic Variation in COMT and MMP2

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Erosive tooth wear (ETW) is a multifactorial condition of increasing prevalence in the younger population. Individual differences in matrix metalloproteinases (MMPs) activity due to genetic variation may increase the efficiency of dentin degradation. Besides, the catechol-O-methyltransferase (COMT) variant is predicted to influence dopamine bioavailability, which have been associated with vulnerability to addiction, such as alcohol consumption, and appears to inhibit the activity of MMPs. These variants seem to be interesting targets to explore for modulation of ETW occurrence. This study aimed to explore the association between different ETW phenotypes with MMP2 and COMT single-nucleotide variants, and selected environmental factors. Saliva samples, erosive wear and dental caries experience data, and dietary/behavioral information from 16-18-year-old patients (n= 747) were used. Genotypes were obtained and phenotypes were further analyzed considering diet and behavioral data, using logistic regression as implemented in PLINK, with the assumption that ETW is a complex gene-environment model. All genotyping data were in Hardy-Weinberg equilibrium. When comparing individuals' erosion-free with those with mild erosion, an association was found with COMT rs6269 ($p = 0.02$). The comparison between erosion-free individuals with individuals with severe ETW also showed an association with COMT rs6269 under a recessive model ($p = 0.03$). Logistic regression showed that in the presence of less common alleles of MMP2 rs9923304 and COMT rs6269, ETW were more likely to occur when individuals drank alcohol. The GG genotype of COMT rs6269 was associated with the presence of lower ($p = 0.02$) and higher ($p = 0.02$) caries experience when individuals with ETW only in enamel were compared with individuals with ETW involving dentin. The results support a role of gene in ETW, particularly when there is exposure to alcohol drinking.

POSTER #22

Contribution of Post-natal Prx1 Expressing Cells to Periodontal Regeneration in Mouse Molars

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Background: Post-natal skeletal stem cells expressing Paired related homeobox 1 (pnPrx1 cells) are known to play an important role in bone regeneration. It was reported that pnPrx1 cells are present within the continuously regenerating periodontal ligament (PDL) of the mouse incisor and are involved in the formation of the mouse molars' PDL. However, the potential involvement and contribution of pnPrx1 cells to periodontal regeneration in mouse molars remain unclear. In the present study, we aimed at identifying the role of pnPrx1 cells within the mouse molar periodontium in periodontal regeneration.

Methods: The contribution of pnPrx1 cells to periodontal regeneration in mouse molars was evaluated by creating a periodontal fenestration defect (~2mm in length, 1 mm in width, and 0.5 mm in depth) in the buccal aspects of the mandible involving mesial and distal roots of the first molar. Defects were created in the test group (Prx1-CreEr-GFP+/TdTomato+) (n=5) and control group (Prx1-CreEr-GFP-/TdTomato+) (n=5) tamoxifen treated mice. All mandibles were harvested at 7- and 30-days post-surgery for histology/fluorescence for detection of TdTomato (red) cells.

Results: TdTomato (red) cells were observed in the newly formed PDL and at the areas of PDL adjacent to and around the periodontal defect of the distal root of the first molar at 7-days post-surgery in test group. Compared to the 7-days post-surgery group, more red cells were observed at 30-days post-surgery in PDL surrounding the distal root and in the newly formed bone. No red cells were observed in control group at 7- or 30-days post-surgery. Incomplete formation of PDL and cementum was observed in both groups throughout the observation period.

Conclusion: pnPrx1 cells contribute to the regeneration of surgically created periodontal defects of the mouse molars. Additional studies are being performed to evaluate whether these cells can be harnessed to foster periodontal regeneration.

POSTER #23

Strontium affects pre-osteoblast and odontoblast mineralization in vitro, and prevents polymethylmethacrylate cement loosening in vivo

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Objectives: The bone tissue provides mechanical support and protection to soft tissues, and regulates the availability and storage of calcium. Conditions like estrogen deficiency, long-term immobilization, and aging are commonly associated with the loss of bone mass and predisposition to fractures. The use of bioactive nanoparticles (NPs) that mimic the structure of bone apatite is a compelling approach to bone healing. Strontium ions (Sr2+) have the ability to control osteoblast activity and affect extracellular matrix (ECM) mineralization. However, how strontium acts on mineralization-competent cells are unclear. In the present work, we hypothesized that Sr2+ affects mineralization in two ways: (1) at a molecular level by regulating indirectly osteogenic genes, and (2) by regulating secretion and molecular composition, hence, the function of matrix vesicle (MV). These vesicles are thought to play a major role in the initiation of ECM mineralization.

Methods: We used alizarin red staining, TNAP activity, and qRT-PCR to check the NPs´ potential in inducing mineralization of the pre-osteoblast MC3T3-E1 cell line. Subsequently, we compared by computerized micrography, histology, qRT-PCR, and mechanical test rabbits´ femur implanted with polymethylmethacrylate cement loaded and unloaded with the NPs. Using an odontoblastic cell line (17IIA11), we analyzed the Sr2+ effect upon MVs secretion (by nanoparticle tracking system) and protein composition (by Western blot).

Results: Our results demonstrate that the NPs in which 90 % of Ca2+ ions were substituted by Sr2+ (NanoSr 90%) upregulate TNAP activity and increase ECM mineralization. Moreover, NanoSr 90% increase the mRNA levels of markers of osteoblast differentiation: Runx2, Ocn, and Sp7. In vivo, studies showed that NanoSr 90% increases BMP2 and OCN gene expression. The mechanical test (push-out) revealed that porous cement loaded with the NPs increases the maximum strength of the interface bone/cement de-bonding. On the molecular level, Sr2+ stimulates Erk1/2 and CREB signaling and represses MVs secretion. However, MVs secreted in the presence of Sr2+ are enriched in proteins engaged in calcium and phosphate regulation.

Conclusion: Our data suggest that Sr2+ modulates mineralization at the gene expression and MVs secretion level, hence its use as a potential bioactive agent could be applied in the bone regeneration field.

POSTER #24

Elucidating the role of keratin 75 in enamel using Krt75^{tm1Der} knock in mouse model

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Keratin 75 (K75) was recently discovered in ameloblasts and enamel organic matrix. Carriers of A161T substitution in K75 present with the skin condition Pseudofollicullitis barbae. This mutation is also associated with high prevalence of caries and compromised structural and mechanical properties of enamel. Krt75tm1Der knock-in mouse (KI) with deletion of Asn159, located two amino acids away from KRT75A161Tcan be a potential model for studying the role of K75 in enamel and the causes of the higher caries susceptibility associated with KRT75A161T mutation. To test the hypotheses that KI enamel is more susceptible to a simulated acid attack (SAA), and has altered structural and mechanical properties, we conducted in vitro SAA experiments, microCT, and microhardness analyses on 1st molars of one-month-old WT and KI mice. KI and WT hemimandibles were subjected to SAA and contralateral hemimandibles were used as controls. Changes in enamel porosity were assessed by immersion of the hemimandibles in rhodamine, followed by fluorescent microscopy analysis. Fluorescence intensity of KI enamel after SSA was significantly higher than in WT, indicating that KI enamel is more susceptible to acid attack. MicroCT analysis of 1st molars revealed that while enamel volumes were not significantly different, enamel mineral density was significantly lower in KI, suggesting a potential defect of enamel maturation. Microhardness tests revealed that in KI enamel is softer than in WT, and potentially less resilient to damages. These results suggest that the KI enamel can be used as a model to study the role of K75 in enamel.

POSTER #25

Comparative study on the osteogenic potential of subchondral and fibrocartilage cells of the Temporomandibular Joint

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INTRODUCTION: The Temporomandibular Joint (TMJ) is the unique ginglymo diarthrodial synovial joint of the body, and a vital component of the stomatognathic system. Despite the limited regenerative ability of cartilage, recent studies on goats have demonstrate that the TMJ cartilage, which is histologically defined as fibrocartilage, possess an innate regenerative ability in vivo. These studies have determined that the mandibular condylar fibrocartilage interface of the TMJ contains a heterogeneous cell population that includes stem cells/progenitor cells. PURPOSE: This study aims to explore the osteogenic potential of the cell populations of the fibrocartilage and subchondral bone of the TMJ, and go further on understanding the tissue biology of these compartments. This work seeks to better understand the cells involved in subchondral bone remodeling and fibrocartilage regeneration in the context of TMJ tissue engineering.

METHODS: Cells from the surface and cartilage layers (separately) were compared to osteoblast from the mandibular condyle and to bone marrow cells for the osteogenic potential. Cells were cultured in 2D for 21 days in media containing inorganic phosphate and 10% FBS. Then all cultures were stained for alkaline phosphatase, alizarin red and Von Kossa staining for mineral deposition.

RESULTS: Results showed that all three cell types were able to deposit copious amounts of mineral. A non-osteogenic media control was used.

CONCLUSIONS: We demonstrate that the subchondral bone and fibrocartilage compartments of the TMJ condyles contain a heterogeneous cell population that include progenitor cells. These populations showed different mineralization activity after cultured under osteogenic condition. We conclude that a putative subchondral stem cells can be harnessed through tissue engineering to guide subchondral bone mineralization and remodeling, as well as fibrocartilage regeneration of the TMJ condyles.

POSTER #26

Lack Of Gravity Lowers The Number The Skeletal Stem Cells Of The Calvaria Without Imparing Calvarial Bone Regeneration.

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BACKGROUND: Various studies report that exposure to microgravity results in elevated bone resorption and reduced bone formation and regeneration. However, not much is known about the responsible cellular and molecular mechanisms. The mouse calvaria, with its suture enriched of skeletal stem cells (SSCs), represents an ideal model to study SSCs and their role in bone homeostasis and regeneration. Therefore, we analyzed the effects of spaceflight on the mouse calvarial sutures and their content of SSCs, and on the regeneration of a 0.5 mm (in diameter) bone defect, artificially created on the right parietal bone.

MATERIALS AND METHODS: Sixteen 8-week old female C57BL/6 mice were randomly distributed in two groups: one group of 8 mice that did not undergo surgery (untreated mice) and one group of 8 mice that, under anesthesia, underwent a surgery to create the 0.5 mm bone defect. After 45 days in space, mice returned to Earth and calvarial tissues and long bones were collected and analyzed by means of microcomputed tomography (μCT) and single cells RNA sequencing (scRNA-seq). The same treatments and analyses were performed on sixteen ground control mice.

RESULTS: μCT analysis of the calvaria showed no significant differences in bone mass between the spaceflight and the ground control mice. On the contrary, and consistent with what has been previously reported, long bones exhibited significant lower bone mass in spaceflight mice. ScRNA-seq data indicate that lack of gravity is associated to a lower level of SSCs of the calvarial sutures, whereas the uCT quantification of the bone regenerated in the calvarial defects shows that equal regeneration occurs in spaceflight and ground control mice.

CONCLUSIONS: This study indicates that, during spaceflight, while the number of the SSCs of the sutures is significantly reduced, bone homeostasis (bone mass) and bone regeneration of the calvarial bones are not impacted. The differences with the well documented loss of bone mass and impaired bone regeneration observed in the long bones highlights a significant spaceflight dichotomy between the flat bones and the long bones.

POSTER #27

Integration of tooth root organoids with rodent mandibular bone

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Challenges in regenerating the tooth root lie in controllably rebuilding the multiple diverse constituent tissues in a spatially organized manner and achieving functional integration with surrounding bone. The dental pulp and periodontal ligament (PDL) each contain a unique population of stem/progenitor cells (DPSCs and PDLSCs, respectively). By co-culturing these cells in a scaffold-free system, we have leveraged their innate capabilities to self-assemble a tooth root organoid. In these constructs, DPSCs localize to the center and PDLSCs localize to the periphery, mimicking their natural positions within the tooth root. Hematoxylin and eosin (H&E) staining shows a solid, cellular structure with a distinct fibrous tissue at the periphery with elongated nuclei. Constructs exhibit a striated mineral pattern similar to the natural tooth root with a central soft tissue surrounded by a mineralized layer, all enclosed in a second peripheral soft tissue. Extracellular matrix protein expression patterns are also similar to a natural tooth root, with periodontal ligament associated protein-1 staining localized to the peripheral soft tissue, cementum protein-1 most strongly localized to the portion of the mineral formed by PDLSCs, and dentin sialophosphoprotein most strongly localized to the portion of the mineral formed by DPSCs. This data provides compelling evidence of an anatomically organized tooth root organoid with pulp-, dentin-, cementum-, and PDL-like layers. Now, we are investigating the capacity for these organoids to become functionally integrated with bone by placing tooth root organoids in rodent mandibular bone fragments and either culturing ex vivo for 2 weeks or placing subcutaneously in a nude mouse for 4 weeks. In the ex vivo system, peripheral fibers re-orient and become integrated with the bone in a manner similar to Sharpey's fibers. microCT data from subcutaneously implanted samples shows that these tissues maintain their organized mineral pattern, and H&E indicates that vasculature has infiltrated the central soft tissue, similar to what is seen in the dental pulp. Altogether, this data shows that tooth root organoids have the capacity to functionally integrate with bone, providing both a novel model system to controllably study tooth-bone interactions and a promising candidate for a biological dental implant.

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