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Center of Regenerative Medicine & Musculoskeletal Research Center present

MUSCULOSKELETAL BIOLOGY & REGENERATION MEETING

Featuring keynote lectures from Kristi Anseth & Vicki Rosen

ERIC P. NEWMAN EDUCATION CENTER, WUSM

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Musculoskeletal Biology and Regeneration Meeting

EPNEC Conference Center, WUSTL, St. Louis MO

May 5-6, 2022

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ORGANIZING COMMITTEE

Primary Organizers	**Farshid Guilak, PhD** Professor | Department of Orthopaedic Surgery Co-Director | Center of Regenerative Medicine Director of Research | Shriners Hospitals for Children St. Louis **Matthew Silva, PhD** Julia and Walter R. Peterson Orthopaedic Research Professor | Department of Orthopaedic Surgery, Co-Director | Musculoskeletal Research Center
	Aaron Johnson, PhD Assistant Professor Department of Developmental Biology **Spence Lake, PhD** Associate Professor Department of Mechanical Engineering & Materials Science **Gabriel Mbalaviele, PhD** Professor Division of Bone & Mineral Diseases **Audrey McAlinden, PhD** Associate Professor Department of Orthopaedic Surgery **Erica Scheller, DDS, PhD** Assistant Professor Division of Bone & Mineral Diseases
Administrative Support	**Angela Bowman PhD** Executive Director, Center of Regenerative Medicine **Kamilla McGhee** Administrative Coordinator, Musculoskeletal Research Center

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All events will take place in the auditorium unless otherwise noted

THURSDAY | May 5, 2022

11:45 am – 12:20 pm	**Registration** (lobby), **poster set-up** (Great Room A)
12:20 pm	**Welcome and Opening Remarks** **Regis O'Keefe, MD, PhD** Fred C Reynolds Professor and Head | Department of Orthopedic Surgery Center of Regenerative Medicine Division of Biology and Biomedical Sciences Institute of Clinical and Translational Sciences Siteman Cancer Center Washington University in St. Louis
12:25 pm	**Keynote Introduction** **Farshid Guilak, PhD** Professor | Department of Orthopaedic Surgery Co-Director | Center of Regenerative Medicine Director of Research | Shriners Hospitals for Children St. Louis Washington University in St. Louis
12:30 pm	**Keynote Lecture: Kristi Anseth, PhD** Tisone Professor and Distinguished Professor University of Colorado | Boulder, CO *"Engineering Bioresponsive and Adaptable Materials for Regenerative Medicine"*
1:35 – 3:03 pm	**Session I – ENGINEERING TISSUE REPAIR** *Session Moderator:* ***Natasha Case***
1:35 pm	**Warren Grayson, PhD** Professor Johns Hopkins University | Baltimore, MD *"Autologous Regeneration of Midfacial Bone in Large Animals"*
2:02 pm	**Stephanie Bryant, PhD** Professor University of Colorado | Boulder, CO "Tissue-Mimetic Hydrogel Composites for Musculoskeletal Tissue Engineering"
2:29 pm	**Nate Huebsch, PhD** Assistant Professor Washington University | St. Louis, MO *"Designing Alginate Hydrogels to Exploit Integrin Signaling for Musculoskeletal Tissue Engineering"*

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2:51 pm	**Ning Gao** The Institute of Materials Science & Engineering Washington University | St. Louis, MO *"Enriching MSC Paracrine Effects by Enhancing N-Cadherin Interaction For Ischemic Limb Regeneration"*
3:15 – 4:00 pm	**POSTER SESSION** (Great Room A)
4:10 – 5:20 PM	**Session 2 – MECHANISMS OF REGENERATION** *Session Moderator:* ***Sade Williams Clayton***
4:10 pm	**Bruno Peault, PhD** Professor University of California at Los Angeles | Los Angeles, CA and the University of Edinburgh (UK) *"Mesenchymal Stem Cells, From Natural Niches to Tissue Repair and Remodeling"*
4:37 pm	**Ken Muneoka, PhD** (virtual) Professor College of Veterinary Medicine and Biomedical Sciences Department of Veterinary Physiology and Pharmacology Texas A&M University | College Station, TX *"A Digital approach to Regeneration and Regenerative Medicine"*
5:04 pm	**Feini (Sylvia) Qu** Instructor Washington University | St. Louis, MO *"Development vs. Regeneration: Skeletal Patterning and Outgrowth of the Murine Digit Tip"*
	DINNER | Third Degree Glass Factory 5200 Delmar Blvd, St. Louis, MO 63108
6:00 pm	Cocktails & Glass blowing demonstration
7:00 pm	Dinner

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FRIDAY | May 6, 2022

7:00 – 8:00 am	**BREAKFAST** | *Main lobby*
8:15 – 9:30 am	**Session 3 – TRANSLATIONAL APPROACHES IN REGENERATIVE MEDICINE** *Session Moderator:* ***M. Farooq Rai***
8:15 am	**Nenad Bursac, PhD** Professor of Biomedical Engineering, Cell biology, and Medicine Duke University | Durham, NC *"Modeling Rare Skeletal Muscle Diseases in a Dish"*
8:42 am	**David Brogan, MD, MSc** Assistant Professor Orthopaedic Surgery Washington University | St. Louis, MO *"Intra-operative Imaging of Nerve Injuries: A Paradigm Shift"*
9:04 am	**Hua Shen, PhD** Orthopaedic Surgery Washington University | St. Louis, MO *"Metabolic Regulation of Intrasynovial Flexor Tendon Repair"*
9:18 am	**Jennifer Brazill, PhD** BMD Washington University | St. Louis, MO *"SARM1 Drives Type 1 Diabetes-Associated Bone Suppression and Fragility"*
9:32 am	**Ryan Potter** Orthopaedic Surgery Washington University | St. Louis, MO *"Systemic VEGFA Ablation Blunts Locomotive Deficts and Intradiscal Innervation Following Lumbar Intervertebral Disc Injury"*
9:46 am	**Xiaohong Tan** Biomedical Engineering Washington University | St. Louis, MO *"Dual Peptide Functionalized Alginate Hydrogels to Modulate Nucleus Pulposus Cell Phenotype"*
9:58 – 10:15 am	**BREAK**

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10:15 am – 12:00 pm	**Session 4 – SKELETAL STEM CELLS AND REPAIR** *Session Moderator:* ***Nicole Gould***
10:15 am	**Matt Greenblatt, MD, PhD** Associate Professor Weill Cornell Medical College | New York City, NY *"A Skeletal Stem Cell Basis for Lineage Selection in Bone Homeostasis and Repair"*
10:42 am	**Celine Colnot, PhD** (virtual) Research Associate Professor INSERM U955-Mondor Biomedical Research Institute Paris Est-Creteil University | Créteil, France *"Skeletal Stem/Progenitor Cells in Periosteum and Skeletal Muscle Coordinate Endochondral Ossification During Bone Regeneration"*
11:09 am	**Kareem Azab, PhD** Associate Professor Radiation Oncology - Div. of Cancer Biology, Department of Radiation Oncology Biomedical Engineering - Washington University | St. Louis, MO Adjunct Clinical Instructor and Preceptor at Saint Louis College of Pharmacy. Founder and CSO at Cellatrix LLC; Founder at Targeted Therapeutics LLC; Co-Founder of CovACE Nanotechnology LLC, Co-Founder of AdaptXRT LLC *"3D Tissue-Engineered Bone Marrow Model as a Tool to Predict Therapeutic Efficacy in Cancer Patient Response"*
11:31 am	**Neda Rashidi** Mechanical Engineering and Material Science Washington University | St. Louis, MO *"Modulation of Human Adipose Stem Cell Collagen Synthesis by Mechanosensing of Substrate Architecture Through the PIEZO1 Ion Channel"*
11:45 am	**Wei Zou, PhD** Pathology and Immunology Washington University | St. Louis, MO *"Car Cells Express BMP Inhibitors Negatively Regulating Bone Formation In Vivo"*
12:00 – 1:00 pm	**LUNCH** | Main Lobby

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1:00 – 2:30 pm	**Session 5 | SYSTEMIC INFLUENCES ON REPAIR AND REGENERATION** *Session Moderator:* ***Natalia Harasymowicz***
1:00 pm	**Philipp Leucht, PhD** Associate Professor NYU Grossman School of Medicine | New York City, NY *"Skeletal Stem Cell Aging: Is it reversible?"*
1:27 pm	**Benjamin Levi, MD** Dr. Lee Hudson-Robert R. Penn Chair Division Chief of General Surgery Director, Center for Organogenesis & Trauma Associate Professor in Surgery University of Texas Southwestern Medical Center | Dallas, TX *"The Power of One: Investing in One Cell and One Lab Member at a Time"*
1:54 pm	**Jie Shen, PhD** Assistant Professor Orthopaedic Surgery Washington University | St. Louis, MO *"Fracture Nonunion: New Insights into Mechanism and Therapy"*
2:16 pm	**Kunjan Khanna, PhD** Orthopaedic Surgery Washington University | St. Louis, MO *"TMEM178 Negatively Regulates IL-1β Production Through Inhibition of NLRP3 Inflammasome"*
2:30 pm	**Xiao Zhang** Department of Medicine Washington University | St. Louis, MO *"Neural Contributions to Leptin-Mediated Bone Marrow Adipocyte Catabolism"*
2:50 – 3:40 pm	**POSTER SESSION** | Great Room A
3:45 pm	**Keynote Introduction** **Matthew Silva, PhD** Julia and Walter R. Peterson Orthopaedic Research Professor | Department of Orthopaedic Surgery, Co-Director | Musculoskeletal Research Center Washington University | St. Louis, MO

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3:50 pm	**Keynote Speaker: Vicki Rosen, PhD** Professor Harvard School of Dental Medicine | Boston, MA *"Cues for Enhancing Musculoskeletal Regeneration From Studying BMP Signaling"*
4:50 pm	**Closing Remarks** **Matthew Silva, PhD** Julia and Walter R. Peterson Orthopaedic Research Professor | Department of Orthopaedic Surgery, Co-Director | Musculoskeletal Research Center Washington University | St. Louis, MO

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Turn the page for

Speaker Biographies

KRISTI ANSETH
PhD

keynote

Tisone Professor and
Distinguished Professor

University of Colorado

Boulder, CO

Kristi.Anseth@colorado.edu

BIO

Dr. Anseth's research group is interested in the development of polymeric biomaterials that can interface with cells and promote tissue regeneration and repair. From a fundamental perspective, they seek to decipher the critical extracellular matrix (ECM) signals that are relevant for tissue development,regeneration, and disease and then design materials that integrate these signals. From an applied perspective, they use this knowledge to engineer materials that can promote tissue regeneration in vitro and in vivo. Dr. Anseth's talk will illustrate their recent efforts towards the synthesis of new hydrogel chemistries for 4D cell culture and regenerative medicine, and how one can dynamically control bio-chemical and biophysical properties through orthogonal, photo-chemical click reaction mechanisms. Some specific examples will include the design of hydrogels that promote musculoskeletal tissue regeneration, materials-directed growth of intestinal organoids from a single stem cell, and super-swelling matrices to visualize cell-matrix interactions with unprecedented resolution. These efforts will then be placed in the broader context of designing precision biomaterials to address demands for patient specific products and treatments.

TALK TITLE

"Engineering Bioresponsive and Adaptable Materials for Regenerative Medicine"

KAREEM AZAB
PhD

session 4

Associate Professor

Radiation Oncology - Div. of Cancer Biology,
Department of Radiation Oncology
Biomedical Engineering
Washington University

Adjunct Clinical Instructor and Preceptor at Saint Louis College of Pharmacy.
Founder and CSO at Cellatrix LLC; Founder at Targeted Therapeutics LLC
Co-Founder of CovACE Nanotechnology LLC
Co-Founder of AdaptXRT LLC

St. Louis, MO

kareem.azab@wustl.edu

BIO

Dr. Azab earned Pharmacy degree, M.Sc. in Medicinal Chemistry and Ph.D. in Pharmaceutical Sciences, from The Hebrew University of Jerusalem, focusing on development of novel drug delivery systems for targeted delivery of chemo and radiotherapy. In addition, he completed his post-doctoral training Harvard Medical School/ Dana-Farber Cancer Institute, studying cancer cell biology and immunology, focusing on tumor micro-environment. The research in Dr. Azab's Lab has a multi-disciplinary translational approach, involving pharmaceutical sciences, medicinal chemistry, cancer biology, immunotherapy, and tissue engineering. He specializes in development of drug delivery systems to improve the specificity and efficacy of cancer therapy; and has special interest in the role of tumor microenvironment in drug resistance, and metastasis. He also focuses on development of ex vivo 3D tissue engineered cancer models for drug development and personalized medicine. Dr. Azab has co-authored over 100 peer-reviewed papers, and mentored over 50 trainees (Post-doctoral, PhD, MSc and Undergraduate Students), and his research was translated into 5 clinical trials, 15 patents, and 4 start-up companies.

TALK TITLE

"3D Tissue-Engineered Bone Marrow Model as a Tool to Predict Therapeutic Efficacy in Cancer Patient Response "

DAVID BROGAN

MD, PhD

session 3

Assistant Professor

Washington University

St. Louis, MO

brogand@wustl.edu

BIO

Dr. David Brogan is an Assistant Professor of Orthopedic Hand and Microsurgery at Washington University in St. Louis. A native of Texas, he completed his undergraduate studies in Biomedical Engineering at Vanderbilt University in Nashville, TN. After graduation, he was awarded a Marshall Scholarship from the British Parliament to study for an MSc in Medical Engineering and Physics at King's College London, followed by an MSc in International Health Policy at the London School of Economics. He then went on to obtain his MD at Washington University in St. Louis, followed by training in Orthopedic Surgery at the Mayo Clinic and fellowship training in Hand Surgery at Duke University. In his current role at Washington University, his clinical practice focuses on care and reconstruction of mangled limbs, as well as brachial plexus reconstruction. He runs the Orthopedic Nerve Research Lab in collaboration with Dr. Christopher Dy and has funding as a Co-investigator on multiple grants, including the US Department of Defense and National Institutes of Health. His research focuses on functional nerve imaging as well as modulation of molecular pathways to inhibit Wallerian degeneration.

TALK TITLE

"Intra-operative imaging of nerve injuries:
A Paradigm Shift "

STEPHANIE BRYANT
PhD

session 1

Professor

Univeristy of Colorado

Boulder, CO

stephanie.bryant@Colorado.edu

BIO

Stephanie J. Bryant is the Thomas F. Austin Faculty Fellow and Professor of Chemical and Biological Engineering, Director of the Materials Science & Engineering Program and member of the BioFrontiers Institute at the University of Colorado at Boulder. She is a fellow of the American Institute for Medical and Biological Engineering and serves as the Associate Editor for Biotechnology and Bioengineering and Current Osteoporosis Reports.

TALK TITLE

"Tissue-Mimetic Hydrogel Composites for Musculoskeletal Tissue Engineering"

NENAD BURSAC
PhD

session 3

Professor of Biomedical Engineering, Cell biology, and Medicine

Duke University

Durham, NC

nenad.bursac@duke.edu

BIO

Dr. Nenad Bursac is a Professor of Biomedical Engineering, Cell Biology, and Medicine at Duke University. During his PhD and postdoctoral work at MIT and JHU, he developed first engineered mammalian heart tissues and methods to study cardiac arrhythmias in a dish. Currently, Dr. Bursac's research involves design of high-fidelity human microphysiological systems to study pathophysiology of striated muscles in vitro and development of novel cell- and gene-based therapies for cardiac and skeletal muscle regeneration in vivo.
Dr. Bursac has authored over 120 publications and mentored over 60 trainees. He is a recipient of the Stansell Family Distinguished Research Award and Stem Cell Innovation Award, a fellow of AIMBE and BMES,and an Associate Editor of Science Advances.

TALK TITLE

“Modeling Rare Skeletal Muscle Diseases in a Dish”

CELINE COLNOT
PhD

session 4

Research Associate Professor

INSERM U955-Mondor Biomedical Research Institute
Paris Est-Creteil University

Créteil, France

colnotc@gmail.com

BIO

Céline Colnot is a Research Associate Professor/ Director of Research at INSERM and a group leader at Mondor Biomedical Research Institute-INSERM U955-Paris Est Creteil University. She completed her PhD in Paris University in 1998 and her postdoctoral training at University of California, San Francisco where she became Assistant Professor until 2010. Her research focuses on the role of skeletal stem/progenitor cells in bone repair and diseases. The laboratory has expertise in mouse models of bone repair, primary skeletal stem cell culture, cellular and molecular analyses of skeletal lineages. The group characterized skeletal stem/ progenitor cells within periosteum and skeletal muscle that contribute to bone regeneration and uses scRNAseq technology to understand the heterogeneity of these cell populations. The projects are funded by French National Research Agency and NIH.

TALK TITLE

"Skeletal Stem/Progenitor Cells in Periosteum and Skeletal Muscle Coordinate Endochondral Ossification During Bone Regeneration"

WARREN GRAYSON
PhD

session 1

Professor

Johns Hopkins University

Baltimore, MD

wgrayson@jhmi.edu

BIO

Dr. Warren Grayson is a Professor and Vice-Chair for Faculty Affairs in the Department of Biomedical Engineering at Johns Hopkins University. Prior to joining Johns Hopkins, he did his post-doctoral training at Columbia University and PhD at Florida State University. He is an elected fellow of the American Institute for Medical and Biomedical Engineering and has also been recognized by the National Academy of Medicine as an Emerging Leader in Health and Medicine.

TALK TITLE

"Autologous Regeneration of Midfacial Bone in Large Animals"

MATHEW GREENBLATT
MD, PhD

session 4

Associate Professor

Weill Cornell Medical College

New York City, NY

mag3003@med.cornell.edu

BIO

Matthew Greenblatt is an Associate Professor of Pathology at Weill Cornell Medical College where his lab works to identify new types of skeletal stem cells and determine their role in driving skeletal disease processes. He also practices as a clinical pathologist and serves as the associate director of the pathology residency training program, overseeing physician scientist and clinical pathology training.

TALK TITLE

"A Skeletal Stem Cell Basis for Lineage Selection in Bone Homeostasis and Repair"

NATHANIEL HUEBSCH

PhD

session 1

Assistant Professor

Washington University

St. Louis, MO

nhuebsch@wustl.edu

BIO

Nate completed his BS in Bioengineering from the University of California, Berkeley and his PhD through the Harvard-MIT Division of Health Sciences and Technology. He then trained as a postdoctoral fellow at the Gladstone Institute of Cardiovascular Disease and the University of California, Berkeley where he held fellowships from the NIH and the California Institute of Regenerative Medicine. He joined the department of Biomedical Engineering at Washington University in Saint Louis in 2018. He is a 2021 recipient of the Young Innovator Award from the Cellular and Molecular Bioengineering journal. His group's research is funded by the American Heart Association and the National Institutes of Health.

TALK TITLE

“Designing Alginate Hydrogels to Exploit Integrin Signaling for Musculoskeletal Tissue Engineering”

PHILIPP LEUCHT
MD

session 5

Associate Professor

NYU Grossman School of Medicine

New York City, NY

Philipp.Leucht@nyulangone.org

BIO

Philipp Leucht is an orthopaedic surgeon-scientist specializing in orthopaedic trauma surgery at NYU Langone Health in New York City and an Associate Professor of Orthopaedic Surgery and Cell Biology at the NYU Robert I. Grossman School of Medicine. He is the Director of the NYU Regenerative Medicine Program and the Director of Research for the Department of Orthopaedic Surgery at NYU Langone Health.
Dr. Leucht's research focuses on skeletal stem cell biology with special focus on aging, inflammation and regeneration. He directs an NIH-funded research group and published extensively on bone biology and orthopaedic trauma. He completed his residency and fellowship training at Stanford University.

TALK TITLE

"Skeletal Stem Cell Aging: Is it reversible?"

BENJAMIN LEVI
MD

session 5

Dr. Lee Hudson-Robert R. Penn Chair
Division Chief of General Surgery
Director, Center for Organogenesis & Trauma
Associate Professor in Surgery

University of Texas Southwestern Medical Center

Dallas, TX

Benjamin.Levi@UTSouthwestern.edu

BIO

Benjamin Levi, M.D., holds the Dr. Lee Hudson-Robert R. Penn Chair in Surgery and Plastic and Reconstructive Surgery and serves as Chief of the Division of General Surgery and Director of the Center for Organogenesis and Trauma Research (CORT) at University of Texas Southwestern/Parkland Hospital. He specializes in acute and reconstructive burn surgery, scar reconstructive surgery and surgical critical care. He got his undergraduate degree at Washington University in Spanish and Biology followed by Medical School at Northwestern University. He did his Plastic Surgery Residency at University of Michigan and Fellowship in Surgical Critical Care and Burn Surgery at Massachusetts General Hospital. He started his career as a surgeon scientist at University of Michigan where he was on faculty for 6 years and directed the Burn/Wound and Regenerative Medicine Laboratory before joining the UT Southwestern faculty in 2020. Dr. Levi has a large research program, currently funded by the National Institutes of Health, 4 awards and Department of Defense to focus on stem cell biology, heterotopic ossification, muscle fibrosis, tissue regeneration and wound healing and repair. He is the author of more than 130 scholarly articles, including in Science Translational Medicine, Proceedings of the National Academy of Sciences, and Nature Communications, as well as chapters in a number of core textbooks in Surgery. He also has significant interest in Positive Intelligence and invests heavily in his division and laboratory team members to support and enhance mental fitness and wellness programs.

TALK TITLE

"The Power of One: Investing in One Cell and One Lab Member at a Time"

KEN MUNEOKA
PhD

session 2

Professor
College of Veterinary Medicine and Biomedical Sciences
Department of Veterinary Physiology and Pharmacology

Texas A&M University

College Station, TX

kmuneoka@cvm.tamu.edu

BIO

Dr. Muneoka received his doctoral and postdoc training at UC Irvine with Dr. Susan Bryant on salamander limb regeneration. In 1986 he started his lab as Assistant Professor at Tulane University with a focus on regeneration in higher vertebrates. From1993 to 2005 Dr. Muneoka served as Chair of the Department of Cell and Molecular Biology at Tulane University and moved his lab to Texas A&M University in 2015. Dr. Muneoka has served as a member of the US Army Science Board, the Eunice Kennedy Shriver NICHD Council, and the VA Office of Regeneration Research Programs Advisory Board. Dr. Muneoka's research is focused on illuminating a road map for tackling the problem of regenerative failure in mammals. His research has been funded by the NIH and DoD.

TALK TITLE

“A Digital approach to Regeneration and Regenerative Medicine”

BRUNO PEAULT
PhD

session 2

Professor

University of California
at Los Angeles and the
University of Edinburgh (UK)

Los Angeles, CA

bpeault@mednet.ucla.edu

BIO

Bruno Péault, PhD, is Professor and Chair of Vascular Regeneration at the University of Edinburgh, UK, and Professor of Orthopaedic Surgery at the University of California at Los Angeles. His laboratory has identified perivascular cells at the origin of mesenchymal stem cells and studies the phenotypic and functional heterogeneity of these presumptive MSCs, and role thereof in organ regeneration and fibrosis. B. Peault's other interest is developmental hematopoiesis; he served earlier as a Professor of Pediatrics and co-director of the Stem Cell Research Center at Children's Hospital in Pittsburgh, as Research Director at CNRS and department head at INSERM in Paris.

TALK TITLE

"Mesenchymal Stem Cells, from Natural Niches to Tissue Repair and Remodeling"

FEINI (Sylvia) QU

VMD, PhD

session 2

Instructor

Washington University

St. Louis, MO

feini.qu@wustl.edu

BIO

Dr. Feini (Sylvia) Qu trained as a veterinarian-scientist at the University of Pennsylvania, graduating with a Doctor of Veterinary Medicine (V.M.D.) and a Ph.D. in Bioengineering in 2017. She then moved to Washington University in St. Louis, where she was an NIH F32 NRSA postdoctoral fellow with Dr. Farshid Guilak and is now a research instructor in the Department of Orthopaedic Surgery.

TALK TITLE

"Development vs. Regeneration: Skeletal Patterning and Outgrowth of the Murine Digit Tip"

VICKI ROSEN
PhD

keynote

Professor

Harvard School of Dental Medicine

Boston, MA

vicki_rosen@hsdm.harvard.edu

BIO

My first position as an independent investigator was at Genetics Institute, where my project was to identify factors present in bone that were responsible for bone formation. This idea, named bone morphogenetic protein (BMP) by Marshall Urist, remained an ill-defined concept for many years. My colleagues and I isolated the first BMPs genes and reported on their activities in 1988, and BMP2 received FDA approval for enhancing bone repair in 2002. I then moved to Harvard School of Dental Medicine, where I am Professor and Chair, Department of Developmental Biology. Research in my lab is focused on understanding how BMPs affect development, maintenance and repair of musculoskeletal tissues.

TALK TITLE

"Cues for Enhancing Musculoskeletal Regeneration From Studying BMP Signaling"

JIE SHEN
PhD

session 5

Assistant Professor
Orthopaedic Surgery

Washington University

St. Louis, MO

shen.j@wustl.edu

BIO

Dr. Jie Shen is an Assistant Professor in the Department of Orthopaedic Surgery at Washington University in St Louis. As a cartilage biologist with specialization in osteoarthritis (OA) and fracture repair, Dr. Shen's interests span aspects of bone and cartilage research, and are mainly focused on injury, repair, and regeneration of musculoskeletal tissues with the goal to understand the progenitor cell population, signals, and role of inflammation and aging on tissue injury and regeneration at the cellular and molecular level. By using of state-of-the-art methodologies, including genetic animal models, unbiased sequencing and cell/tissue engineering, Dr. Shen is to apply his multidisciplinary expertise in inflammation, metabolism and epigenetics, in musculoskeletal systems to advance the understanding of the pathological mechanisms and development of preventive and management strategies as well as personalized biologic therapies for OA and fracture nonunion.

TALK TITLE

"Fracture Nonunion: New Insights into Mechanism and Therapy"

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Abstracts

(Alphabetically by first author)

IMAGING AND RESOLVING SPATIOTEMPORAL COALESCENCE OF THE NEUROSKELETAL NICHE DURING BONE REMODELING

Alec T Beeve[1,2], Anna Li[1], Jennifer R Brazill[2], Erica L Scheller[1,2]
[1]Department of Biomedical Engineering, Washington University in St. Louis, St. Louis, MO
[2]Division of Bone & Mineral Diseases, Department of Internal Medicine, Washington University School of Medicine, St. Louis, MO

Various cell types and niche components coalesce at localized regions of the bone surface to orchestrate skeletal remodeling. The spatial restriction and temporal coordination of cellular crosstalk within this niche is critical for normal skeletal development, adaptation, and repair. Yet, feature quantification from long bone histology relies mainly on global methods that average skeletal metrics across an entire sample. This global data lacks spatial context inherently important to identify local cellular populations that directly modulate bone remodeling. We hypothesize that spatial analysis of long bone cross-sections will reveal novel intercellular relationships at bone remodeling sites, specifically between nerves and skeletal cells. Skeletal cells can secrete nerve guidance factors that may increase their prevalence at remodeling surfaces. In turn, localized innervation may provide neuronal factors that regulate skeletal metabolism. To study neuroskeletal crosstalk, we developed a pan-neuronal reporter mouse and optimized a histology workflow to obtain dynamic histomorphometry and nerve densities in the same fixed, frozen sections. Along with a custom computational tool called RadialQuant, we will use these sections to quantify and correlate nerve densities and mineral apposition on the same bone surface. For validation of our tool, we demonstrate known variations in apposition along the periosteum and endosteum. We also apply RadialQuant to parse contributions of the sciatic and femoral nerves to local tibial innervation via selective and combined neurectomy. With these new tools, we hope to explore local cellular crosstalk during bone remodeling, generating new hypotheses to develop novel skeletal therapeutics.

DEVELOPMENT OF MICRORNA-MEDIATED CARTILAGE PROGENITOR CELL-BASED OSTEOCHONDRAL TISSUE CONSTRUCTS

Austin Bell-Hensley[1], Hongjun Zheng[2], Jin Liu[2], Farshid Guilak[1,2,3,4], Audrey McAlinden[2,4,5]

[1]Department of Biomedical Engineering, Washington University, St. Louis, MO, USA
[2]Department of Orthopaedic Surgery, Washington University, St. Louis, MO, USA
[3]Center for Regenerative Medicine, Washington University, St. Louis, MO, USA
[4]Shriners Hospital for Children – St. Louis, St. Louis, MO, USA
[5]Department of Cell Biology & Physiology, Washington University, St. Louis, MO, USA

Degenerative cartilage ailments (e.g. osteoarthritis) cause damage to articular joints that native tissue cannot repair due to low cell proliferation and the avascular nature of the tissue. Tissue engineering constructs that incorporate both cellular and scaffold components have shown promise for treating these joint diseases. Progenitor cells that are widely used tend to develop fibrocartilage or hypertrophic cartilage which are not suited for the articular environment. A less common cell type, the cartilage progenitor cell (CPC), has the ability to differentiate into articular-like or hypertrophic chondrocytes, osteoblasts, or adipocytes. Here, microRNA overexpression was used to guide CPC differentiation down articular-like or hypertrophic chondrocyte lineages. MicroRNAs (miRs) are small non-coding RNAs that prevent translation of target protein-coding mRNAs. Because of their binding mechanism, each miR can target tens to hundreds of mRNAs in a given cell type which gives them a unique advantage for regulating complex processes like differentiation. In this investigation, the roles of miR-138 and the miR-181a/b-1 cluster were studied in CPC chondrogenesis. Further, CPCs were seeded onto clinically relevant demineralized human bone scaffolds. CPC seeding density and seeding conditions were varied to optimize CPC differentiation down skeletal lineages. In addition, a culture system that simultaneously exposed different regions of the seeded scaffold to osteogenic media and chondrogenic media (a “bi-culture system”) was employed to determine if CPC differentiation could be spatially guided. These results suggest the reparative potential for CPC-laden scaffolds in orthopaedic injuries.

POINT MUTATIONS IN *DNMT3A* CAUSE SKELETAL OVERGROWTH & CORTICAL BONE THINNING

Austin Bell-Hensley[1], Diana Christian[2], Hongjun Zheng[3], Audrey McAlinden[3,4,5], Harrison Gabel[2]

[1]Department of Biomedical Engineering, Washington University, St. Louis, MO, USA
[2]Department of Neuroscience, Washington University School of Medicine, St. Louis, MO, USA
[3]Department of Orthopaedic Surgery, Washington University, St. Louis, MO, USA
[4]Department of Cell Biology & Physiology, Washington University, St. Louis, MO, USA
[5]Shriners Hospital for Children – St. Louis, St. Louis, MO, USA

Overgrowth and intellectual disability (OGID) in humans is typified by height and/or head circumference > 2 standard deviations larger than the mean as well as intellectual disability. Recent studies have identified mutations in the DNA methyltransferase 3A (DNMT3A) gene as causative for a subset of OGID. DNMT3A is a critical regulator for DNA methylation during embryonic and early postnatal development. Homozygous mutations in *Dnmt3a* in mice leads to severe runting and poor survival beyond 1 week of age. However, heterozygous point mutations in the DNMT3A gene result in mice that have similar phenotypes to their human counterparts. In the present study, we show that two different point mutations in DNMT3A (R878H and P900L) cause skeletal overgrowth in mice. Others have found that these mice present with behavioral impairments, paralleling human OGID symptoms. Importantly, we have found that these mutations cause cortical bone to become thinner and weaker in skeletally mature mice – a phenotype that has not been monitored in OGID patients. This distinction is important for improving both clinical assessments as well as the quality of life for OGID patients. These studies also provide new information on a potential role for DNMT3A in regulating post-natal bone development.

SARM1 DRIVES TYPE 1 DIABETES-ASSOCIATED BONE SUPPRESSION AND FRAGILITY

Jennifer M. Brazill[1], Kristann L. Magee[1], Ivana R. Shen[1], Alec T. Beeve[1,2], Gretchen A. Meyer[3,4], Aaron DiAntonio[5,6], Jeffrey Milbrandt[5,6], and Erica L. Scheller[1,2]

[1]Division of Bone and Mineral Diseases, Department of Medicine, Washington University School of Medicine, St. Louis, MO
[2]Department of Biomedical Engineering, Washington University, St. Louis, MO, USA
[3]Department of Orthopaedic Surgery, Washington University School of Medicine, St. Louis, MO
[4]Program in Physical Therapy, Washington University School of Medicine, St. Louis, MO
[5]Department of Developmental Biology, Washington University School of Medicine, St. Louis, MO
[6]Needleman Center for Neurometabolism and Axonal Therapeutics, Washington University School of Medicine, St. Louis, MO

Neuroskeletal crosstalk can directly influence bone growth, homeostasis, and regeneration. Diabetic peripheral neuropathy (DPN) is a common complication of type 1 (T1D) and type 2 diabetes, and is routinely associated with bone loss and fracture. Sarm1 (sterile α and TIR motif-containing protein-1) is an NADase highly expressed in the nervous system that initiates axon degeneration upon a range of insults, including DPN. We hypothesized that Sarm1-dependent DPN disrupts neuroskeletal signaling and suppresses bone formation in T1D, leading to onset and progression of diabetic bone disease. To test this, we used a streptozotocin (STZ)-induced insulin-dependent model of T1D. T1D-associated impairments in bone formation and strength emerged as early as 3-weeks post induction of T1D. Knockout of Sarm1 prevented these deficits in females in a sex-dependent matter, despite comparable changes in body and muscle mass, hyperglycemia, and hypoinsulinemia. Interestingly, males exhibited Sarm1-dependent susceptibility to DPN, whereas females did not develop clinical symptoms of neuropathy in 15-weeks of T1D. This highlights the potential for Sarm1 activation to drive sub-degenerative axon dysfunction that disrupts neural regulation of bone formation. To isolate cellular mechanisms of Sarm1 in diabetic bone disease, we are now probing Sarm1-mediated bone transcriptomic responses, neuron-specific inactivation of Sarm1, and skeletal innervation patterns at sites of bone accrual during the onset of T1D-bone suppression. Our work identifies Sarm1 as a novel central mediator of diabetic skeletal disease and informs the therapeutic potential for clinical Sarm1 inhibitors currently under development to restore bone and nerve health in T1D.

POSTER LOCATION # 5

MICRORNA EXPRESSION PROFILE IN MANDIBLES FROM NORMOTENSIVE AND SPONTANEOUSLY HYPERTENSIVE RATS WITH PERIODONTAL DISEASE

Victor Gustavo Balera Brito[1,2], Carlos Ferreira Santos[3], Flavia Lombardi Lopes[4], Audrey McAlinden[2], Sandra Helena Penha Oliveira[1]

[1] Department of Basic Sciences, São Paulo State University (Unesp), School of Dentistry, Araçatuba, SP, Brazil
[2] Department of Orthopaedic Surgery, Washington University School of Medicine, St Louis, MO, USA
[3] Department of Biological Science, Bauru School of Dentistry, University of São Paulo, SP, Brazil
[4] Department of Production and Animal Health, São Paulo State University (Unesp), School of Veterinary Medicine, Araçatuba, SP, Brazil

Periodontal disease (PD) is an inflammatory disease affecting the tooth-supporting tissues; comorbidities, such as hypertension, can exacerbate disease progression. We previously showed that Spontaneously Hypertensive Rats (SHR) present with increased PD-induced alveolar bone loss, increased inflammatory burden, and altered expression of osteoblast and osteoclast markers. MicroRNAs (miR) are important epigenetic regulators that suppress the expression of target mRNAs. There is limited information on the role of miRs in the alveolar bone in the context of PD and hypertension. The goal of this study was to evaluate the miR profile in the mandible body from Wistar (normotensive) and SHR with PD. PD was induced in 10-week-old male Wistar rats and SHR via bilateral silk ligature in the first inferior molars. After 15 days, RNA was isolated from the mandibles for microRNA array analysis (Affymetrix® miRNA Array 4.1; n = 3). In healthy controls, 11 downregulated miRs were identified in SHR compared to normotensive rats (fold change ≥2, adj. p-value <0.01). Comparison of the hypertensive and normotensive groups with PD to their respective healthy controls identified 5 downregulated miRs shared between those comparisons. In addition, 18 differentially-expressed (DE) miRs were identified in the normotensive group, and 4 DE miRs were found in the hypertensive group. This study provides the first miRNA expression profiles associated with PD in normotensive and hypertensive rats. Data from this array will guide future studies in identifying miR candidates that modulate periodontal homeostasis and may help explain the increased alveolar bone loss associated with the hypertensive phenotype.

Imaging and Mechanical Testing Services offered by Washington University Structure and Strength Core for Routine and Innovative Assessment of Musculoskeletal Structures and Function.

Brodt MD, Migotsky N, Tang SY, Silva MJ

Washington University Musculoskeletal Research Core B, Washington University in Saint Louis

Core B specializes in bone imaging and mechanical testing, along with a variety of techniques for non-mineralized tissues. We have two MicroCT and a digital x-ray system for *in-vivo* and *ex-vivo* use. The MicroCT and the x-ray box can be used for longitudinal evaluation of bone changes *in-vivo*. These systems are complemented by the specimen MicroCT scanner that can also be used for *ex-vivo* specimens. Imaging allows the quantification of bone morphology (geometry) and bone density in both cortical and cancellous bone. Mechanical testing will allow the evaluation of function in the tissue of interest, e.g. bone strength. The combination of imaging and mechanical testing will thus allow the characterization of differences in outcome or phenotype due to treatment or genotype, with attribution to structural or material changes.

Another x-ray imaging system, operated in partnership with the WashU Center for Cellular Imaging, allows us to use 3D X-Ray Microscopy (XRM) to examine the microstructural features of bone at sub-micron resolution. We recently investigated 8 outbred strains of mice and found a 57% difference in osteocyte lacunae density between the highest and lowest strains. XRM allowed us to visualize and quantify the lacunae in 3D at resolution not possible with other imaging modalities or histology. The use of the XRM also allowed us to detect morphological differences between individual lacunae and microporosity.

Finally, the use of contrast-enhancement allows MicroCT based evaluation of nonmineralized soft tissues through the use of selective radiopaque agents. For example, the whole joint structure can be visualized at high-resolution by incubating the tissue in phosphotungstic acid (PTA) or polyoxometalates (POM). Another example is the use of osmium tetroxide to allow spatial quantitation of bone marrow fat.

DEFECTS IN BONE QUALITY AND OSTEOCYTE LACUNAR DENSITY ARE MITIGATED BY THE SYSTEMIC ABLATION OF RAGE IN A MOUSE MODEL OF TYPE 2 DIABETES

Kaitlyn S. Broz[1], Remy E. Walk[1], and Simon Y. Tang[1]
[1]Washington University in St Louis, St Louis, MO
broz@wustl.edu

Disclosures: None

Type 2 diabetes (T2D) patients present with bone mineral density-discordant spinal fractures that may be due to compromised bone quality. Hyperglycemia in T2D promotes the accumulation of advanced glycation end-products (AGEs) which adversely affects bone quality and mechanics.[1,2] AGEs also activate the receptor for AGEs (RAGE) to impair bone cell function.[3,4,5] We thus investigated the effects of systemic RAGE ablation on spinal bone quality and homeostasis in a mouse model of T2D. 6-8 month old female mice with homozygous mutations in the leptin receptor (db/db), with and without systemic RAGE signaling (RKO), and respective wild-type (wt) littermates were used for this study (4 groups; n=6-8). The db/db and db/db;RKO animals developed diabetes with similar levels hyperglycemia and elevated hemoglobin A1c. Consistent with diabetes in humans, higher BV/TV was noted in db/db (+72% vs wt; $p = 0.022$) and db/db;RKO (+77% vs RKO; $p= 0.011$). A fluorometric assay revealed increased AGEs in db/db mice (+90.2% vs wt, $p < 0.005$), while AGEs levels in db/db;RKO were unaffected by diabetes (vs RKO; $p = 0.90$). X-ray (3D, 455.8 nm voxel size) and light (2D) microscopy showed that db/db mice have reduced osteocyte lacunar density compared to wt (3D: $p = 0.07$; 2D: $p = 0.003$). Dynamic histomorphometry resulted in similar mineral apposition rates in all groups ($p > 0.7$), confirming RAGE ablation appears to affect lacunar density and osteocytes, but not mineralizing osteoblasts. Comparisons were made using multi-way ANOVA. Loss of RAGE signaling maintained osteocyte density and protected against the hyperglycemia-mediated accumulation of AGEs in T2D, suggesting that osteocytes may play a critical role in maintaining the bone matrix during disease.

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Tissue-Mimetic Hydrogel Composites for Musculoskeletal Tissue Engineering

Bryant, Stephanie J

Tissue engineering and regenerative medicine offer exciting alternative therapies to tissue and organ transplantation. However, many challenges remain. An ideal therapeutic approach would direct stem cell differentiation, allow for new tissue growth, and restore function. To this end, our approach combines soft biomimetic hydrogels with stiff 3D printed structures fabricated by stereolithography which are capable of supporting the mechanical forces *in vivo*. The soft biomimetic hydrogels are designed with extracellular matrix analogs and growth factors tethered into a degradable synthetic polymer network to direct local differentiation of stem cells. A major focus of our research is on designing soft biomimetic hydrogels that promote chondrogenesis and osteogenesis by the incorporation of growth factors (i.e., TGFβ3, IGF-1, and BMPs) and tissue-specific ECM analogs (i.e., chondroitin sulfate, hydroxyapatite) for cartilage, bone, and osteochondral tissue regeneration with the ultimate goal of treating injuries in articulating joints and the growth plate.

CARBONIC ANHYDRASE 2 MAINTAINS CHONDROCYTE METABOLIC HOMEOSTASIS

Lei Cai, Mingming Yan, Xin Duan, Regis J. O'Keefe, M. Farooq Rai

Department of Orthopaedic Surgery, Washington University School of Medicine, St. Louis, MO

Here, we investigated the role of carbonic anhydrase II (encoded by *CA2*), the most efficient isoform in the CA family of zinc-containing metalloenzymes, in chondrocyte metabolism. CA2 expression was measured in human and mouse osteoarthritic cartilage. RNA-seq was used to probe biological processes altered by lentivirus-mediated CA2 knockdown. Chondrocytes were cultured under normoxia or hypoxia after CA2 knockdown. Cell metabolism was studied by measuring extracellular lactate production, glucose consumption, intracellular ADP/ATP, pH_i, ROS and glycolysis. The effect of CA2 knockdown on anabolic and catabolic markers was measured following exposure to IL-1β. Effect of CA2 was examined on cell proliferation, cell-cycle, colony formation, and migration. Results showed that CA2 was highly expressed in osteoarthritic cartilage. RNA-seq analysis revealed enrichment of glycolysis, apoptosis and TNF signaling in CA2-deficient cells. CA2 expression was 10-fold higher under hypoxia and its knockdown reduced extracellular lactate production, exacerbated ADP/ATP ratio, impaired glycolysis, decreased glycolytic capacity and lowered the expression of glycolysis rate-limiting enzymes, but did not affect the pH_i and ROS. CA2 deficiency disturbed chondrocyte anabolic and catabolic equilibrium. CA2 knockdown suppressed chondrocyte migration and proliferation and arrested cell-cycle. Forced expression of CA2 stabalized chondrocyte metabolism. In summary, we uncover a novel mechanistic role of CA2 in chondrocyte metabolism and inflammation. These findings indicate that CA2 is required for the maintenance of chondrocyte metabolic homeostasis. Future work is needed to further illuminate functional role of CA2 in osteoarthritis development.

SINGLE CELL RESOLUTION IDENTIFICATION OF INFILTRATING IMMUNE CELL SUBTYPES FOLLOWING INTERVERTEBRAL DISC INJURY

Sade W. Clayton[1], Aimy Sebastian[2], Nicholas R Hum[2], Remy E. Walk[1], Garrett W Easson[1], Rachana Vaidya[1], Gaby G Loots[2], Simon Y. Tang[1]
Washington University in St. Louis, St. Louis, MO[1]
Lawrence Livermore National Laboratory, Livermore, CA[2]

Intervertebral disc (IVD) degeneration impairs spinal function and contributes to debilitating low back pain that accelerate with aging and trauma. There are no disease modifying treatments that restore function, and little is known about the regenerative mechanisms of the IVD. Successful healing in connective tissues rely on well-orchestrated immune cell subtypes to infiltrate damaged tissue and initiate regeneration. However, the identities of these immune cells following an acute IVD injury remain relatively unknown. Thus, we aim to identify the infiltrating immune cells and their functions after IVD injury. Following tissue harvest and cell extraction, single cell RNA sequencing was performed on control (noninjured) and injured (bilateral 30G needle puncture) caudal IVDs (CC) 7 days post injury in 12 wk old female C57/BL6 mice. Each sample contained 15 IVDs pooled from 3 mice, and 5 control (CC12/13- CC16/17) and 5 Injured (CC5/6- CC9/10) IVDs were collected from the same animal. Cluster analysis identified 9 distinct clusters amongst 6 cell populations: annulus fibrosus, nucleus pulposus, immune cells, endothelial cells, pericytes, and stem-like cells. Interesting, the differentially expressed genes in the immune cell clusters revealed the upregulation monocyte/macrophage markers (Cd68, Cd14), and the downregulation of dendritic cell (Cd83) and B lymphocyte markers (Cd79a/b, Cd19). Future experiments will determine the trajectories of immune cell subtypes during the early stages of healing. We hypothesize that certain subtypes of immune cells have important, distinct roles during specific timepoints of the inflammation stage of healing that influence regeneration of injured IVDs.

PROLIFERATION OF OSTEOBLAST LINEAGE CELLS IN THE FIRST 7 DAYS POST FRACTURE IS REQUIRED FOR NORMAL HEALING IN MICE

Andre F. Coello Hernandez[1,2], Jennifer A. McKenzie[1], Katherine R. Hixon[1], Anna N. Miller[1], Matthew J. Silva[1,2]
[1]Dept. of Orthopaedic Surgery, [2]Dept. of Biomedical Engineering, Washington University in St. Louis, St. Louis, MO
a.coellohernandez@wustl.edu

ABSTRACT:
Atrophic nonunions are an inadequate formation of an osteochondral callus leading to failed fracture healing. Treatments for atrophic nonunions require a better understanding of their biology. A key feature of fracture healing is periosteal cell proliferation in the first 2 weeks. We investigated proliferation in fracture healing in mice expressing a thymidine kinase (TK+) 'suicide gene' in the 3.6Col1a1 promoter, active in periosteal osteoblast lineage cells. Proliferating TK+ cells die when given ganciclovir (GCV), without affecting non-dividing cells. We hypothesized fracture healing is impaired when giving GCV for 3, 7, or 14 days post fracture (DPF). We performed a full femur fracture and fixation on 12-week-old TK+ mice and their wildtype (WT) littermates. Following fracture, mice were dosed daily with GCV for 3, 7, or 14 days until sacrifice at day 21. Weekly radiographs monitored healing and found 90% of TK+ mice receiving GCV for 3 days had a fully bridged callus at 21 DPF, while TK+ mice receiving GCV for 7 and 14 days showed 57% and 25% bridging, respectively. µCT analysis found TK+ mice had progressively smaller total callus volume with increasing GCV dosing duration, and lower bone volume compared to WT in 7 and 14 day GCV dosing groups. Day 21 histology showed all WT mice had fully bridged calluses, regardless of GCV dosing. TK+ mice receiving GCV for 3 days also had fully bridged calluses. In contrast, TK+ mice dosed for 7 and 14 days showed progressively less mineralized calluses with more cartilage and fibrous tissue present, indicating impaired fracture healing. We conclude that proliferation of osteoblast lineage cells in between 3 and 7 DPF is required for normal fracture healing in mice.

IDENTIFYING DRIVERS OF AGING AMONG GENES DIFFERENTIALLY EXPRESSED WITH AGE

Ariella Coler-Reilly[1,2], Sandeep Kumar[1], Toshifumi Sugatani[1], Zachary Pincus[3,4], Erica L. Scheller[1,3], Tim R. Peterson[1], and Roberto Civitelli[1].

[1]Division of Bone and Mineral Diseases, Department of Medicine, Washington University School of Medicine, St. Louis, MO, USA

[2]Medical Scientist Training Program, Washington University School of Medicine, St. Louis, MO, USA

[3]Department of Biomedical Engineering, Washington University School of Medicine, St. Louis, MO, USA

[4]Departments from Genetics and Developmental Biology, Washington University School of Medicine, St. Louis, MO, USA

Aging is the primary risk factor for major chronic illnesses plaguing the developed world, including cardiovascular disease, osteoporosis, and dementia. To combat these conditions at the source, researchers have turned their attention to deciphering the mechanisms of aging. Dozens of studies have emerged comparing gene expression in young and old samples, identifying numerous genes differentially expressed with age. However, these studies only describe associations, failing to distinguish drivers of aging from the multitude of downstream passengers and compensatory geroprotective pathways. To address this critical flaw, we are developing a workflow to characterize the effects of differentially expressed genes on healthy lifespan, stem cell function, and cellular senescence. Using the NCBI Gene Expression Omnibus, I located 25 datasets comprising samples of various tissues taken from healthy, untreated adult mammals at two distinct ages. I performed a meta-analysis of these datasets and produced a ranked list of genes, ordered by the number of datasets in which the gene was differentially expressed with age. To functionally characterize the top-ranked genes, I selected two complementary experimental systems. First, effects on lifespan are assessed by subjecting *C. elegans* worms to post-developmental RNA interference of the corresponding ortholog. Second, primary human mesenchymal stem cells are transfected with a plasmid carrying the corresponding DNA or shRNA, and markers of normal function and cellular senescence are used to screen for rejuvenating effects. Our study is expected to produce insight into the relationship between differential gene expression and aging phenotypes and reveal novel therapeutic targets.

POSTER LOCATION # 19

LEPTIN IS A KEY MEDIATOR IN FAT-CARTILAGE CROSSTALK

Kelsey H. Collins[1,2,3], Erica V. Ely[1,2,3], Kristin L. Lenz[1,2,], Arin K. Oestreich[1,2,3], Bo Zhang[3,4], Farshid Guilak[1,2,3]

[1]Department of Orthopaedic Surgery, Washington University in St. Louis School of Medicine
[2]Shriners Hospitals for Children, St. Louis
[3]Center of Regenerative Medicine, Washington University in St. Louis School of Medicine
[4]Department of Developmental Biology, Washington University in St. Louis School of Medicine

Fat free lipodystrophic (LD) mice are protected from cartilage damage in an injury induced osteoarthritis model, implicating fat-cartilage crosstalk. Mouse embryonic fibroblast (MEF)-derived fat implants restore 15-20% of circulating leptin and reintroduce cartilage damage in LD mice. However, it is unknown if secreted leptin from MEF derived fat drives cartilage degradation. To answer this question, LD mice (adiponectin-DTA) and littermate DTA/+ mice (n=12-19/group) were given one of the following types of fat transplant: wildtype MEF (MEF), leptin deficient heterozygous MEF (MEF-OB/+) or leptin knockout MEF (MEF-OB/OB). Mice were challenged with unilateral destabilization of the meniscus (DMM) at 16 weeks, pain assessments were made at 27 weeks, and mice were sacrificed at 28 weeks of age. All types of fat transplants were prepared for bulk sequencing (n=3-5/group). Knee joints were scored histologically. MEF-OB/OB transplanted LD mice had less cartilage damage compared to controls and were protected from increased pressure-pain hyperalgesia and allodynia with DMM ($p<0.05$). Bulk RNA seq of the MEF transplanted fat revealed close clustering with MEF-OB/OB and DTA/+ visceral adipose, suggesting similarities to native adipose despite leptin deficiency. Interestingly, there were 1747 upregulated and 2710 downregulated genes in MEF-OB/OB vs MEF fat. Upregulated GO Biological Processes in MEF-OB/OB in were involved in neutrophil activation, immune responses, and low density lipoprotein particle binding, along with decreased voltage-gated potassium channel activity. Together, this work suggests the absence of leptin in MEF-OB/OB fat alters immune responses in fat and prevents cartilage damage in the knee following injury.

SIZE-DEPENDENT SOLUTE DIFFUSION THROUGH SYNOVIAL EXPLANTS: AN EXPERIMENTAL-COMPUTATIONAL APPROACH

Alexandra L. Davis[1], Milad Rohanifar[1], Young Guang[1], Thomas M. McGrath[1], Christine T.N. Pham[2], James A.J. Fitzpatrick[1,3], Lori A. Setton[1,4]

[1]Department of Biomedical Engineering, Washington University in St. Louis, St. Louis, MO
[2]Division of Rheumatology, Washington University School of Medicine, St. Louis, MO
[3]Center for Cellular Imaging, Washington University School of Medicine, St. Louis, MO
[4]Department of Orthopedic Surgery, Washington University School of Medicine, St. Louis, MO

The synovium is a thin, multilayer tissue that separates the diarthrodial joint space from systemic circulation; its draining lymphatic and blood vessels rapidly clear drugs from the synovial joint space, hindering the benefits of intra-articular delivery. Drug clearance has been studied in pre-clinical models and human subjects, but comparing quantitative parameters such as half-life and diffusivity remains challenging due to unknown boundary conditions and material properties as well as system geometries that vary across studies. We previously developed a finite element model of synovium with fixed permeability, porosity, and material properties, which could be used with experimental diffusion data to determine urea's intrinsic effective diffusivity (D_{eff}) through synovial explants. Here, we directly measured synovium's compressive properties and hydraulic permeability. We also studied how molecular weight (MW) influences the diffusion of model uncharged solutes in synovium. As expected, D_{eff} depended on fluid pressure and decreased as MW increased in human and porcine synovium. We then calculated solute half-lives in synovium that increased with solute MW; this trend generally follows *in vivo* results reported elsewhere. This suggests that *in vitro* studies in synovial tissue could predict some aspects of drug transport *in vivo*.

THE ROLE OF THE OSTEOCLAST IN PEDIATRIC OSTEOMYELITIS

Jennifer L. Davis[1], Saumya Bhagat[1], Linda Cox[1], Luke O'Connor[1], Chun Wang[1], Jenna R[2]. Petronglo, Jim E. Cassat[2-6], Gabriel Mbalaviele[1], Deborah J. Veis[1,7,8]
[1]Musculoskeletal Research Center, Division of Bone and Mineral Diseases, Washington University School of Medicine, St. Louis, MO, USA.
[2]Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, Nashville, TN, USA
[3]Department of Biomedical Engineering, Vanderbilt University, Nashville, TN, USA
[4]Department of Pediatrics, Division of Pediatric Infectious Diseases, Vanderbilt University Medical Center, Nashville, TN, USA
[5]Vanderbilt Institute for Infection, Immunology, and Inflammation (VI4), Vanderbilt University Medical Center, Nashville, TN, USA
[6]Vanderbilt Center for Bone Biology, Vanderbilt University Medical Center, Nashville, TN, USA
[7]Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO, USA.
[8]Shriners Hospitals for Children, St. Louis, MO, USA

Hematogenous osteomyelitis (HOM) due to infection with *S. aureus* is common in children and mainly occurs in areas of high tissue turnover such as the metaphyses. Eradication of established bacterial in bone remains a clinical challenge. While osteoblasts, osteocytes, osteoclasts (OC) all may harbor *S. aureus*, we recently demonstrated that *S. aureus* can replicate within OC. Based on these data, we hypothesized that elimination of the OC as a niche for bacterial colonization and growth will reduce the burden of infection. To reduce the number of OCs, we employed a murine anti-RANKL antibody (10mg/kg, subcutaneous). Dosing trials revealed durable inhibition of serum TRAP for 4-28 days following a single injection in young, naïve mice. Applying this to a model of HOM, 6 wk old mice were given either control IgG or anti-RANKL antibody 4 days prior to tail vein inoculation of a bioluminescent *S. aureus* clinical isolate, and infection was monitored by bioluminescent imaging (BLI) for 2 weeks. Serum TRAP levels remained low in infected mice with RANKL blockade, suggesting effective inhibition of osteoclastogenesis. Hindlimb BLI signals (peak and endpoint) were lower in the anti-RANKL vs. IgG group. Infection incidence also trended lower (50% vs 71% of legs at risk). Infection severity was remarkably decreased following OC inhibition, with 43% of IgG animals presenting with high BLI vs 0% with anti-RANKL 2 weeks post-infection. MicroCT analysis showed maintenance of bone in the anti-RANKL group vs bone loss in the IgG controls. Lower serum IL-18 in the anti-RANKL group indicated less inflammasome activation with OC blockade. Collectively, our results position OCs as novel and active players in the progression of pediatric HOM.

MICROFLUIDIC FABRICATION AND CHARACTERIZATION OF RADIOPAQUE BARIUM SULFATE POLYETHYLENE GLYCOL-BASED HYDROGEL MICROSPHERES

Ether Dharmesh, Samuel Stealey, Silviya Petrova Zustiak
Department of Biomedical Engineering, Parks College, Saint Louis University

Currently, catheter embolization procedures used in prostatic hyperplasia and liver cancer treatments employ non-opaque microspheres with a tracer dye, which can result in off-target embolization. The goal of this study was to fabricate monodisperse, radiopaque polyethylene glycol (PEG)-based barium sulfate hydrogel microspheres that can be easily tracked via microcomputed tomography (microCT) during embolization procedures. The hydrogel microspheres were fabricated using 4-arm PEG-Acrylate macromer and PEG-dithiol crosslinker solutions in a custom-designed microfluidic chip that allowed for on-chip mixing and gelation of a timed-gelation system, greatly improving bead fabrication throughput and reproducibility. Microspheres were loaded with 1 μm barium sulfate particles and bovine serum albumin to aid in barium suspension. Microspheres were imaged both before and after washing with a buffer using an inverted microscope to measure microsphere diameter and degradation. Settling of barium sulfate solutions was measured qualitatively in microcentrifuge tubes. Monodisperse, barium sulfate loaded-hydrogel microspheres were produced, the size of which could be controlled by modulating the inlet flow rates of the dispersed and continuous phases. The results show that the opacity and stiffness of the barium sulfate hydrogels increased with increased barium sulfate concentration. Furthermore, the swelling ratio, mesh size, and gelation time of hydrogels decreased with increased barium sulfate concentration. Future experiments will evaluate the radiopacity under microCT, degradation properties, and injectability of the hydrogel microspheres to further explore their application in catheter embolization procedures.

KIF26B KNOCKDOWN PREVENTS OSTEOGENESIS OF STEM CELLS AND ATTENUATES ECTOPIC CALCIFICATION IN MICE

Xin Duan, Mingming Yan, Lei Cai, Robert Brophy, Matthew Silva, Farshid Guilak, M. Farooq Rai

Department of Orthopaedic Surgery, Washington University School of Medicine, St. Louis, MO

Ectopic calcification is an osteogenic process that leads to the formation of inappropriate bone within soft tissues, with limited understanding of underlying mechanism. Using a population genetics approach, we identified an association of the kinesin superfamily member 26b (Kif26b) with ectopic calcification in mice, consistent with a GWAS study that identified KIF26B as a severity locus for heterotopic ossification in patients with hip osteoarthritis. However, little is known about the mechanistic role of KIF26B in ectopic calcification. Here, we studied functional role of KIF26B in osseous and chondrogenic differentiation of stem cells and in a murine model of intra-articular ectopic calcification. We found that KIF26B knockdown arrested osteogenesis of stem cells and promoted chondrogenesis. Moreover, KIF26B knockdown significantly decreased cell viability and proliferation and induced cellular apoptosis. Mechanistically, loss of osteogenesis was reverted by the addition of a Wnt agonist demonstrating a role of KIF26B in canonical Wnt/β-catenin signaling. Finally, intra-articular delivery of Kif26b shRNA in B6-129SF2/J mice significantly hampered the development of intra-articular ectopic calcification at 8 weeks after injury compared with mice treated with non-target scrambled shRNA. In summary, these observations highlight that KIF26B plays a crucial role in ectopic bone formation by repressing osteogenesis, but not chondrogenesis, potentially via modulating Wnt/β-catenin signaling. These findings establish KIF26B as a critical determinant of the osteogenic process in pathologic endochondral bone formation and an actionable target for pharmacotherapy to mitigate ectopic calcification (and heterotopic ossification).

TRPV4 INHIBITION PROTECTS AGAINST THE INVERTEBRAL DISC DEGENERATION CAUSED BY SUSTAINED LOADING

Garrett Easson[1], Alireza Savadipour[1,3], Leanne Iannucci[4], Spencer Lake[1,4], Farshid Guilak[1-4]
Simon Tang[1,2]
[1]Department of Mechanical Engineering, Washington University in St. Louis
[2]Department of Orthopaedic Surgery, Washington University – School of Medicine
[3]Shriner's Hospital for Children – St. Louis
[4]Department of Biomedical Engineering, Washington University in St. Louis

Numerous factors contribute to intervertebral disc (IVD) degeneration including abnormal mechanical loading. Abnormal loading, such as sustained loading or overloading, initiates an upregulation of inflammatory factors which then initiate or accelerate the degenerative process. The molecular mechanisms responsible for transducing mechanical stimuli into this inflammatory response are still not fully understood. The ion channel, TRPV4, is a strong candidate for mechanotransduction in the IVD. The role of TRPV4 in IVD mechanical loading was evaluated in whole tissue IVD culture (n=8/group). IVDs underwent sustained loading for 24 hours of which half were treated with the TRPV4-antagonist, GSK205. After 14 days of culture, IVDs were histologically scored for degeneration using a standardized system. Compressed IVDs exhibited significant degeneration compared to the non-loaded IVDs ($p<0.05$). The compressed IVDs with concurrent TRPV4 inhibition were significantly less ($p<0.05$) degenerated than the IVDs with intact TRPV4 signaling. Compressed IVDs also exhibited characteristics of degeneration including the rounding of annulus fibrosus (AF) cells, AF fiber mis-alignment, the loss of the boundary the AF - nucleus pulposus (NP) boundary, and doublet cells in the IVD endplates. The inhibition of TRPV4 completely prevented degenerative changes in the end plates as well as partially protecting the IVDs against degenerative changes to the AF and the AF / NP interface. Inhibition of TRPV4 did not protect against degeneration that was observed in the NP. These findings demonstrate the role of TRPV4 transducing damaging mechanical loading of the IVD into degeneration.

GENOME ENGINEERED IPSCS AS A SYSTEM TO DISENTANGLE ADIPOSE TISSUE SIGNALING IN OSTEOARTHRITIS

Erica V. Ely[1,2,3], Ruhang Tang[1,2,3], Farshid Guilak[1,2,3], Kelsey H. Collins [1,2,3]

[1]Department of Orthopaedic Surgery, Washington University in St. Louis School of Medicine
[2]Shriners Hospitals for Children, St. Louis
[3]Center of Regenerative Medicine

Introduction: Obesity is a key risk factor of osteoarthritis (OA), with obese patients representing >60% of people with OA. While adipose tissue has been shown to be an important mediator of cartilage[1], the complex secretome of adipocytes is context-dependent, making it difficult to determine which signaling factors are involved in OA progression. The goal of this study was to bioengineer designer adipose implants using murine induced pluripotent stem cells (iPSCs) as a system to facilitate deconstruction of adipokine signaling.
Methods: iPSCs were generated using an established protocol[2,3] and treated with adipogenic media, control expansion media, or transduced with a control Pparg lentivirus. Additionally, we used CRISPR-Cas9 to create Leptin Knockout and Ccl2-IL1Ra iPSCs, which function as tools for gene knockout and drug delivery. Engineered cells were compared to native adipocytes, and morphology was determined in monolayer culture with BODIPY/DAPI and Oil Red O (ORO) staining. Gene expression profiles of iPSC-derived adipocytes were generated using RT-qPCR. Outcomes were assessed by one-way ANOVA ($n=4-6$, $p<0.05$).
Results: iPSCs demonstrate robust adipogenesis by ORO and BODIPY on days 5-14 with adipogenic media compared to expansion media, which was similar to Pparg lentivirus. Peak gene expression for key adipogenic markers was observed on day 11. Leptin knockout iPSCs showed failed adipogenesis; however, adding 500ng/ml mouse leptin to the medium rescued the adipogenic phenotype. Ccl2-IL1Ra iPSCs demonstrate robust adipogenesis and maintain the ability to secrete IL-1Ra.
Conclusion: We created adipocytes from non-edited and CRISPR-Cas9 genome-edited iPSCs in a virus-free manner. Additionally, we found a critical role for leptin as a mediator of iPSC growth and adipogenesis. Designer adipose tissue implants will be applied to disentangle the role of adipokines in cartilage damage and to develop therapeutic fat implants with this and other engineered cell lines[3] to deliver autoregulated cell-based anti-inflammatory mediators.

References: 1. Collins+ *PNAS* 2020 2. Diekman et al *PNAS* 2012 3. Brunger et al *Arthritis & Rheumatology* 2017 4.Tashiro+ *Stem Cells* 2009

BONE-DERIVED DKK1 SUPPORTS HEMATOPOIETIC STEM CELLS DURING TUMOR PROGRESSION

Emily Eul[1], Roberta Faccio[1]
[1]Department of Orthopaedics, Washington University School of Medicine, St. Louis, MO, USA

Hematopoiesis, the process by which blood and immune cells are generated from hematopoietic stem and progenitor cells (HSPCs), occurs in the bone marrow, and is altered at the onset of primary tumors to favor the generation of pro-tumorigenic immune populations. The mechanisms behind these immunological alterations are not fully understood. Dickkopf-related protein 1 (Dkk1), a Wnt/β-catenin signaling inhibitor, is highly produced in the bone, and is best known for suppressing bone formation and increasing bone resorption. Dkk1 has also been shown to directly and indirectly affect HSPCs at steady state and under stress conditions, but it is unclear if Dkk1 alters hematopoiesis during cancer progression. To assess changes in HSPC frequencies during tumor progression, female C57BL/6 mice were orthotopically injected with EO771 breast cancer cells. HSPC populations were detected in the bone marrow by flow cytometry 14 or 30 days after tumor injection. We observed increases in HSPC populations during tumor progression, and saw similar results using the B16F10 melanoma model. Additionally, we administered Dkk1 neutralizing antibody to EO771 tumor bearing mice (10mg/kg every 48 hrs), and saw a striking decrease in HSPC frequencies at the 30 day time point but no changes on day 14. Findings correlated with reduced tumor growth and decreased numbers of tumor promoting immune populations. Further work using osteoblast lineage specific Dkk1 overexpressing mice (2.3kbCol1α1Dkk1Tg) showed increases in HSPC frequencies and tumor growth compared to controls. All together these results suggest that bone-derived Dkk1 might drive alterations to hematopoiesis that support the generation of a tumor promoting immune landscape.

ADULT-DERIVED OSTEOLINEAGE (OSTERIX +) CELLS AS A NEW REGULATOR OF BREAST CANCER TUMORIGENESIS

Giulia Furesi[1], Roberta Faccio[1]

[1]Department of Orthopedics, Washington University School of Medicine, St. Louis, MO, USA

Osterix (Osx) is an osteogenic marker required for osteoblast differentiation. Although its expression was thought to be limited to bone, we identified $CD45^{neg}Osx+$ cells in primary tumors at extra-skeletal sites. However, the origin, identity, and function of these extra-skeletal Osx+ cells are unknown.
By using Osx-cre;TdTomato reporter mice orthotopically injected with PyMT breast cancer cells, we confirmed by IF the presence of elongated $CD45^{neg}TdT^{Osx}+$ cells, sharing the morphology of tumor supportive cancer-associated fibroblasts (CAF). RNA-seq and FACS analyses of tumor-infiltrating $CD45^{neg}TdT^{Osx}+$ cells confirmed the expression of CAF markers, such as a-SMA, Fsp1, and Fap, but also of osteoblast-related genes. To elucidate their role in tumor progression, $CD45^{neg}TdT^{Osx}+$ cells were sorted from PyMT mammary tumors and co-injected with tumor cells at 1:2 or 1:1 ratio into WT recipient mice. Strikingly, $CD45^{neg}TdT^{Osx}+$ cells significantly increased tumor growth compared to mice injected with tumor cells alone, in all conditions tested. To determine if tumor-infiltrating $CD45^{neg}TdT^{Osx}+$ cells derive from the bone marrow, we investigated their presence in circulation and observed a 5-fold increase in their numbers in tumor-bearing mice compared to controls. Next, we performed bone marrow transplantation using lethally irradiated WT mice transplanted with BM from $TdT^{Osx}+$ mice. The resulting chimeric mice were orthotopically inoculated with PyMT cells, and $TdT^{Osx}+$ were detected at tumor site, suggesting that they originate from BM-derived Osx+ cells. In sum, our data demonstrate the presence of osteolineage Osx+ cells in extra-skeletal tumors, which have tumor-promoting effects and may represent a new subset of CAFs.

POSTER LOCATION # *(podium talk only)*

ENRICHING MSC PARACRINE EFFECTS BY ENHANCING N-CADHERIN INTERACTION FOR ISCHEMIC LIMB REGENERATION

Ning Gao[1], Zhongting Liu[1], Ya Guan[1], Ting Zhong[1], Gretchen Meyer[2], Jianjun Guan[1]
[1]The Institute of Materials Science & Engineering, Mechanical Engineering & Materials Science
[2]Program in Physical Therapy
Washington University in St. Louis, St. Louis, MO 63130

Peripheral artery disease (PAD) is a progressive atherosclerotic disorder that affects more than 200 million people worldwide. The end-stage manifestation and most severe form of PAD is critical limb ischemia (CLI). Stem cell therapy has been considered as a promising approach for treating CLI. However, the low survival rate or impaired function of transplanted cells due to anoikis will compromise the therapeutic efficacy. N-cadherin, as a calcium-dependent cell-cell adhesion protein, is involved in the adhesion, paracrine effect, and differentiation of mesenchymal stromal cells. In this work, a thermosensitive biodegradable hydrogel poly(NIPAAm-co-MAPEG-co-AOLA-co-NAS) was designed, which conjugated the N-Cadherin mimic peptide HAV to promote cell-matrix interaction. This conjugation of HAV functionalized the hydrogel (Gel-HAV) with the ability to promote the expression of N-Cadherin in Induced Pluripotent Stem Cells (iPSC)-derived MSCs (iMSCs), and therefore, increase attachment triggered paracrine effect and cell survival. The growth factors collected from iMSCs cultured in Gel-HAV could accelerate myoblasts and endothelial cells migration in vitro, which contributed to a faster myotube formation and tube formation. To further reveal its clinical outcomes, mouse CLI model was applied, and blood flow, vessel density, muscle function, and other relative indications were detected. The delivery of iMSCs encapsulated in this functionalized pNMAN hydrogel (Gel-HAV) showed a great outcome in improving angiogenesis and myogenesis. Further studies also indicated that iMSCs delivered with Gel-HAV could promote the activation of skeletal muscle satellite cells, and therefore, accelerate myogenesis.

METABOLIC REGULATION OF INTRASYNOVIAL FLEXOR TENDON REPAIR

Richard H Gelberman[1], Ryan Lane[1], Shelly E Sakiyama-Elbert[2], Stavros Thomopoulos[3], and Hua Shen[1],
[1]Washington University in St. Louis, St. Louis, MO, [2]University of Texas, Austin, TX, [3]Columbia University, New York, NY

Avascular intrasynovial flexor tendons exhibit minimal cellular and vascular responses and variable healing outcomes after injury and repair. In contrast, extrasynovial flexor tendons show robust cellular and vascular responses and negligible postoperative complications. Based on these observations and our prior findings, we hypothesized that the divergent healing outcomes between the two flexor tendon regions are in part due to their differences in metabolic signature and shifting the metabolic signature of intrasynovial tendons toward that of extrasynovial tendons can improve intrasynovial tendon healing. To test the hypothesis, we compared canine intrasynovial and extrasynovial flexor tendon metabolism and tenogenic potency. Healthy instrasynovial flexor tendons expressed three times more *PDK1* but much less *SCX* and *IGF1* than extrasynovial tendons. *PDK1* encodes a pyruvate dehydrogenase kinase that inhibits the conversion of pyruvate generated from glycolysis to acetyl-coA and thereby ATP production via oxidative phosphorylation (OXPHOS). Consistently, intrasynovial tendon cells produced 60% of ATP via glycolysis, whereas less than 40% of ATP was generated through this pathway in extrasynovial tendon cells. Inhibition of PDK1 with dichloroacetate (DCA) successfully increased ATP production via OXPHOS in intrasynovial tendon cells. Therefore, we used DCA to assess the effect of metabolic reprogramming on intrasynovial tendon healing in a canine flexor tendon injury and repair model. Oral DCA administration doubled Acetyl-CoA content, reduced inflammatory genes *TLR4* and *CD86* expression, and increased tenogenic genes *SCX*, *IGF1*, and *TGFB3* expression in intrasynovial flexor tendons 7 days after tendon repair. Histologically, DCA increased neovascularization, promoted tendon cell proliferation, and improved tendon reunion compared to untreated tendons. These findings supported a beneficial effect of regulating glucose metabolism in improving early intrasynovial tendon healing after repair.

COMPRESSIVE STATIC LOADING OF DIABETIC MURINE INTERVERTEBRAL DISCS CAUSES ACCELERATED DEGENERATION AND IS RESCUED BY TRPV4 INHIBITION.

Christian Gonzalez, BS[1,2], Simon Tang, PhD, MSCI[1,2]

[1] Dept of Biomedical Engineering, Washington University in St. Louis, St. Louis, MO, USA

[2] Dept of Orthopaedic Surgery, Washington University in St. Louis, St. Louis, MO, USA

Type-2 diabetes (T2D) is a disease which promotes inflammatory response and degeneration in IVDs, primarily mediated through AGE-RAGE signaling. Continuous AGE accumulation via hyperglycemic effects in T2D has been shown to lead to stiffening of the IVD, implicating degenerate T2D discs as being more prone to mechanical degradation than endogenous discs. Endogenous IVDs are already prone to mild mechanical degradation in the presence of compressive static loading conditions, signifying that loading may accelerate degeneration in T2D. Biological mechanotransduction of loading is primarily controlled by Ca^{2+} ion channel TRPV4, which has been shown to be an effective therapeutic target in mitigating loading induced degeneration. This relationship between AGEs, T2D-induced degeneration, and supraphysiological static load is important to understand for clinical settings, especially in the context of obesity and diabetes as comorbidities. Based on this understanding, our central hypothesis of is that loading-induced IVD inflammation and degeneration is accelerated in the presence of diabetes and can be mitigated by inhibition of mechanotransductive ion channel TRPV4. An *in vitro* organ culture study was conducted on coccygeal IVDs of db/db;NFκB-GFP-luc+/- mice to examine this hypothesis. Histological analysis indicated the relationship of static loading, diabetes, and TRPV4 to be along the lines of that suggested in the central hypothesis. Longitudinal assays of inflammatory cytokines VEGF-A and IL-6 yielded mixed results, indicating that static loading and modulation of TRPV4 may have influence on downstream inflammatory effects of diabetes.

PROLIFERATION OF EARLY AND LATE OSTEOBLASTS IS REQUIRED FOR CALLUS FORMATION FOLLOWING FULL FEMUR FRACTURE

Nicole R. Gould[1], Jennifer A. McKenzie[1], Katherine R. Hixon[1], Andre F. Coello Hernandez[1], Leyi Chen[1], David M. Ornitz[2], Matthew J. Silva[1]

[1]Department of Orthopaedic Surgery, Washington University in St. Louis, St. Louis MO

[2]Department of Developmental Biology, Washington University in St. Louis, St. Louis MO

Atrophic non-union following fracture is the permanent failure of a broken bone to properly heal due to inadequate formation of a mineralized callus. Atrophic non-unions arise from a failure in biology, whether that is a lack of osteoprogenitor cells or poor vascularity. Our lab previously found that proliferation of 3.6Col1A1-expressing periosteal cells is essential for fracture healing. Single cell RNAseq of early fracture calluses shows that 3.6Col1A1-expressing cells constitute multiple cell populations. Therefore, it is difficult to determine which cell populations are critical for proper fracture healing. To address this, we developed a novel mouse model where the ROSA26 promoter drives expression of thymidine kinase (TK), a “suicide gene”, which is preceded by a floxed stop cassette. Breeding to any Cre recombinase mouse line drives tissue-specific expression of TK. Treating ROSA-TK;Cre-positive mice with ganciclovir (GCV), a competitive inhibitor of guanosine, causes apoptosis in replicating TK-expressing cells. Here, we present initial results from ROSA-TK mice crossed with two osteoblast-lineage Cre mice: Osx-CreERT2 and Ocn-Cre. Two weeks after full femur fracture stabilized with an intramedullary pin, ablation of replicating pre-osteoblasts using Osx-CreERT2 and mature osteoblasts with Ocn-Cre significantly impairs fracture healing. X-rays show significantly fewer fully bridged calluses in ROSA-TK;Cre-positive mice treated with GCV compared to mice treated with vehicle. After euthanasia, μCT shows significantly smaller callus volume and histology shows a stark lack of woven bone within the fracture callus. These data support that proliferation of pre- and mature osteoblasts are necessary for proper callus formation and fracture healing.

Interplay of Genotype and Substrate Stiffness in Driving the Hypertrophic Cardiomyopathy Phenotype in iPSC-Micro-Heart Muscle

Jingxuan Guo, David R. Schuftan, Daniel Simmons, Huanzhu Jiang, **Nathaniel Huebsch***.

Hypertrophic Cardiomyopathy (HCM) shares many similarities with non-inherited cardiac hypertrophy induced by pressure overload. This suggests a potential role for environmental factors in tissues with mutation-induced changes to develop phenotypes associated with HCM. Here, we assessed heterozygous loss of function of Myosin Binding Protein C ($MYBCP3^{+/-}$), combined with mechanical loading to drive changes in action potential morphology, calcium transients and contractility.

Isogenic control (WTC) and $MYBCP3^{+/-}$ iPSC were differentiated into cardiomyocytes using small molecule manipulation of Wnt signaling. The cardiomyocytes were seeded into ‘‘dog bone’’ shaped stencil molds to form micro-heart muscle. To mimic changes in myocardial stiffness stemming from pressure overload, we varied the rigidity of the substrates micro-heart muscle contract against. Stiffnesses consist neonatal (0.4 kPa), healthy (15 kPa) and fibrotic (65 kPa). Substrates were embedded with a thin layer of fluorescent beads to track contractile force; parent iPSC were engineered to express the genetic calcium indicator, GCaMP6f to study calcium dynamics. Tissues were stained with voltage sensitive dye to assess action potential waveforms. High speed video microscopy was used to quantify contractility, calcium handling and action potential of micro-heart muscles.

Environmental stiffness triggered physiological adaptation for both genotypes. At soft 0.4 kPa, similar contractility and electrophysiology were seen. At 15 kPa, substrate stiffness led to hypercontractility together with prolonged calcium upstroke and action potential duration for $MYBCP3^{+/-}$ tissues, however, this difference is less substantial at 65 kPa fibrotic conditions. This work suggests that mimicking non-genetic, environmental factors *in vitro* is key to unveiling genotype-phenotype relationships observed *in vivo* to allow physiologically relevant studies in human cells.

SINGLE-CELL RNA SEQUENCING OF THE SYNOVIUM REVEALS DISTINCT JOINT PHENOTYPES IN AGING-, INJURY-, OR OBESITY-INDUCED MODELS OF OSTEOARTHRITIS

Natalia S. Harasymowicz[1,2], Neda Rashidi[1,2], Kristin Lenz[1,2], Gwendalyn Krekeler[1,2], Ruhang Tang[1,2], Kathleen Dobbs[1,2], Farshid Guilak[1,2]
[1]Department of Orthopaedic Surgery, Washington University, St. Louis, MO, USA;
[2]Shriners Hospitals for Children – St. Louis, MO, USA
*Email: harasymowiczn@wustl.edu

Osteoarthritis (OA) is a family of diseases characterized by degradation of cartilage, alterations in bone structure, and inflammation and fibrosis of the synovium and joint fat pad. We used single-cell RNA sequencing (scRNA-seq), flow cytometry, microCT, and histological techniques to study the effects of obesity on knee joint tissues from two mouse OA models: post-traumatic OA (PTOA) and aging OA. All animal procedures were approved by the IACUC at WUSTL. Four-week-old male mice were fed either Control or high-fat diet (HFD). One group received DMM surgery on the left hindlimb at 16 weeks of age and was sacrificed at 28 weeks of age. Another group of mice was aged up to 52 weeks with no surgical intervention. Standard histological and bone microstructure assessments were conducted. To assess immune cell changes, synovial pouches were collected and subjected to collagenase digestion, flow cytometry sorting (based on $CD45^+$ marker), and scRNA-seq (10x Genomics). Statistical analysis was performed using the Mann-Whitney U test or Two-Way ANOVA, $p<0.05$. We found that aging and PTOA models are characterized by distinct molecular, cellular, and architectural changes, with obesity affecting disease progression differently. Specifically, in the aging model, obesity mildly increased cartilage damage, lowered trabecular bone quality in the tibia, and increased pro-senescent T-cells. However, in the PTOA model, obesity demonstrated increases in macrophage content and fibrosis, significant cartilage loss, and both femoral and tibial trabecular/subchondral bone microstructure alterations. Our results align with clinical studies suggesting multifactorial etiology of OA in patients and suggest that future approaches in OA therapies.

A HIGH-RISK OSTEOARTHRITIS MUTATION IN *COL6A3* ALTERS CHONDROCYTE MECHANOBIOLOGY

Zainab Harissa[1,2], Amanda Dicks[1, 2], Niek G Bloks[3], Shaunak Adkar[1,2], Nancy Steward[1,2], Yolande F M Ramos[3], Ingrid Meulenbelt[3], Farshid Guilak[1,2]

[1]Washington University, Saint Louis, MO, [2]Shriners Hospitals for Children, Saint Louis, MO, [3]Leiden University Medical Center, Leiden, The Netherlands

zharissa@wustl.edu

Disclosures: ZH (N), AD (N), SA (N), NGB(N), NS(N), YFMR(N), IM (N), FG (Cytex -3A,4)

Osteoarthritis (OA) is a multifactorial disease involving the contribution of genetic and environmental risk factors that lead to the degradation of articular cartilage. Chondrocytes are surrounded by a pericellular matrix (PCM) distinguished primarily by collagen type VI (COL6). COL6 maintains the PCM's mechanical properties, which have been shown to regulate chondrocyte mechanotransduction. Recent studies identified variants in *COL6A3,* a component of COL6, to be associated with severe OA. We used human iPSCs to examine the hypothesis that a high-risk *COL6A3* mutation alters the chondrocyte's normal metabolic response to loading as a potential mechanism linking this mutation to severe OA. hiPSCs were gene-edited using CRISPR-Cas9 to introduce the *COL6A3* mutation R1504W for the OA variant rs144223596. *COL6A3* mutant and wildtype (WT) hiPSCs were differentiated into chondrocytes and subjected to physiological levels of dynamic mechanical loading or hypoosmotic stimuli. WT and mutant hiPSC-derived chondrocytes had no significant difference in GAG production or chondrogenic gene expression, indicating a similar chondrogenic capacity. *COL6A3*-mutant chondrocytes displayed a reduced anabolic response to mechanical loading reflected by the decreased expression of mechanosensitive genes, and a higher Ca^{2+} response to hypoosmotic stimuli compared to WT chondrocytes. This suggests that the *COL6A3* mutation R1504W causes alterations in PCM properties that lead to dysfunctional mechanical signal transduction. Further studies are needed to directly measure PCM mechanical properties in WT and *COL6A3* mutant chondrocytes, as well as transcriptomic analysis of signaling pathways that may be altered in response to loading.

DO ORIGINS MATTER? A NOVEL PERIOSTEAL CELL POPULATION CONTRIBUTING TO CRANIOFACIAL SKELETAL REGENERATION

Mohamed G. Hassan[1], Erica L. Scheller[1,2,3]

[1]Division of Bone and Mineral Diseases, Department of Medicine, Washington University School of Medicine, St. Louis, MO, USA.
[2]Department of Biomedical Engineering, Washington University, Saint Louis, MO, USA.
[3]Department of Cell Biology and Physiology, Washington University, Saint Louis, MO, USA.

Abstract

Unlike the rest of the body, the craniofacial skeleton originates from two distinct embryogenic tissues: neural crest and mesoderm. Beside bone, neural crest cells also give rise to most of the peripheral nervous system and to several cell types, including Schwann cells, cardiovascular smooth muscle cells, skin pigment cells, and more. The regenerative potential of neural-crest derived bone was previously found to be higher than adjacent mesoderm-derived bone. However, the fundamental molecular and cellular mechanisms underlying this result remain unclear. Using advanced imaging techniques and FACS sorting, we provide the first evidence for a unique p75 neurotrophin receptor-positive (p75-NTR+) periosteal cell population covering only neural crest-driven bone. We found that these cells form a dense network specifically over the surface of the neural crest-derived bone in adult mice. We hypothesize that this p75-NTR+ cells may contribute in the enhanced regeneration capacity of the neural crest-driven bone. Using a calvarial defect mouse model, we are planning to profile the p75-NTR+ cells using RNAseq to identify differences in basal gene expression and predicted measures of pathway activation and interactions with neighboring skeletal cells in intact calvarial bone, and during the process of skeletal regeneration. Furthermore, we are in the process of generating an inducible transgenic mouse that will allow us to perform pulse-chase lineage tracing of p75-NTR+ cells in addition to tracking of the endogenous p75-NTR expression during the regenerative process. We expect that the characterization of these cells and their mechanism of action will inform methods to enhance craniofacial skeletal regeneration.

POSTER LOCATION # 43

REFLECTANCE QUANTITATIVE POLARIZED LIGHT IMAGING FOR DYNAMIC MICROSTRUCTURAL ANALYSIS OF ORTHOPAEDIC SOFT TISSUES

Leanne E. Iannucci[1], Spencer P. Lake[1,2,3]

[1] Biomedical Engineering, Washington University in St. Louis
[2] Mechanical Engineering and Materials Science, Washington University in St. Louis
[3] Orthopaedic Surgery, Washington University in St. Louis

There are over 33 million cases of musculoskeletal injuries reported annually, with approximately 50% involving soft tissue structures like tendon and ligament. In many cases, injury is caused by an accumulation of microdamage or progressive degeneration to the collagenous extracellular matrix. Unfortunately, due to limitations associated with many imaging modalities, this damage often goes undetected until a traumatic injury event occurs concomitantly with a loss of tissue function. Our objective is to develop a reflectance based quantitative polarized light imaging (rQPLI) technique that permits real-time, dynamic analysis of collagen microstructure in these commonly injured tissues. First, we evaluated the sensitivity of rQPLI to the detection of important extracellular matrix properties associated with musculoskeletal tissues (e.g., alignment, crosslinking, density, opacity). We compared data obtained with rQPLI with a previously characterized transmission mode polarimetry technique as well as to other optical imaging modalities (e.g., second harmonic generation, optical coherence tomography). Finally, we demonstrated the utility of rQPLI to evaluate the onset and progression of biologically mediated tendon damage by monitoring polarimetry outcomes during enzyme-mediated degeneration of tendon. Data from these studies help to establish the utility of rQPLI as a tool for real-time dynamic analysis of collagenous tissues under complex, clinically relevant environments.

POSTER LOCATION # 45

NONDESTRUCTIVE EVALUATION OF IN SITU BONE QUALITY BY USING SINGLE AND SUCCESSIVE IMPACT MICRO INDENTATIONS

Authors: Babak Jahani[1], Donald A. Aboytes[1], James M. Jin[2], Simon Y. Tang[1, 2]

[1]Department of Orthopaedic Surgery, Washington University in St. Louis, St. Louis, MO, USA

[2]Department of Biomedical Engineering, Washington University in St. Louis, St. Louis, MO, USA

The fracture resistance of bone is derived from its hierarchical collagen-mineral crystal network, and any deterioration in this network will increase fracture risk. DEXA is the current gold standard for determining fracture risk by means of assessing bone mineral density. Yet there are many BMD-discordant fractures across populations, suggesting that evaluating bone density alone is not sufficient to accurately assess fracture risk. There is thus a need to evaluate bone quality in vivo through novel mechanical testing methods. This study aimed to evaluate the capability of impact micro indentation on cortical bone in corroborating fracture toughness of denatured bone. Six bovine femurs were locally sourced and cut in half in the transverse direction, with one half treated in boiling water for 3 hours while the other half was kept at -20C. The anterior side of each bone was indented at 20C, and the resistance against single and successive indentations at the bone surface was measured. Notched beams were then machined from the anterior side and then loaded to failure in three-point bending. Boiled bone exhibited reduced fracture toughness (-60.2% $p < 0.0001$) and resistance to a single indentation (-14.7%, $p < 0.05$). Inspection of the slopes of successive indentations detected impaired toughening behavior due to boiling (-16.5%, $p < 0.001$). Linear mixed-effects models were used for comparisons. The reduction in fracture toughness, which may be the best available measure of fracture resistance, was corroborated by periosteal micro-indentation on the intact whole bone. Moreover, successive indentations at the same site yielded additional insights on the toughening behavior of bone and could be clinically important.

Title: EPIGENETIC DYSREGULATION DURING PROGRESSION OF HIP FAI: A LOSS OF CARTILAGE HOMEOSTASIS

Authors: Tomoyuki Kamenaga, Jie Shen, Robert Brophy, Regis O'Keefe, John Clohisy and Cecilia Pascual-Garrido

Affiliation: Washington University School of Medicine, St. Louis, MO

Introduction: Femoroacetabular impingement (FAI) is a leading cause of hip osteoarthritis (OA). Epigenetic changes are associated with OA progression. Specifically, DNA methyltransferase 3B (DNMT3B) plays a critical role on cartilage homeostasis by attenuating catabolic gene expression. This study aimed to examine the catabolic state of articular chondrocyte (ACs) during OA progression in hip FAI disease, particularly, assess the expression levels of *DNMT3B* and *ABAT* (a downstream of DNMT3B). **Methods:** Full-thickness cartilage samples were collected from the impingement zone. Six patients underwent hip preservation surgery for cam FAI (early-FAI) and 6 patients underwent total hip replacement for advanced OA secondary to cam FAI (late FAI-OA). As a non-disease (ND) group, five healthy samples were procured from hip joint cadavers. The explants were cultured in unstimulated conditions or catabolic stimulus (IL1β). Histological analysis were performed with safranin-O/fast-green. Gene expression was analyzed via qPCR for *GAPDH*, *DNMT3B*, *ABAT*, *MMP-13*, and *COL10A1*. Methylation specific PCR was performed to assess the methylation status at *ABAT* promoter. **Results:** Cartilage samples in early-FAI and late FAI-OA showed a histological OA phenotype and increased expression of catabolic markers compared to ND group (*MMP13*; ND vs early $p=0.004$, ND vs late $p<0.001$, *COL10A1,* ND vs early $p<0.001$, ND vs late $p<0.001$). RT-PCR confirmed reduce expression of *DNMT3B* (ND vs early $p<0.001$, early vs late $p=0.016$) and *ABAT* overexpression (ND vs early $p<0.001$, early vs late $p=0.035$) with advanced disease. Additionally, there was hypomethylation at the *ABAT* promoter during end stage of disease. IL1β stimulus accentuated the *ABAT* promoter hypomethylation and led to the further overexpression of *ABAT* and catabolic markers in early-FAI and late FAI-OA ACs. **Conclusion**: Catabolic and epigenetic molecule expressions varied suggesting a less catabolic phenotype for early stage of disease. Sustained inflammation induced *ABAT* promoter hypo-methylation resulting in a catabolic phenotype.

TITLE: PPARγ PROMOTER DEMETHYLATION RESCUES ARTICULAR CHONDROCYTES FROM CATABOLIC STATE IN HIP FEMOROACETABULAR IMPINGEMENT

Authors: Tomoyuki Kamenaga, Jie Shen, Robert Brophy, Regis O'Keefe, John Clohisy and Cecilia Pascual-Garrido
Affiliations: Washington University School of Medicine, St. Louis, MO

Introduction: Femoroacetabular impingement (FAI) is a leading cause of hip osteoarthritis (OA). Peroxisome proliferator-activated receptor-gamma (PPARγ) is widely express in chondrocytes and is essential for cartilage homeostasis. This study aimed to investigate the PPARγ expression and methylation status at the PPARγ promoter during OA progression in hip FAI. **Methods:** Full-thickness cartilage samples were collected from the impingement zone. Six patients underwent hip preservation surgery for cam FAI (early FAI) and 6 patients underwent total hip replacement for advanced OA secondary to cam FAI (late FAI-OA). As a non-disease (ND) group, 6 healthy samples were procured from hip joint cadavers. The explants were cultured in unstimulated conditions or catabolic stimulus (IL1β) with or without DNA demethylating agent 5Aza. Histological analysis was performed with safranin O/fast green. Gene expression was analyzed via qPCR for *GAPDH*, *DNMT3A, PPARγ, MMP13,* and *COL10A1*. Methylation specific PCR was performed to assess the methylation status at *PPARγ* promoter site. **Results:** Cartilage samples in early-FAI and late FAI-OA displayed histological OA phenotype and increased expressions of *MMP13* and *COL10A1*. As the OA progresses, overexpression of *DNMT3A* (ND vs early $p<0.001$, early vs late $p=0.003$) and hypermethylation at the *PPARγ* promoter (ND vs early $p=0.012$, early vs late $p=0.024$), and concomitant *PPARγ* suppression (ND vs early $p=0.010$, early vs late $p=0.027$) were observed. IL1β stimulus exhibited further increased of *PPARγ* promoter methylation for early (34% to 57%, $p=0.015$) and late stages (48% to 79%, $p=0.001$) and suppressed *PPARγ* expression, but treatment with 5Aza brought the level of methylation back to 37% ($p=0.033$) and 46% ($p<0.001$) and prevented IL1β induced PPARγ suppression in early-FAI and late FA-OA ACs. **Conclusion**: PPARγ expression was gradually suppressed via promoter hypermethylation during OA progression in hip FAI. PPARγ promoter demethylation rescued ACs from catabolic state in hip FAI disease.

SUSTAINED RELEASE OF SMALL MOLECULES FROM NANOCOMPOSITE HYDROGELS

Mariam Khachani, Samuel Stealey, *Silviya P Zustiak

Biomedical Engineering Program, Parks College of Engineering, Saint Louis University, Saint Louis, MO 63103, USA

To improve the efficacy of therapeutic treatment, sustained and localized delivery of small molecules is desired. Because of their biocompatibility and capacity to retain therapeutic bioactivity, hydrogels have been widely exploited for delivery applications. Traditional hydrogel delivery methods are limited by large initial burst release and quick diffusion-controlled small molecule release. Two nanoclays with different crystallographic structures (disks and nanotubes) which have negatively charged surface, provide the potential for electrostatic adsorption of charged molecules, offering an extra lever to control and sustain therapeutic delivery. The clays were added to polyethylene glycol (PEG) hydrogels form nanocomposite delivery devices. When clay particles were added to PEG hydrogels, the release of model small molecules was greatly reduced compared to PEG-only hydrogels. The creation of complexes between silanols (Si-OH) groups and positively charged small molecules prolonged the release of small molecules, thereby limiting release due to passive diffusion. Absorption experiments were performed to investigate the effect of small molecule contact time on the formation of complexes to better understand the interaction between silanol groups and small molecules. Because of the strong contact between the positively charged small molecule and the negatively charged clay surface, >90% adsorption of small molecules was observed within the first 5 minutes. Therefore, nanocomposite hydrogels containing clay particles have great potential for use as therapeutic delivery devices because of their sustained and prolonged release patterns that can improve efficacy of drug delivery.

POSTER LOCATION # *(podium talk only)*

TMEM178 NEGATIVELY REGULATES IL-1β PRODUCTION THROUGH INHIBITION OF NLRP3 INFLAMMASOME

Kunjan Khanna[1,2], Hui Yan[1,2,3], Gabriel Mbalaviele[4], Roberta Faccio[2,5]

1. These authors contributed equally and are listed in alphabetical order
2. Department of Orthopedics, Washington University in St Louis , MO, USA
3. Current address: Key Laboratory for Animal Disease-Resistance Nutrition of China Ministry of Education, Ministry of Agriculture and Rural Affairs and Sichuan Province, Animal Nutrition Institute, Sichuan Agricultural University, Chengdu 611130, China
4. Department of Medicine, Washington University in St Louis, MO, USA
5. Shriners Hospital for Children, St Louis, MO, USA

Excessive IL-1β levels have been reported in patients with systemic juvenile idiopathic arthritis (sJIA) and Cytokine storm syndrome (CSS). The release of bioactive IL-1β requires inflammasome activation. High IL-1β levels are found in sJIA and CSS patients with mutated and unmutated inflammasome components, raising questions on the mechanisms of IL-1β regulation in these disorders. Here, we report that Tmem178, an endoplasmic reticulum transmembrane protein modulating intracellular calcium fluxes and a negative regulator of macrophage inflammatory responses, controls NLRP3 inflammasome activation and IL-1β secretion. Mechanistically, Tmem178 modulates the NLRP3 inflammasome pathway through its interactions with the store operated calcium entry component Stim1. In vivo, *Tmem178*$^{-/-}$ mice injected with LPS have elevated levels of IL-1β, a response that is NLRP3-dependent. Furthermore, the premature death of *Tmem178*$^{-/-}$ mice in the virus-dependent model of CSS was delayed following administration of an inhibitor of inflammasome signaling. Importantly, downregulation of Tmem178 expression levels in macrophages exposed to plasma from sJIA patients correlates with increased IL-1β release. Our work suggests that downregulation of Tmem178 levels could serve as a new biomarker to identify sJIA patients that could benefit from receiving drugs targeting inflammasome signaling.

POSTER LOCATION # 51

ENGINEERED SELF-REGULATING MACROPHAGES FOR TARGETED ANTI-INFLAMMATORY DRUG DELIVERY

Molly Klimak[1,2,3], Amanda Cimino[1,2,3], Lara Pferdehirt[1,2,3], Farshid Guilak[1,2,3]
[1]Dept. of Orthopaedic Surgery, [2]Dept. of Biomedical Engineering, Washington University in St. Louis, [3]Shriners Hospitals for Children – St. Louis

Rheumatoid arthritis (RA) is a systemic, autoimmune joint disease characterized by the inappropriate regulation of inflammatory signaling factors. Macrophages act as one of the key drivers of this imbalance, accelerating progression of RA through the continued perpetuation of chronic inflammation. Therefore, the goal of this study was to develop a macrophage-based therapeutic to mitigate NF-κB mediated inflammation by delivering IL-1 receptor antagonist (IL-1Ra), in an auto-regulated manner. Bone marrow derived macrophages were lentivirally transduced with a synthetic NF-κB inducible promoter upstream of either *Il1rn* (IL-1Ra) or *Luciferase* (LUC) transgenes. In response to IL-1β, macrophages produced significant amounts of IL-1Ra or luciferase in a time and dose-dependent manner. To assess if IL-1Ra macrophages could protect cartilage from cytokine-induced degradation, IL-1Ra or LUC macrophages were co-cultured with murine iPSC-derived chondrocytes in the presence of IL-1β for 48 hours. Non-treated chondrocytes showed no differences between LUC and IL-1Ra macrophage co-cultures. However, with IL-1β treatment, miPSC derived chondrocytes showed significant sGAG loss and increased inflammatory gene expression. This IL-1β-induced degradation and inflammatory activation was reduced in co-cultures with IL-1Ra macrophages which produced significant amounts of IL-1Ra in response to IL-1. These findings demonstrate the successful development of engineered macrophages that possess the ability for controlled, auto-regulated production of IL-1Ra based on NF-κB signaling that can mitigate the effects of the inflammatory cytokine IL-1β.

ROLE OF PERIOSTIN EXPRESSING CELLS IN INTRAMEMBRANOUS BONE REGENERATION

Frank C. Ko[1], Rong Xie[1], Brandon Willis[2], Chundo Oh[1], Di Chen[3], D. Rick Sumner[1]

[1]Rush University Medical Center, [2]UC Davis Mouse Biology Program, [3]Shenzhen Institute of Advanced Technology

Intramembranous bone regeneration plays an important role in orthopaedic and dental procedures such as fracture healing and implant placement. Despite a widespread recognition of the importance of intramembranous bone regeneration in these clinical procedures, its underlying mechanisms have not been well explored. We have previously profiled temporal transcriptome changes of regenerating bone, and discovered that an increase in periostin gene expression preceded increases in osteogenic genes. We therefore further sought to determine the role of periostin expressing cells (PECs) in intramembranous bone regeneration. We used a genetic mouse model that allows tamoxifen-inducible fluorescent labeling of PECs (Postn-creERT;tdTM). These reporter mice underwent surgical ablation of the femoral bone marrow cavity, a well-established intramembranous bone regeneration model. We also depleted PECs using Postn-creERT;iDTR mice. These mice also underwent surgical bone marrow ablation. We found that in intact bones, fluorescently labeled PECs were largely restricted to the periosteal surface of cortical bone and were absent in the sbone marrow. However upon surgical disruption of bone marrow, PECs were found within the regenerating tissue of this space 7 days post-op even though the cortical bone remained intact. The source of PECs appeared to be heterogenous, including the periosteal surface, and pericytes or endothelial cells within the bone marrow cavity. Depletion of PECs at the time of surgery impaired intramembranous bone regeneration. These data suggest an important role of PECs in intramembranous bone regeneration and may lead to novel therapeutic interventions to accelerate fracture healing or enhance implant fixation.

MINERALIZED COLLAGEN SCAFFOLD PORE STRUCTURE ENHANCES IMMUNOMODULATORY POTENTIAL OF MESENCHYMAL STEM CELLS

Vasiliki Kolliopoulos, Brendan A.C. Harley
University of Illinois at Urbana-Champaign, IL

Critical-sized craniomaxillofacial (CMF) defects require surgical intervention to promote healing. Our lab has developed a class of mineralized collagen scaffolds to promote mesenchymal stem cell (MSC) osteogenic differentiation and subsequent bone regeneration in the absence of exogenous growth factors. MSCs can also endogenously secrete immunomodulatory factors to alter macrophage (MΦ) recruitment and phenotype towards more pro-inflammatory M1 or anti-inflammatory M2. Others suggest that the inflammatory status of MSCs may lead to the production of disparate combinations of biomolecules that influence macrophage polarization. We describe the immunosuppressive behavior of MSCs cultured in mineralized collagen scaffolds based on their initial inflammatory status: licensed MSCs vs. MSCs in basal media. We report the combined effect of MSC inflammatory status as well as the structural and compositional properties of the scaffold on MΦ polarization via analysis of MSC secretome and co-culture experiments over 7 days of culture. MSCs exhibited increased metabolic activity in anisotropic scaffolds while licensed MSCs in all scaffold groups secreted more immunomodulatory cytokines compared to basal MSCs, though scaffold anisotropy induced the greatest production. We then examined MSC-MΦ interactions using indirect and direct co-cultures with and without inflammatory stimulation. While the MΦ secretome directly influences MSC metabolic activity, MΦ-MSC co-cultures increase MSC production of OPG and immunomodulatory factors. These results show that licensed MSCs displayed a higher capacity to polarize MΦ toward an M2 phenotype with the effects strongly influenced by scaffold pore anisotropy and composition.

A CARRAGEENAN–BASED HYDROGEL CONFINEMENT APPROACH TO INCREASE COLLAGEN DEPOSITION DURING IN VITRO OSTEOCHONDRAL TISSUE DEVELOPMENT

Joseph Krebs (1), Alyssa Brown (1), Samuel Stealey (1), Austin Krohn (1), Silviya P. Zustiak (1,2), Natasha Case (1)

(1) Biomedical Engineering Program, School of Engineering, Saint Louis University, St. Louis, MO, USA
(2) Department of Physiology and Pharmacology, School of Medicine, Saint Louis University, St. Louis, MO, USA

The successful development of a tissue-engineered osteochondral replacement for joint tissue regeneration will address a significant clinical need. Current *in vitro* approaches produce a tissue that is deficient in a fibrillar collagen-rich extracellular matrix and has insufficient tissue properties. Achieving abundant ECM deposition during *in vitro* culture is challenging, due in part to insufficient assembly and retention of fibrillar collagen. Macromolecular crowding has been shown to enhance the processing of type I procollagen, leading to significant increases in fibrillar collagen assembly and accumulation during *in vitro* culture of a variety of cell types. The confinement approach, while more difficult to implement within the *in vitro* culture environment, has the advantage of avoiding any interactions with the growing tissue layer. Additionally, the “confined” environment can be more easily controlled than the “crowded” environment, since the confinement approach is based upon a macromolecular barrier rather than macromolecules in solution. Limited evidence suggests that a confinement approach can be used to improve *in vitro* collagen deposition. In this study, we developed a carrageenan-based hydrogel confinement approached. It was found that an approach using a 5% w/v kappa-CR gel increased collagen accumulation by 1.9-fold over the control condition after 7 days of application in human MG-63 bone cell cultures. Additionally, computational modeling of oxygen and glucose transport within the confinement culture model was performed to complement the experimental results and to provide insights about nutrient concentration spatial and temporal variability within the culture system.

IKK-B INHIBITION FACILITATES INTRASYNOVIAL TENDON HEALING IN A CANINE FLEXOR TENDON INJURY AND REPAIR MODEL

Ryan A. Lane[1], Hua Shen[1], Shelly E. Sakiyama-Elbert[2], Richard H. Gelberman[1], Stavros Thomopoulos[3]

[1]Washington University School of Medicine, St. Louis, MO, [2]University of Texas, Austin, TX, [3]Columbia University, New York, NY

The outcomes of intrasynovial flexor tendon repair are inferior to those of extrasynovial tendon repair with respect to the recovery of digital motion and the incidence of complications, including the formation of extensive adhesions and rupture of the repair. Compared to extrasynovial tendons, intrasynovial flexor tendons express higher levels of pro-inflammatory genes and complement proteins after injury and repair. We hypothesized that systemic delivery of ACHP, an IKK-β inhibitor, following flexor tendon repair would attenuate the inflammatory response, resulting in improved healing. 22 adult female canines flexor digitorum profundus (FDP) tendons were transected and repaired. Animals were divided into 3-day (n=10) and 7-day (n=12) treatment groups and euthanized at 3 and 14 days after repair, respectively. Changes in tendon gene expression were determined via TaqMan RT-PCR. Tendon structure and cellularity were assessed histologically with pentachrome staining. Gross assessment of repairs detected no FDP ruptures and minimal gaps between distal and proximal stumps in all ACHP-treated tendons, compared to 1 rupture and 2 moderately gapped untreated repairs. At 3 days after repair, a marked decrease in fibrovascular adhesions was observed in ACHP-treated repairs compared to untreated repairs. Gene expression analysis showed no significant changes in the expression of *IL-1β* (p=0.18) and *IL-6* (p=0.32). However, analysis of tendon sections revealed a marked reduction in epitenon thickness in ACHP-treated samples compared to control samples (N=3/group). At 7 days, CBC detected an 18% reduction in WBC count (p=0.003) in treated animals compared to controls. At 14 days, expression of tenogenic genes *SCX* and *TNMD* and tendon matrix genes *COL1A1* and *COL3A1* were significantly increased. A trending treatment effect was observed in *TGF-β3* expression based on a two-way ANOVA (p=0.06). Using a clinically relevant large animal model, this study revealed a new therapeutic strategy for intrasynovial flexor tendon repair.

LOADING-INDUCED BONE FORMATION IS MEDIATED BY *WNT1* INDUCTION IN OSX-LINEAGE CELLS

Authors: Lisa Lawson[1-2], Nicole Migotsky[1-2], Christopher Chermside-Scabbo[1-2], John Shuster[1-2], Kyu Sang Joeng[3], Roberto Civitelli[2,4], and Matthew Silva[1-2]

[1] Department of Orthopaedic Surgery, Washington University School of Medicine, St Louis, MO
[2] Musculoskeletal Research Center, Washington University in St Louis, St Louis MO
[3] McKay Orthopaedic Research Laboratory, Department of Orthopaedic Surgery, University of Pennsylvania, Philadelphia, PA
[4] Division of Bone and Mineral Diseases, Washington University in St Louis, St Louis, MO

Skeletal loading stimulates bone formation, in part through the Wnt/Lrp signaling pathway. Recently, we reported that Wnt ligands produced by Osx-lineage cells play a crucial role in mediating the bone anabolic response to mechanical loading. Here, we show that *Wnt1* specifically mediates the bone anabolic response to tibial loading. Relative to sex-matched controls, OsxCreERT2; Wnt1F/F knockouts had a 65 to 94% reduction in Ps.rBFR/BS, indicating that *Wnt1* was required for the periosteal response to strain-matched loading. RT-qPCR indicated that *Wnt1* was 6.6-fold higher in the loaded vs non-loaded bones of Wnt1F/F mice, concomitant with enhanced *Bmp2*, *Col1a1*, and *Bglap* expression in the loaded tibia ($p<0.001$). By comparison, loading failed to increase *Wnt1*, *Bmp2*, *Col1a1*, and *Bglap* in the bones of *Wnt1* knockouts, indicating that *Wnt1* induction was required for the upregulation of *Bmp2*, *Col1a1*, and *Bglap* in the bone. In sum these data show that loading-induced *Wnt1* expression in the Osx-lineage is required for the bone anabolic response to tibial loading.

DICKKOPF-1 INDUCES BREAST CANCER PROGRESSION BY LIMITING T CELL INFILTRATION INTO THE TUMOR MICROENVIRONMENT

Seunghyun Lee[1], Biancamaria Ricci[1], and Roberta Faccio[1]
[1] Department of Orthopedics, Washington University School of Medicine, St. Louis, MO, USA

Dickkpof-1 (Dkk1), a soluble Wnt/β-catenin inhibitor, has been implicated in bone loss in postmenopausal osteoporosis, arthritis, and multiple myeloma. However, high levels of Dkk1 are also observed in mice bearing primary tumors at extraskeletal sites with no evidence of metastatic dissemination to the bone. We recently reported that Dkk1 supports tumor progression by creating an immune-suppressive environment. However, how Dkk1 orchestrates its tumor-promoting effects remains unclear. In this study, we used mice orthotopically injected with luminal B ER+ PyMT and triple-negative 4T1 cell lines. We found that Dkk1 is upregulated in all models and its neutralization reduces tumor growth. Although Dkk1 was barely expressed in these tumor lines, elevated expression of Dkk1 was detected from osteoblasts (OBs) in bone and cancer-associated fibroblasts (CAFs) in the tumor stroma. To address the role of bone- versus tumor stroma-derived Dkk1 during tumor progression, we deleted Dkk1 from OBs (OsxCre;Dkk1fl/fl), and from CAFs (aSMACreERT2;Dkk1fl/fl). The growth of orthotopically injected PyMT cells was reduced in all mouse models, suggesting that Dkk1 can act both systemically and locally to modulate tumor progression. Interestingly, FACS analysis showed that targeting Dkk1 did not change the immune composition in the bone marrow, spleen, or at the tumor site. However, IHC showed infiltration of T cells in the center of the tumor mass in CAF-Dkk1cKO or anti-Dkk1-treated mice, while T cells remained at the edges of the tumors in the controls. In summary, we provide evidence that Dkk1 exerts local and systemic effects to promote tumor progression by modulating the infiltration of immune cells into the tumor microenvironment.

THE LEPTIN-MEDIATED CENTRAL NERVOUS SYSTEM CONTROLS OF SKELETAL METABOLISM

Anurag Majumdar[1], Xiao Zhang[1, 2], Erica L Scheller[1, 2, 3]

[1]Division of Bone and Mineral Diseases, Washington University, Saint Louis, MO, USA
[2]Department of Biomedical Engineering, Washington University, Saint Louis, MO, USA
[3]Department of Cell Biology and Physiology, Washington University, St. Louis, MO, USA

Leptin is a key regulator of adipose tissue metabolism and is often elevated in conditions of excess fat storage and obesity. Chronic exposure to high levels of leptin can also cause neural desensitization to the hormone promoting leptin resistance. Our lab has previously shown that the delivery of leptin into the brain causes the rapid depletion of adipose tissue, particularly bone marrow adipose tissue (BMAT). This experiment aimed to identify the regions of neural activity within the brain in response to chronic leptin administration that may be responsible for the catabolism of BMAT. Mice were implanted with an osmotic pump that delivers leptin into the brain through intracerebroventricular injection at a constant rate of 10 or 100 ng/hr over 3-days or 9-days. Mice in all leptin treatment groups were noted to have a dose-dependent decrease in body mass, blood glucose level, white adipose tissue, and BMAT mass. C-Fos, a marker of neuronal activity, was upregulated by leptin in regions of the hypothalamus, thalamus, and amygdala by 3-days after leptin treatment. At 9-days, leptin-induced increases in neural activity, as assessed by c-fos, were absent in the amygdala and reduced, but still broadly present, in the hypothalamus compared to three-day groups. These results suggest that regions within the hypothalamus, thalamus, and amygdala may play a critical role in the leptin-mediated depletion of BMAT. In addition, our results suggest that chronic delivery of leptin may induce leptin resistance in select brain regions after a period of 9-days. This work identifies candidate central neural pathways that may mediate the regulation of bone marrow adiposity and energy partitioning within the skeleton.

SYSTEMIC MUSCLE INJURY ACTIVATES A MULTI-ORGAN RESPONSE THAT PROMOTES MUSCLE REPAIR

JENNIFER MCADOW, TIFFANY OU, AARON N. JOHNSON. DEPARTMENT OF DEVELOPMENTAL BIOLOGY, WASHINGTON UNIVERSITY SCHOOL OF MEDICINE

The regenerative response to acute skeletal muscle injury has been extensively investigated, but the molecular and cellular responses to injury are largely unknown. Using zebrafish to study these responses, we developed an acute, systemic injury platform that targets muscle cell ablation to render the organism immobile within 48 hours of injury. And the injured fish, astonishingly, will regain full mobility within 2 weeks due to the regenerative nature of zebrafish. Using a forward genetic screen we identified alleles that prevent systemic muscle regeneration completely. We uncovered alleles that specifically disrupt posterior muscle regeneration while anterior muscle regeneration remains unaffected; similarly, we have uncovered alleles that disrupt dorsal muscle regeneration but not ventral muscle regeneration. Systemic muscle repair is thus controlled by a body-wide regenerative response as well as by spatially restricted regenerative responses. To explain spatially constricted regenerative pathways, we performed ScSeq to identify muscle stem cell populations with different anterior-posterior positional identities in injured fish. After injury, multiple known pro-regenerative pathways were enriched, and our bioinformatics study reveals that the muscle lineage communicates with a variety of unexpected cells types during systemic repair, including oligodendrocytes and vascular epithelia. These studies argue systemic injury activates a multi-organ response that promotes muscle repair. Our aims are identifying the signaling networks that sense and respond to systemic muscle injury.

DEGENERATION OF THE INTERVERTEBRAL DISC RELIES ON COMMON METABOLIC PATHWAYS DESPITE DIFFERING MODELS

Mulati Mieradili[1], Shambhavi Bhagwat[1], Kya Vaughn[1], Evan Polsky[1], Matthew Silva[1], Regis O'Keefe[1], Matthew L. Goodwin[1]
[1]Washington University, Department of Orthopedic Surgery, St Louis, MO

Disc degeneration (DD) is the key component involved in many common spinal pathologies. The mechanisms driving degeneration remain poorly understood. Earlier work has shown that elevated intracellular lactate drives degeneration, once thought to be purely mechanical. Here, we present murine (IVD) degeneration injury models designed to elucidate the role of lactate and its transport in DDD. F mice (C57BL/6J, n=108) randomly assigned 1 of 4 IVD models: group 1) lumbar-disc poke 2) tail-disc poke 3) spinal instability 4) sham/control group. Mice were euthanized at 2, 4, and 8 weeks. IVD degeneration was evaluated using histological, immunofluorescence (IF) and QPCR analysis. Groups 1-3 IF results showed DD characterized by loss of NP cells (gradual decrease in expression of GLUT1 and CA3 (NP marker)) and the gradual increase in matrix components (increased expression of Collagen X and MMP13) in the NP, but sham mice showed no significant DD. There was decreased expression of Slc16a3, a lactate transporter, Slc16a8 was upregulated. Both the NP and end plate (EP) of sham mice maintained strong expression of MCT4, while the NP and EP in injured mice had markedly reduced expression of MCT4. Expression of HK2 glycolytic enzyme was reduced. Our results introduce novel evidence supporting a common molecular pathway whereby discs degenerate after direct injury or instability. Importantly, we show that loss of MCT4 expression plays a key role in degeneration of the IVDs. MCT4 is associated with lactate exportation, and its loss results in elevated intracellular lactate and DD, MCT3 is rarely expressed and may act as a rescue lactate transporter. Lowered HK2 gene expression led to reduced glycolysis and may contribute to DD.

MULTI-SCALE CORTICAL BONE PHENOTYPES FROM FEMALE AND MALE MICE VARY ACROSS EIGHT INBRED STRAINS

Nicole Migotsky[1], Surabhi Kumar[1], Matthew J. Silva[1]
[1]Washington University in St Louis, St. Louis, MO

Osteoporosis is a large and growing public health problem affecting more than 54 million Americans over the age of 50. While this low bone mass disease is moderately heritable, little is known about the influence of genetics on cortical bone traits. Our goal in this study was to investigate how bone parameters across different length scales are genetically modulated. We quantified whole-bone morphology, cortical mechanical properties, estimated cortical material properties, and lacunar morphology of eight genetically diverse inbred mouse strains. All measured bone properties, across all length scales, vary significantly with genetic background (strain effect) and for a large majority of properties this effect is sex dependent (strain-sex interaction). Additionally, all but four traits have a broad sense heritability above 60%, above or in the range of heritably for cancellous microstructure, implicating a genetic contribution to cortical bone properties across length scales. At the whole-bone scale, bone area and ultimate force vary significantly with strain, sex, have a strain-sex interaction and are strongly correlated with each other ($r = 0.95$). While bone area and strength individually vary significantly with strain and sex, however, the relationship between the two appears strain and sex independent, indicating that structure-strength relationships are well conserved in the species *mus musculus*. At the micro-scale, osteocyte lacunar density ranges from 46,700/mm^3 (A/J males) to 84,500/mm^3 (129S1 females), a 57% difference. Total porosity (lacunar plus vascular porosity) and median lacunar size both vary significantly between mouse strain, sex, and have a sex-strain interaction. Median lacunar volume is moderately positively correlated with total lacunar volume density ($r = 0.65$). Additionally, we showed that bone properties correlate across length scales with larger bones (higher bone area) having larger lacunae (higher median lacunar volume). This work motivates future studies to investigate the gene loci modulating these relationships.

CELL ADHESIVE AND GROWTH FACTOR PEPTIDE MIMETICS COOPERATIVELY INFLUENCE OSTEOGENIC ACTIVITY OF MESENCHYMAL STEM CELLS IN 3D CULTURE

Sydney Neal[1]*, Era Jain[1], Xiaohong Tan[1], Hannah Graf[1,2], Rama Balasubramaniam[1], Nathaniel Huebsch[1,3]

[1] Washington University in St. Louis, MO, USA (nhuebsch@wustl.edu) [2] Eberhard-Karls University Tübingen, Germany [3] Center for Regenerative Medicine, Washington University in St. Louis

Regenerative medicine applications of growth factors typically require supraphysiological doses to achieve clinically meaningful benefits. This leads to dangerous off target effects, such as ectopic bone growth and nerve damage caused by recombinant human BMP-2 (rhBMP2). Matrix immobilized growth factor mimicking peptides have been studied as potential substitutes that would prevent off-target effects of growth factors. However, these short peptides often lack the potency of full-length growth factors. During normal development, growth factors work together with ECM to cause tissue formation at much lower growth factor levels. We hypothesize that combining immobilized cell adhesive peptides with growth factor mimetic peptides will enhance the potency of short peptide mimetics of rhBMP2. We grafted cell adhesive cyclo-RGD (cRGD) and growth factor mimetic BMP-2 knuckle epitope (KE) via two orthogonal click chemistries: strain promoted azide-alkyne cycloaddition (SPAAC; for cRGD) and maleimide-thiol for the knuckle epitope (KE) of BMP2 onto alginate polymers. FRET assays on fluorophore surrogates suggest that when KE and cRGD were coupled to the same polymer, their separation was 6.3± 0.7 nm. Clonally derived mouse mesenchymal stem cells (MSCs) were exposed either to rhBMP2 or KE. ALP assays and preliminary RUNX2 staining demonstrated that immobilized KE peptide was comparable to rhBMP2 in osteogenic potency. We also observed a trend towards higher osteogenic potency when cRGD and KE were presented in close proximity to one another by being conjugated to the same polymer molecules.

MATERNAL HIGH-FAT-HIGH-SUGAR DIET IMPAIRS BONE BUT NOT CARTILAGE INTEGRITY IN AGED OFFPRING

Arin K. Oestreich[1,2], Natalia S. Harasymowicz[1,2], Alireza Savadipour[1,2], Mohammed Kuziez[1,2], Kelle H. Moley[1], Farshid Guilak[1,2]

1. Washington University School of Medicine, Saint Louis, MO 63110
2. Shriners Hospitals for Children, St. Louis, MO 63110

The developmental origins of health and disease hypothesis proposes that environmental exposures and insults such as an unbalanced maternal diet during critical windows of fetal development can program the long-term health of the aging offspring. Maternal high-fat high-sugar diet (HFHS) increases the risk of the adult offspring developing cardiometabolic disease. However, the impact of maternal diet on offspring musculoskeletal health is less understood. We hypothesized maternal HFHS diet would promote spontaneous cartilage and bone changes characteristic of osteoarthritis in 1 year old offspring. Female C57BL/6J mice were fed either a HFHS or control chow diet for 6 weeks before mating. Offspring were weaned to a control chow diet and raised to 1 year of age. Knee joints were histologically evaluated for osteoarthritis and synovial inflammation using established scoring systems. A hallmark of osteoarthritis is matrix degeneration and reduced compressive modulus of the pericellular matrix of chondrocytes. To test the mechanical properties of the pericellular matrix, we performed collagen VI immunofluorescence-guided atomic force microscopy. Finally, bone structure and strength were assessed using micro-computed tomography and 3-point bending. Although offspring did not show signs of osteoarthritis, we found a decrease in trabecular number, connectivity, bone volume per total volume, and bone mineral density in the tibial metaphysis of male offspring from HFHS fed dams. Radii of female offspring from HFHS dams had decreased cortical thickness along with decreased post-yield displacement. Together this data suggests maternal HFHS diet impairs bone, but not cartilage in aged offspring.

IDENTIFING THE PIEZO1-RESPONSIVE GENE REGULATORY NETWORK FOR USE IN A NOVEL MECHANOGENETIC SYSTEM

Daniel Palmer[1,2], Robert Nims[1,2], Bo Zhang[1], Farshid Guilak[1,2]
[1]Washington University School of Medicine [2]Shriners Hospitals for Children—Saint Louis

Damage to articular cartilage initiates a degenerative cascade of biochemical and biomechanical changes resulting in progressive tissue damage. Inflammatory cytokine interleukin-1 (IL-1) upregulates expression of the mechanosensitive ion channel Piezo1, producing hypersensitivity to high strain and increasing the likelihood of further tissue damage. This IL-1/Piezo1 interaction presents a novel target for self-regulated drug delivery: a mechanogenetic approach could be used to create a synthetic gene circuit which produces IL-1 receptor antagonist (IL-1Ra) in response to Piezo1 activation. To find the Piezo1-responsive gene regulatory network, porcine chondrocytes were nucleofected with *Piezo1*-targeting or non-targeting control siRNAs or left non-transfected. All groups were cast in 2% agarose and cultured for three days. Next, all groups were either subjected to quasi-static compressive loading to 80% strain at a rate of 1.77% strain/min, chemical activation of Piezo1 with Yoda1, or standard media with a dimethyl-sulfoxide vehicle control. RNA was harvested from the samples and underwent bulk RNA sequencing. RT-qPCR confirmed that *IL6*, *ARL15*, *ACYP1*, and *RDH14* were significantly ($p<0.05$) upregulated by hyperphysiologic loading to 80% strain, with *IL6*, *ARL15*, and *ACYP1* significantly responding to Piezo1 activation specifically by Yoda1. Notably, none of these genes significantly responded to lower magnitudes of strain, indicating a specificity towards selective Piezo1 activation at supraphysiologic strain magnitudes. These genes will be used as the basis of mechanogenetic gene circuits that are responsive to Piezo1 activation for the development of self-regulating cell-based drug delivery systems.

IDENTIFYING THE ROLE OF FASCICULAR ELASTIN IN TENDONS USING MURINE KNOCKOUT MODELS

Shawn N. Pavey, Jeremy D. Eekhoff, Spencer P. Lake

The elastin protein provides elastic behavior to many biological tissues, including tendon, where it is assembled into elastic fibers with the help of Fbln5 during development. Elastic fibers form a dense mesh structure between fascicles, which has been shown to influence shear properties, but more sparsely align themselves along collagen fibers within a fascicle, the effects of which are unclear. We hypothesize fascicular elastin mediates collagen fiber engagement in order to reduce fatigue damage accumulation. Elastin's effect is hypothesized to be stronger in energy-storing tendons such as the Achilles tendon (AT) than in more positional tendons such as the tibialis anterior tendon (TBAT). Mouse tendons are structurally similar to single fascicles from larger animals, making murine knockout (KO) models an excellent way to test our hypothesis. Analysis of $Fbln5^{-/-}$ KO tendons showed a dysregulation of elastic fibers and corresponding alteration of stress-strain curves with higher stiffness values at higher strains in the AT (but not the TBAT). Polarized-light imaging further indicated a greater change in degree of linear polarization (DoLP) throughout stress-relaxation in $Fbln5^{-/-}$ ATs, suggesting elastin plays a significant role in determining collagen fiber alignment. A new knockout model, $Prx1Cre/Eln^{fl/fl}$, is also being investigated as a complete KO of elastin in the extremities, rather than its dysregulation, hopefully leading to more pronounced differences and altered fatigue properties. Validation of this model is currently underway, though promising preliminary results show an absence of elastic fibers in two-photon imaging and decreased elastin expression.

MODELING TUMOR-BONE INTERACTIONS IN ADULT T CELL LEUKEMIA WITH HTLV-1 INFECTED PERIPHERAL BLOOD CELL LINES

Authors:

POKHREL N [1], PANFIL A [2], HABIB H [1], RAUCH D [3], SPRUNG R [4], GILMORE P [4], QIANG Z[4], MALONE J [4], TOWNSEND R [4], WEILBAECHER K [3] and VEIS D[1,5,6]

[1] Division of Bone & Mineral Diseases, Musculoskeletal Research Center, Washington University School of Medicine, Saint Louis, MO, USA; [2] Veterinary Biosciences, The Ohio State University, Columbus, Ohio, USA; Division of Bone & Mineral Diseases, Musculoskeletal Research Center, Washington University School of Medicine, Saint Louis, MO, USA; [3] Division Molecular Oncology, Washington University School of Medicine, Saint Louis, MO, USA; [4] Division of Endocrinology, Washington University School of Medicine, Saint Louis, MO, USA; [5] Shriners Hospitals for Children, St. Louis, MO USA; [6] Department of Pathology and Immunology, Washington University School of Medicine, Saint Louis MO

Adult T cell Leukemia (ATL), caused by infection of human CD4+ T cells with HTLV-1, is associated with osteolytic lesions and hypercalcemia. HTLV-1 infected T cells (HTLV/T) produce exosomes, previously shown to facilitate infection. We hypothesized that these exosomes also mediate bone loss. HTLV/T were generated by co-culturing lethally irradiated HTLV-1 producer cells with human peripheral blood mononuclear cells (hPBMCs). Exosomes were isolated from supernatants and fluorescently labelled with PKH-26. Mouse bone marrow macrophages (mBMMs) and hPBMCs were cultured in osteoclastogenic conditions with supernatant or exosomes from HTLV/T, then stained for TRAP. Supernatants from HTLV/T cells were variable in their ability to stimulate OC differentiation, but showed similar effects on murine and human cultures. Expression of RANKL and OPG mRNAs by these HTLV/T was also variable, but we found no correlation between OC numbers and the RANKL/OPG ratio. Isolated exosomes were taken up by mBMMs, and carried most of the OC stimulatory activity of supernatants. LC-MS/MS followed by statistical analysis (R package) of proteins from exosomes from high and low osteoclastogenic HTLV/T cell lines showed distinct compositions. Select HTLV/T lines were injected into one tibia of immunodeficient NCG mice. In a pilot experiment, intratibial injection of HTLV/T clones with high *in vitro* OC activity led to systemic bone loss and spread of human T cells to contralateral tibia and spleen. Further work is needed to determine if the exosomes they secrete are responsible for induction of osteoclasts in vivo, and which exosome cargoes are most important for bone effects.

SYSTEMIC VEGFA ABLATION BLUNTS LOCOMOTIVE DEFICTS AND INTRADISCAL INNERVATION FOLLOWING LUMBAR INTERVERTEBRAL DISC INJURY

Ryan S. Potter[1,2], Remy E. Walk[1,3], Donald Aboytes[1], Munish C. Gupta[1], Alec T. Beeve[3,4], Erica Scheller[3,4], Lori A Setton[1,3], Simon Y. Tang[1,2,3] 1. Department of Orthopedic Surgery, Washington University in St. Louis 2. Department of Mechanical Engineering and Materials Science, Washington University in St. Louis 3. Department of Biomedical Engineering, Washington University in St. Louis 4. Department of Medicine, Division of Bone and Mineral Diseases, Washington University in St. Louis

INTRODUCTION: Chronic Lower back pain (LBP) is a disabling condition with the intervertebral disc (IVD) as the most common pain generator[1,2]. Innervation of the IVD has been observed in both animals and humans and is likely the pathoanatomical mechanism for LBP[3,4]. Vascular Endothelial Growth Factor A (VEGFA) is critical in neurogenesis and has been associated in painful behavior[5,6]. Using an inducible Cre-ERT2/loxP system driven by ubiquitin (UBC), we examined the effects of VEGFA removal on the intradiscal innervation and locomotion following a lumbar puncture (LP) injury. **METHODS**: 4-6mo. old mice (M=18; F=20) underwent SHAM and LP surgeries. VEGFA KO (n=21) and WT Cre-;$VEGFA^{f/f}$; (n=17) received either LP or SHAM procedures. The animals then received 2 doses of tamoxifen (100 µg /g) on post-op days 3 and 4[7]. Locomotive function and pain sensitivity was performed using Open Field (Omnitech, USA), Rotarod and an E-Von Frey (Bioseb, France) with immunohistochemistry (IHC) performed using PGP9.5, on midsagittal sections of the IVD and validated with RFP-$Na_v1.8$ tissues. Nerve infiltration was graded (0-3) by the depth and penetrance of the PGP9.5+ nerve structures into the annulus fibrosus. Linear mixed effects models (Sidak's posthoc test). **RESULTS**: The pre-TAM, pre-op mice were no different on the Rotarod ($p=0.85$). Following LP surgery and TAM-administration, the VEGFA-null mice exhibited improved latency on the Rotarod ($p=0.004$). Open field and Von Frey showed no differences pre- and post- op. SHAM animals exhibited no PGP9.5 in the IVD, and the WT animals exhibited extensive PGP9.5+ staining in LP and adjacent IVDs ($p=0.004$). The VEGFA-null animals showed reduced PGP9.5 staining compared to the WT ($p=0.02$). **DISCUSSION**: Ablation of VEGFA following the LP injury allowed to animals to regain their pre-operative locomotive performance on the Rotarod. We also observed reduced intradiscal innervation in the VEGFA-null animals. Future work will need to account for tissue-specific sources of VEGFA. **SIGNIFICANCE**: VEGFA removal improved motor function and reduced IVD innervation in a mouse model of IVD injury and could be a viable therapy for LBP. **ACKNOWLEDGEMENTS**: The study is supported by the NIH. **REFERENCES**: [1] Airaksinen et al 2006 [2] Simon et al 2014 [3] Millecamp and Stone 2018 [4] Binch et al 2015 [5] Lai et al 2016 [6] Carmeliet and Almodóvar 2013 [7] Buetterman et al 2019

POSTER LOCATION # *(podium talk only)*

MODULATION OF HUMAN ADIPOSE STEM CELL COLLAGEN SYNTHESIS BY MECHANOSENSING OF SUBSTRATE ARCHITECTURE THROUGH THE PIEZO1 ION CHANNEL

Neda Rashidi[1,2*], Natalia S. Harasymowicz[1,2], Alireza Savadipour[1,2], Farshid Guilak[1,2]
Washington University in St. Louis[1], Shriners Hospitals for Children - St. Louis[2]
n.rashidi@wustl.edu

Mechanosensitive ion channels located in the cell membrane provide a mechanism by which cells sense external mechanical cues such as ECM (extracellular matrix) architecture. Activation of these channels triggers mechanical signaling pathways that can regulate critical cellular processes. This cell-ECM interaction can play a vital role in pathologies such as fibrosis, in which cells produce excessive ECM proteins, leading to tissue dysfunction. However, in the progression of conditions such as soft tissue fibrosis, the fundamental cellular mechanisms connecting mechanosensitive ion channels to ECM synthesis are not fully understood. Here, we hypothesized that the mechanosensitive ion channel Piezo1 senses substrate architecture to regulate collagen synthesis and fibrous tissue formation in human adipose stem cells (ASCs). We investigated role of Piezo1 in the regulation of collagen synthesis in ASCs on aligned vs. non-aligned cell-adhesive micropatterns in 2D *in vitro* model as well as in a 3D model using highly aligned vs. random polycaprolactone substrate fibers. Piezo1 knockdown upregulated collagen synthesis in both 2D and 3D models, and this response was sensitive to the matrix architecture and was only significant for aligned cells. Our results are consistent with previous studies showing ECM architecture plays an important role in providing critical signals that can induce transcriptional changes in the profibrotic gene expression of ASCs. Finally, ASCs sense substrate architecture via Piezo1 mechanotransduction to modulate collagen production. Varying Piezo1 activity may provide a means of inhibiting or enhancing collagen production for adipose tissue engineering and disease modeling for adipose tissue fibrosis.

ABROGATING THERAPY-INDUCED SENESCENCE DRIVEN BONE LOSS TO IMPROVE QUALITY OF LIFE

Ganesh Kumar Raut[1], Zhangting Yao[1], Tom Cole[1], Xianmin Luo[1], Qihao Ren[1] and Sheila A. Stewart[1,2,3,4*]

[1]Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, MO 63110, USA. [2]Department of Medicine, Washington University School of Medicine, St. Louis, MO 63110, USA. [3]Siteman Cancer Center, Washington University School of Medicine, St. Louis, MO 63110, USA. [4]ICCE Institute, Washington University School of Medicine, St. Louis, MO 63110, USA.

Abstract

Despite the central role chemotherapy plays in prolonging survival, the toxicities associated with its use can negatively impact quality of life and in some cases, be so severe that patients forego further life preserving treatments. Therefore, it is critical that we understand the mechanisms that drive these toxicities and develop approaches to mitigate their severity. Recently we demonstrated that chemotherapy-induced senescence drives bone loss by both limiting mineralization of new bone and increasing bone resorption. To establish whether senescent bone resident cells or systemic responses to chemotherapy drove therapy-induced bone loss, we used a vossicle model in which neonatal vertebral bones (L4 and L5) were transplanted from 4-day old wildtype or *INKATTAC* pups (allow to selectively kill senescent cells) into wildtype or *INKATTAC* adult mice. Using this approach, we found that the elimination of senescent cells in donor vossicles protects from chemotherapy-induced bone loss, indicating that senescent bone resident cells are responsible for therapy-induced bone loss. To understand the mechanism(s) that contributed to therapy-induced bone loss, we used scRNA-seq to determine which bone resident cells underwent senescence in response to chemotherapy and how their gene expression was impacted. Using this approach, we found that adipocytes, fibroblast, chondrocytes and osteoblasts underwent senescence as evidenced by expression of p16, p21 and loss of Mki67 and displayed evidence of a senescence associated secretory phenotype (SASP), which we postulate contributes to therapy-induced bone loss. Collectively, our data indicated that chemotherapy causes bone loss via senescence and can be protected by eliminating senescent cells.

SINGLE CELL RNA-SEQUENCING REVEALS UNIQUELY EXPRESSED GENES AND HETEROGENOUS CELLS IN THE RAT MODEL OF INTERVERTEBRAL DISC DEGENERATION

Milad Rohanifar[1], Deepanjali S. Patil[1], Garrett Easson[2], Sade Clayton[2], Frank Lee[1], Liufang Jing[1], Marcos N. Barcellona[1], Julie E. Speer[1], Jordan J. Stivers[2], Simon Y. Tang[2], Lori A. Setton[1,2]

[1]Department of Biomedical Engineering, Washington University in St. Louis
[2]Department of Orthopedic Surgery, Washington University School of Medicine, St. Louis, Missouri USA

Intervertebral disc (IVD) degeneration is characterized by changes in cell-mediated activity that drives anatomic changes to IVD structure. In this study, we use single cell RNA-sequencing analysis to reveal distinct cell types and differentially expressed genes that point to a role for novel cells infiltrating into the injured IVDs. Rats (n=8, 8 weeks of age) underwent surgery for exposure and puncture of the L4-5 and L5-6 IVDs via a needle. Rats were recovered postoperatively for either 2 (n=4) or 8 weeks (n=4). At sacrifice, IVD tissues were isolated and pooled from L4-5 and L5-6 (DEG=degenerate), or from L2-3 and L3-4 (CON=control). The separated tissue was digested to obtain IVD cells (51,655 and 60,626 total cell yield from CON and DEG respectively). cDNA libraries were obtained and sequenced (10x Genomics) and data were reduced via quality control filtering. Unsupervised K-means clustering identified chondrocytes-like cells, endothelial cells, and immune cells. The most notable difference between control and degenerated IVDs pointed to increased numbers of immune cells in DEG samples largely at 2 weeks post-injury, but also at the later 8 week timepoint. The immune cell cluster contained the highest number of differentially expressed genes between CON and DEG samples, and sub-clustering analysis revealed the involvement of multiple cell types from the myeloid and lymphoid lineages, most notably macrophages and B cells. Gene ontology analyses reveals the role of upregulated genes (CD72, BLNK, SYK and RAG1) with primary roles in immune system processes. These findings provide the basis to understand the involvement of select subsets of cells of the immune system in mediating disease progression in IVD degeneration.

BAP1 PROMOTES OSTEOCLAST FUNCTION BY MITOCHONDRIAL ACTIVATION AND CYSTINE TRANSPORTER SUPPRESSION.

Nidhi Rohatgi[1], Wei Zou[1], Yongjia Li[1,2], Eric Tycksen[3], Anwesha Dey[4] and Steven L. Teitelbaum[1,5]

1. Division of Anatomic and Molecular Pathology, Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO 63110, USA
2. Department of Pharmacology, Jiangsu University School of Medicine, Zhenjiang, Jiangsu Province, 212013, PR China
3. Genome Technology Access Center, McDonnell Genome Institute, Washington University School of Medicine, St Louis, MO 63110, USA
4. Department of Discovery Oncology, Genentech Inc., South San Francisco, CA 94080, USA.
5. Division of Bone and Mineral Diseases, Department of Medicine, Washington University School of Medicine, St. Louis, MO 63110, USA

Treatment of osteoporosis most commonly diminishes osteoclast number which suppresses bone formation. Bone formation is not suppressed, however, when osteoclast resorptive capacity rather than differentiation is inhibited. We find deletion of deubiquitinase, *BRCA1-associated protein 1 (BAP1),* in myeloid cells (*Bap1*$^{\Delta LysM}$), arrests osteoclast function but not formation. *Bap1*$^{\Delta LysM}$ osteoclasts fail to organize their cytoskeleton which is essential for bone degradation. Consequently, bone mass increases in the mutant mice. The deubiquitinase activity of Bap1 regulates osteoclast function by altering mitochondrial respiration. Unexpectedly, rescue of mitochondrial function alone in *Bap1*$^{\Delta LysM}$ osteoclast is insufficient to normalize osteoclast activity. On the other hand, the cystine transporter, *SLC7a11,* which is increased in Bap1 deficient osteoclast lineage cells, due to enhanced H2Aub occupancy of its promoter, partners with mitochondrial insufficiency to arrest resorptive activity. SLC7a11 appears to maintain cellular ROS levels and fueling the mitochondrial TCA cycle, two components integral for osteoclast function.

SELF-REGULATING ANTI-CYTOKINE HYDROGEL IMPLANTS FOR THE TREATMENT OF RHEUMATOID ARTHRITIS

Leila S. Saleh[a,b,c], Emma Erlich[a], Rafael Sanguinetti Czsepielewski[a], Haleigh Pine[e], Christine T.N. Pham[a], Gwendalyn J. Randolph[a], Farshid Guilak[a,b,c]

[a]Washington University in St. Louis, St. Louis, MO, 63110, USA
[b]Shriners Hospitals for Children, St. Louis, MO, 63110, USA
[c]Center of Regenerative Medicine, Washington University in St. Louis, St. Louis, MO, 63110

Rheumatoid arthritis (RA) is a progressive inflammatory disease which leads to painful joint swelling and degeneration and presents in flares accompanied by varying levels of inflammatory cytokines. Current treatments are limited by the unpredictable nature of these flares. The goal of this study was to develop an implantable cell-based system which can provide on-demand, proportional anti-cytokine therapy for long-lasting RA therapy in a novel model of tumor necrosis factor alpha (TNF-α)-mediated inflammatory arthritis. Using CRISPR-Cas9 genome editing, we have established a murine iPSC line that responds to elevated exogenous TNF-α by synthesizing its inhibitor, soluble TNF receptor 1 (sTNFR1) in a self-regulating manner. These cells were encapsulated in a poly(ethylene glycol) (PEG) hydrogel which maintained differentiation and cell viability, allowed for direct signaling and responsiveness of encapsulated cells, and supported diffusion and export of sTNFR1. Preliminary studies characterizing the RA phenotype in the novel $TNF^{\Delta ARE/+}$ model of arthritis indicate a consistent RA phenotype exemplified by pronounced bone erosion by micro computed tomography and cartilage degradation by histological assessment compared to WT littermate controls. Treatment with anti-TNF antibody mitigated disease severity in 24-week-old mice compared to an isotype antibody control, indicating that the damage is reversible. Finally, we showed that bone erosion is inducible in WT mice through bone marrow transplant from $TNF^{\Delta ARE/+}$ mice. Taken together, these findings suggest that $TNF^{\Delta ARE/+}$ is a suitable model for RA with which to test our cell-laden hydrogel system and which can provide further insight into the role of TNF-α in arthritis.

Developing an Automated Image Analysis Program to Evaluate Elbow Contracture Using Deep Learning Algorithms

Authors/Co-Authors: Shah, Shikha; David, Michael; Liang, Bianca; Bradley, Evan; Movva, Arun; Bacon, Alison; Lake, Spencer

Abstract:

Post-traumatic joint contracture (PTJC) often occurs in the elbow after injury (e.g., dislocation, fracture), causing pain and debilitating loss of motion. To better understand the pathogenesis behind PTJC, we previously developed a rat elbow injury model leading to similar clinical symptoms of PTJC including capsule fibrosis and mild cartilage damage. To further study the changes in cartilage at the cellular level, we utilized immunohistochemistry and histology to examine different components of healthy and injured tissues to determine how PTJC affects structural changes at a microscopic level. Unfortunately, histology-based analyses with large datasets can be extremely time-consuming. In addition, such approaches are generally qualitative in nature and lack the ability to extract sensitive/meaningful quantitative metrics. To improve upon this, machine learning approaches like deep learning with neural networks offer automated methods to classify images and identify/recognize relationships between features. Machine/deep learning has been used to automatically assess cartilage health in joint diseases of the knee; however, the use of these approaches in contracture and arthritis of the elbow is limited. The purpose of this study was to build and train a neural network to recognize and identify chondrocytes in healthy and injured cartilage tissue, then extract meaningful information about spatial patterns and phenotypical clustering. Results have shown that machine learning combined with digital histopathology can fully automate and accelerate cartilage image analysis following an elbow injury, which will provide insights towards better understanding the impact of PTJC on joint tissue health.

EXPLORING THE RELATIONSHIPS BETWEEN SARM1, NEUROPATHY, AND BONE DISEASE IN ADOLESCENTS WITH TYPE 1 DIABETES

Ivana R Shen[1], Jennifer M Brazill[1], Aaron DiAntonio[3,5], Mahshid Mohseni[1], Yo Sasaki[4], Erica L Scheller[1,2]

[1]Division of Bone and Mineral Diseases, Department of Medicine, Washington University School of Medicine, St. Louis, MO, USA

[2]Department of Biomedical Engineering, Washington University, St. Louis, MO, USA

[3]Department of Developmental Biology, Washington University School of Medicine, St. Louis, MO, United States

[4]Department of Genetics, Washington University School of Medicine, St. Louis, MO, United States

[5]Needleman Center for Neurometabolism and Axonal Therapeutics, St. Louis, MO, United States

Type one diabetes (T1D) often comes with life-long nerve and bone damage, which can manifest earlier with poor glycemic control. The early identification and treatment of neuropathy and bone disease is crucial to enhance the quality of life for these patients. Sarm1 is a central mediator of neurodegeneration and diabetic peripheral neuropathy. In addition, we have recently discovered that Sarm1 is a critical contributor to diabetic bone disease in a mouse model of T1D. We hypothesize that inadequate glucose control drives Sarm1 activation in adolescents with T1D, leading to the early onset of nerve damage and bone loss. To test this hypothesis, we plan to enroll 12 control and 24 T1D participants, age 12-18, over the span of one year. T1D participants will be divided into two groups based on HbA1c levels. Current results show that adolescents with poorly controlled T1D have increased body mass. Consistent with previous clinical reports, this was associated with increases in cortical bone parameters as assessed by XtremeCT-II. However, we also observed a concurrent decrease in trabecular bone quality, potentially indicating deterioration of skeletal structural integrity in patients with T1D. Though overt signs of neuropathy were not detected, we observed a trending increase in neuropathy score in the group with poorly controlled T1D. Our preliminary data also showed that Sarm1 can be regulated by pharmaceutical agents in patient derived peripheral blood mononuclear cells. We are currently recruiting more participants into the study and creating multiple regression models to isolate the relationships between Sarm1, neuropathy, and bone quality in young patients with T1D.

IκBζ IS A CENTRAL MODULATOR OF RHEUMATOID ARTHRITIS PATHOGENESIS.

Gaurav Swarnkar, Manoj Arra, Yousef Abu-Amer
Department of Orthopedic Surgery, Washington University in StLouis

Rheumatoid Arthritis (RA) is a chronic inflammatory disease of joints. Elevated levels of pro-inflammatory cytokines in the synovium, is considered hallmark of RA. IL-1β, TNFα, IL-17, IL-6 and other highly abundant cytokines in the RA, promote activation of macrophage, chondrocyte, osteoclasts and effector T cells. As such, current therapies targeting cytokines show limited efficacy owing to cytokine redundancies. NF-κB signaling plays a crucial role in the production and function of these inflammatory cytokines. However, given the essential role of NF-κB in physiologic processes, a more promising approach is to target pathway downstream of NF-κB, which regulate expression of inflammatory cytokines in RA. To this end, we have shown that IκBζ is a unique inflammatory signature of NF-κB, controls transcription of inflammatory cytokines during pathologic conditions only. In fact, deletion of *Nfkbiz*, the gene encoding IκBζ, attenuated production of these factors in various cell types involved in RA. Further, RNA-Seq of $CD11b^{+}$ cell from joints of WT and *LysMcre:Nfkbiz* knockout mice, subjected to serum-induced arthritis, showed decreased expression of *IL-6, IL-17, IL-1β, TNFα* and other inflammatory signals in KO mice. Likewise, using PCR and ELISA we confirmed that deletion of *Nfkbiz* significantly attenuated the expression of *IL-1β, IL-6, IL-17* as well as other inflammatory factors such as *MMPs, Nos2, S100a8/9*. Finally, pharmacological inhibition of IκBζ using Dimethyl Itaconate, significantly decreased expression of inflammatory genes. In summary, our study holds promise to establish IκBζ, unlike other physiologically essential proteins, as an inflammation-specific target for therapeutic intervention in RA.

DUAL PEPTIDE FUNCTIONALIZED ALGINATE HYDROGELS TO MODULATE NUCLEUS PULPOSUS CELL PHENOTYPE

Xiaohong Tan[1*], Era Jain[1], Marcos N. Barcellona[1], Evan Morris[1], Sydney Neal[1], Munish C. Gupta[2], Jacob M. Buchowski[2], Michael Kelly[2], Lori A. Setton[1,2,3], Nathaniel Huebsch[1,3,4]
[1]Department of Biomedical Engineering, [2]Department of Orthopedic Surgery, [3]Center for Regenerative Medicine, [4]Center for Investigation of Membrane Excitability Diseases, Center for Cardiovascular Research, Washington University in St. Louis; [*]*xiaohong.tan@wustl.edu*

Intervertebral disc (IVD) degeneration is a major contributor to disability and the economic burden of musculoskeletal disease. Decreased cellularity and altered phenotype in cells of the nucleus pulposus (NP) are some of the earliest changes observed. Biomaterial and/or cell supplementation to the NP has been widely explored for regeneration of the IVD. We hypothesized that co-presentation of integrin and syndecan binding peptides (cyclic RGD and AG73, respectively) would promote expression of a more biosynthetically active NP phenotype as compared to unmodified alginate and single peptide hydrogel. Primary human NP cells isolated from NP region of to-be-discarded surgical waste of IVD tissues were cultured *atop* and encapsulated in unmodified alginate, cRGD, AG73, or cRGD/AG73 hydrogels. NP-specific phenotype and matrix synthesis were evaluated. Our results illustrated the incorporation of cell-adhesive peptides in alginate hydrogel promoted cell viability, biosynthetic activity, and NP-specific phenotypes over unfunctionalized alginate. Encapsulation of NP cells in gels presenting cRGD/AG73 was linked to an elevated accumulation of new ECM and expression type II collagen and aggrecan after 7 and 21 days of culture compared to alginate and single peptide hydrogel. Similarly, a significantly higher protein expression of brain acid soluble protein 1 (BASP-1) and N-Cadherin were expressed in NP cells in both 2D and 3D. Altogether, these results demonstrate a benefit of incorporating both cell adhesive domains to allow a more biosynthetically active, NP-specific cell phenotype. The results represent a promising step toward understanding how distinct adhesive peptides can be combined to guide NP cell fate in 3D culture.

BILATERAL NEEDLE PUNCTURE OF MOUSE CAUDAL INTERVERTEBRAL DISCS RESULTS IN COMPROMISED STRUCTURE, LOSS OF HYDRATION, AND *DE NOVO* INTRADISCAL INNERVATION

Remy E. Walk[1], Kaitlyn S. Broz[2], Lori A. Setton[1,3], Munish C. Gupta[3], Simon Y. Tang[1-3]
[1]Department of Biomedical Engineering [2]Institute of Material Science and Engineering
[3]Department Orthopaedic Surgery
Washington University in St. Louis, St. Louis, MO
rwalk@wustl.edu

Disclosures: NONE

While intervertebral disc (IVD) degeneration is associated with low back pain, the pathoanatomy of pain is unknown. Needle puncture of the IVD is a useful animal model to induce degeneration and innervation [1,2], and the use of caudal IVDs offer the advantage of ease of access with only a skin wound in addition to the IVD injury. Degeneration of caudal IVDs following puncture has been shown previously [3]; however, there is limited data demonstrating innervation. We evaluate *de novo* intradiscal innervation in the mouse caudal IVD following bilateral puncture. Procedures were approved by WUSM IACUC. 9-month-old C57BL/6 female mice (n = 12) were used. CC4/5 and 5/6 IVDs were punctured bilaterally with a 30G needle, and the CC6/7 served as control. Mice were euthanized 4 weeks after puncture for structure and innervation analyses: microCT (n = 3), Safranin-O (n = 3), and Protein Gene Product 9.5 immunostaining, a neuronal marker (PGP9.5) (n = 6). MicroCT samples were incubated in Ioversol (OptiRay 350, Guebert, St. Louis), a nonionic, hydrophilic contrast agent. PGP9.5 sections were graded on a scale ranging from 0, where no PGP9.5+ structures penetrate the IVD, up to 3, where PGP9.5+ structures penetrate 3 or more lamella of the AF. Student's t-tests were used to determine the effects of injury. All statistics conducted using Prism 9.12. Degeneration of the injured IVDs were readily observable histologically. The whole disc volume was not different between groups, but the NP volume fraction declined by 29% ($p = 0.03$) and NP hydration was reduced ($p = 0.05$) in injured IVDs. PGP9.5 grades were greater in the injured IVDs ($p = 0.02$). Caudal puncture provides an experimentally convenient alternative to lumbar puncture with observable innervation.

References: [1] Lotz and Ulrich JB&JS, 2006 [2] Luoma et al. Spine, 2000 [3] Piazza et al. JOR, 2018 [4] Isa et al. Science Advances, 2018 [5] Orita et al. Spine, 2010

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AIM2 Inflammasome Mediates Chemotherapy-Induced Bone Loss

Chun Wang, Jianqiu Xiao, Kai Sun, Gabriel Mbalaviele
Division of Bone and Mineral Diseases, Washington University School of Medicine, St. Louis, MO

Chemotherapy is widely used as a myeloablative, adjuvant, or neo-adjuvant strategy for the treatment of a variety of solid and hematological malignancies. Despite its success in improving survival rates of cancer patients, chemotherapy causes deleterious side effects, including bone marrow toxicity, osteoporosis, and fracture risk. Neutropenia, which may be the result of NETosis, a form of neutrophil-specific cell death characterized by the release to the extracellular space of decondensed chromatin and granular contents known as neutrophil extracellular traps (NETs), is also one of the most serious hematologic toxicity induced by chemotherapy. The mechanisms that underlie the cytotoxic effects of chemotherapy are not well understood. Since chemotherapeutic drugs such as doxorubicin cause DNA damage and leakage to the cytoplasm, we studied the role of the AIM2 inflammasome (a sensor of mislocated DNA in the cytoplasm) on off-target effects of this drug in a non-tumor-bearing mouse model. We found that neutrophils and macrophages express AIM2. Interestingly, the absence of the AIM2 inflammasome completely prevented doxorubicin-induced bone mass loss in vivo. Mechanistically, doxorubicin induced the secretion of IL-1β and IL-18 (markers of inflammasome activation) by macrophages and neutrophils in vitro as well the formation of NETs, outcomes that were significantly decreased in cells lacking AIM2. Since IL-1β is a potent stimulator of osteoclast-mediated bone resorption, and NETs promote not only inflammation but also neutrophil death, our study has uncovered the AIM2 inflammasome as a novel mechanism through which doxorubicin inflicts damage to bone and causes neutropenia.

POSTER LOCATION # *(podium talk only)*

NEURAL CONTRIBUTIONS TO LEPTIN-MEDIATED BONE MARROW ADIPOCYTE CATABOLISM

Xiao Zhang[1, 2], Kristann L Magee[1], Anurag Majumdar[1], Jennifer M Brazill[1], Erica L Scheller[1, 2, 3]

[1]Division of Bone and Mineral Diseases, Washington University, Saint Louis, MO, USA
[2]Department of Biomedical Engineering, Washington University, Saint Louis, MO, USA
[3]Department of Cell Biology and Physiology, Washington University, St. Louis, MO, USA

The nervous system is the central regulator of energy partitioning and metabolic homeostasis. Within the skeleton, sympathetic and sensory axons are located near the bone marrow adipocytes (BMAds) and trace back to leptin-responsive regions of the brain. However, how the nervous system regulates bone marrow adipose tissue (BMAT) catabolism remains largely unknown. To study the neural regulation of BMAd catabolism, we delivered leptin to the mouse brain through intracerebroventricular (ICV) injection either acutely or chronically. Mice treated with three acute injections of 1.5 µg leptin over 24 hours lost 18% of their total tibial bone marrow adipose tissue (BMAT), whereas chronic 10 or 100 ng/hr ICV leptin treatment caused a dose-dependent, 79-100% decrease in tibial BMAT over 9 days. Leptin treatments in both settings also caused decreases in body and fat mass. The depletion of BMAT in acute leptin treatment was due to decreases in both BMAd size and number, which were rescued by the subcutaneous administration of sympathetic and sensory neurotransmitter inhibitors, respectively. However, in the chronic setting, neither surgical denervation nor chemical sympathectomy rescued ICV leptin-mediated BMAT depletion. Together, these results suggest that ICV leptin induces a rapid, initial phase of BMAd lipolysis and/or apoptosis mediated by the peripheral nervous system, whereas the chronic, centrally-mediated systemic changes drive the catastrophic depletion of BMAT in a later stage. Future work is needed to further interrogate this model and identify the systemic regulators and cellular mechanisms of leptin-induced regulation of BMAT and its consequences for both skeletal and metabolic health.

MICRORNA-181A/B-1 ENHANCES BONE FRACTURE REPAIR VIA REGULATION OF MITOCHONDRIAL METABOLISM AND PDK4 ACTIVITY

Hongjun Zheng[1], Jin Liu[1], Jennifer McKenzie[1], Matthew Silva[1], Audrey McAlinden[1,2]
[1]Washington University School of Medicine, St Louis, MO; [2]Shriners Hospital for Children-St Louis, MO

Introduction: We previously showed that miR-181a/b-1 increased osteogenesis via regulation of the PTEN/PI3K/AKT axis and enhancement of mitochondrial metabolism. This study was designed to determine the effects of miR-181a/b-1 on bone formation in vivo and to identify additional mediators regulated by this miRNA cluster. **Methods**: Lentiviruses expressing miR-181a/b-1(LV-181) or a non-silencing control RNA (LV-NS) were transduced into human cartilage progenitor cells (CPCs). Western blotting and luciferase reporter assays were used to confirm pyruvate dehydrogenase kinase-4 (PDK4) as a target of miR-181a/b-1. The role of PDK4 in regulating osteogenesis and pyruvate dehydrogenase complex (PDC) activity was explored via shRNA technology or a PDK4 inhibitor drug, DADA. Ulnar fractures were created in 12 wk male mice followed by injection of LV-181 or LV-NS into the fracture site. Limbs were harvested at day 14 and processed for X-ray and micro-CT imaging. **Results**: Over-expression of miR-181a/b-1 in CPCs resulted in reduced PDK4 protein. Treatment of CPCs with LV-181, sh-PDK4 or DADA resulted in reduced PDC activity and increased osteogenesis. X-ray and micro-CT imaging revealed formation of a larger repair callus volume in the LV-181 group and higher levels of bone within the callus region compared to control LV-NS group at day 14 post fracture. **Discussion**: Our data indicates that PDK4 is a target of miR-181a/b-1. Suppression of this mitochondrial enzyme may partly explain the effects of this miRNA cluster on enhancing mitochondrial metabolism and osteogenesis in vitro. Importantly, the bone-enhancing effects of miR-181a/b-1 were also demonstrated in an in vivo model of endochondral fracture repair.

BIOENGINEERED EXOSOMES AND OXYGEN RELEASE NANOPARTICLES FOR TREATING CRITICAL LIMB ISCHEMIA IN DIABETIC MICE

Ting Zhong, Xiaolei Nie, Ning Gao, Zhongting Liu, Jianjun Guan

Department of Mechanical Engineering & Materials Science, McKelvey School of Engineering, Washington University in St. Louis, St. Louis, MO, United States

Revascularization represents the primary therapeutic goal for patients with critical limb ischemia (CLI). Yet current approaches have unsatisfied outcomes as they cannot efficiently vascularize the tissues and improve cell survival before vascularization is established. Herein, the ischemia-targeting exosomes (EXO) secreted by human-induced pluripotent stem cell-derived mesenchymal stem cells (iMSCs) and oxygen release nanoparticles (ONP) were developed to address current limitations. In vitro, the survival of human umbilical vein endothelial cells (HUVECs) and mouse myoblast cells (C2C12) under hypoxia (1% O_2) was significantly improved in the EXO/ONP group. The combination of EXO and ONP also promoted tube formation of HUVECs, and myotube formation of C2C12 cells. After delivering by intravenous injection, the ONP and EXO accumulated mostly in the ischemic region of hindlimbs. The EXO and ONP exhibited a synergistic effect in facilitating blood flow recovery and promoting skeletal muscle regeneration.

CAR CELLS EXPRESS BMP INHIBITORS NEGATIVELY REGULATING BONE FORMATION in vivo

Wei Zou[1], Nidhi Rohatgi[1], Matthew J Silva[2], Steven L Teitelbaum[1,3]

1. Division of Anatomic and Molecular Pathology, Department of Pathology and Immunology, Washington University School of Medicine, St. Louis
2. Department of Orthopaedic Surgery, Washington University School of Medicine, St. Louis
3. Division of Bone and Mineral Diseases, Department of Medicine, Washington University School of Medicine, St. Louis

C-X-C motif chemokine 12 (CXCL12)-abundant reticular (CAR) cells are a marrow residing stromal cell believed to be osteoblast progenitors. The mechanism by which CAR cells differentiate into osteoblast is not well known. Our lab has generated an inducible fat deletion mouse model by mating mice expressing an inducible DTR to those bearing adiponectin (ADQ)-Cre. Within 4 days of DTR activation, the systemic bone mass of DTR^{ADQ} mice begins to increase due to stimulated osteogenesis, with a 1,000% enhancement by 10-14 days. This adipocyte ablation-mediated enhancement of skeletal mass reflects bone morphogenetic protein (BMP) receptor activation following the elimination of its inhibitors, Gremlin1 and Chrdl1 by depletion of marrow residing adiponectin-expressing cells. With lineage tracing we found virtually all CAR cells are derived from adiponectin positive lineage cells and DTR activation completely depletes marrow of CAR cells. By sorting CAR cells from Cxcl12-GFP reporter mice, we found they are the principal source of the BMP inhibitors. BMP targeted cells in DTR^{ADQ} mice are early committed osteoblast lineage cells driven by the 3.6Col1a1 promoter likely differentiating into mature osteoblasts characterized by activated 2.3Col1a1 promoter. Taken together, our data show Cxcl12+ CAR cell not only differentiate into osteoblasts, but are also the principal source of BMP inhibitors in marrow negatively regulating bone formation in vivo. Thus, unexpectedly, elimination of CAR cells, which are osteoblast progenitors, rapidly increases bone mass due to BMP activation of more committed osteoblast lineage cells.