Research Mentors

Edward Akelman, M.D.
Vincent Zecchino Professor & Chairman, Department of Orthopaedics
Surgeon-in-Chief, Department of Orthopaedics, Rhode Island Hospital & Miriam Hospital

Joseph J. Crisco, Ph.D.
Henry Frederick Lippitt Professor of Orthopaedic Research
Executive Director for Research
Director, Bioengineering Laboratory, Department of Orthopaedics

Roy Aaron, M.D.
Professor of Orthopaedics
Director, Orthopaedic Program in Clinical/Translational Research
Research Director, Miriam Hospital Joint Replacement Center

Christopher T. Born, M.D., FACS, FAAOS
Intrepid Heroes Professor of Orthopaedic Surgery,
Emeritus Chief, Division of Orthopaedic Trauma Director, The Diane N. Weiss Center for Orthopaedic Trauma

Qian Chen, Ph.D.
Michael G. Ehrlich Professor and Vice Chair for Research, Department of Orthopaedics
Director, NIH Center of Biomedical Research Excellence in Skeletal Health and Repair

Alan Daniels, M.D.
Associate Professor of Orthopaedics
Chief, Adult Spinal Deformity Service, Rhode Island Hospital

Braden Fleming, Ph.D.
Lucy Lippitt Professor of Orthopaedics, Professor of Engineering

Dioscaris Garcia, Ph.D.
Assistant Professor of Orthopaedics (Research), Assistant Professor of Molecular Pharmacology, Physiology and Biotechnology
Co-Director, The Diane N. Weiss Center for Orthopaedic Trauma Research

Chathuraka Jayasuriya, Ph.D.
Assistant Professor of Orthopaedics

Douglas C. Moore, M.S.
Associate Director, Bioengineering Laboratory, Department of Orthopaedics

Brett Owens, M.D.
Professor of Orthopaedics
Director, The Rhode Island Cartilage Repair Center

Wentian Yang, M.D, Ph.D.
Associate Professor of Orthopaedics

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DEPARTMENT OF ORTHOPAEDICS GRAND ROUNDS

RHODE ISLAND HOSPITAL
WARREN ALPERT MEDICAL SCHOOL
OF BROWN UNIVERSITY

STUDENT RESEARCH DAY 2019

WEDNESDAY, MAY 15, 2019
7:00 – 8:00 AM
NURSING ARTS 5

UNIVERSITY ORTHOPEDICS

BROWN Department of Orthopaedics

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7:00 - Welcome Remarks

Joseph J. Crisco, Ph.D
Henry Frederick Lippitt Professor of Orthopaedic Research
Executive Director for Research
Director Bioengineering Laboratory, Department of Orthopaedics

7:05 Wes Durand, Sc.B, M.D '20
"Neural Network Utilization for the Automated Extraction of Schwab Modifiers from Plain Radiographs in Adult Spinal Deformity Patients"

7:10 Meng "Joel" Feng, M.D, Ph.D '19
"Sonic Hedgehog from Human Osteoarthritic Cartilage-Resident Mesenchymal Stem Cells Drives SASP and Paracrine Senescence in Cartilage Aging"

7:15 Shaun Forbes, MA, Ph.D '20
"Bounding the Implications of Non-Compliance in Randomized Controlled Trials in Orthopedic Surgery"

7:20 Jillian Glasser, BA
"A Rapid Visualization Assay for the Detection of Orthopedic Related Infection on Surgical Hardware, Tissue and Synovial Fluid"

7:25 Jacob Jamison, BA
"Effect of Nucleoside Reverse Transcriptase Inhibitors (NRTIs) on Bone Health in Young Adult and Aged Mice"

7:30 Brian McHugh, Sc.M.
"Accuracy of Measurement Systems for Quantifying In Vivo Wrist Kinematics"

7:35 Jake Newberry Sc.M.
"Cartilage Progenitors Stimulate Meniscus Tear Reintegration and Fibrochondrocyte Proliferation to Facilitate Healing in Two Independent Ex-Vivo Models of Meniscus Injury Repair"

7:40 Jeffrey Okewunmi, Sc.B.
"The Effect of Vitamin D3 on Osteochondral Graft Viability Ex Vivo"

7:45 Grace Plassche, Sc.B.
"Novel Enviromimetic Culturing Prototype for the Elucidation of Soil Derived Marine Bacteria"

7:50 Casey Tierney, Sc.M.
"Effects of Experience Level on Head Impact Exposure in Youth Football"

7:55 Brian Vuong, Sc.B.
"Extending the Viability of Fresh Cartilage with a Novel High-Subzero Cryopreservation Approach"

8:00 Jiayu Wei, Ph.D '20
"SHP2 Regulation of Osteoblastogenesis and Bone Mineral Homeostasis"

8:05 Closing Remarks
Joseph J. Crisco, Ph.D.

Student Research Day
May 15, 2019
Project Abstract Descriptions

Name: Wes Durand, Sc.B., M.D. '20 **Graduating Date: May 2020**
Mentor: Alan Daniels, M.D.
Future Plans: 4th Year Medical Student applying to orthopedic surgery residency
Project Title: Neural Network Utilization for the Automated Extraction of Schwab Modifiers from Plain Radiographs in Adult Spinal Deformity Patients

The measurement of sagittal alignment is a key component of patient assessment and operative planning in adult spinal deformity surgery. Machine learning techniques, such as artificial neural networks (ANNs), have shown promise in medical image analysis. We sought to evaluate the potential for ANNs to automate extraction of Schwab modifiers from lateral plain radiographs of adult spinal deformity patients. This was a retrospective analysis of a multi-center, prospectively-defined database of a consecutive cohort of ASD patients. From 1,506 patients, 3,598 and 883 lateral plain radiographs were respectively randomized to training and testing datasets. The primary outcome measures were Schwab modifiers for global alignment as measured by sagittal vertical axis (SVA), pelvic tilt (PT), and pelvic incidence minus lumbar lordosis (PI-LL). Schwab modifiers were analyzed as binary (0 vs. +/++). Pre- and post-operative lateral plain spine radiographs were abstracted as available up to 2-year follow-up. Image preparation and analysis was done using the Julia programming language and the Flux.jl, Images.jl, and Metalhead.jl packages, and a modified DenseNet architecture. Images were randomly divided into training and testing datasets at an 80:20 ratio. A cross-entropy loss function was used. Models for each outcome were trained for 50 epochs. Among Schwab modifier outcomes, AUCs for prediction of 0 vs. +/++ rating were 0.95 for SVA, 0.85 for PI-LL, and 0.78 for PT. The corresponding prediction accuracies and false positive/negative rates (FPR/FNR) for SVA, PI-LL, and PT were 87% (FPR 11%, FNR 16%), 79% (FPR 11%, FNR 35%), and 71% (FPR 28%, FNR 30%), respectively. This study demonstrates the potential for ANNs to automate extraction of binary SVA, PI-LL, and PT from pre- and post-operative lateral plain radiographs of ASD patients. The predictive accuracy and AUC for SVA showed the most promise. The results suggest the potential of ANNs, identify areas for refinement, particularly with regard to perception of non-global alignment metrics.

Name: Meng "Joel" Feng, M.D., Ph.D. '19 **Graduating Date: June 2019**
Mentor: Qian Chen, Ph.D.
Future Plans: Dual M.D. PhD program in Orthopedic Surgery (China)
Project Title: Sonic Hedgehog from Human Osteoarthritic Cartilage-Resident Mesenchymal Stem Cells Drives SASP and Paracrine Senescence in Cartilage Aging

Osteoarthritis (OA) is an aging-associated disease. The abundance of cartilage-resident mesenchymal stem cells with replicative senescence increases during development of OA, while the number of chondrocytes declines in aging cartilage. Senescence-associated secretory phenotype (SASP) promotes extracellular matrix degradation and inhibits tissue regeneration in aging cartilage. Senescent stem cells can transmit limited senescent phenotypes to neighboring cells called paracrine senescence. The molecular basis of OA mesenchymal stem cell aging (OA-MSC) remains an area of active investigation, but the autonomous signaling pathways leading to uncontrolled release of the inflammatory components are not yet understood. Specific deletion of p16INK4a positive chondrocytes does not inhibit the increased production SASP components during physiological aging. It indicated that senescent chondrocytes might not be a major driver accounting for SASP. Therefore, an accurate identification of senescent cell type before and after clinical treatment is required for the development of senolytic drugs. Hedgehog signaling is an important player during OA pathogenesis. In this study, we hypothesized that Sonic Hedgehog (SHH) was synthesized by OA-MSC, which stimulated SASP and drove cartilage aging by spreading senescent characteristics to chondrocytes. Our results indicated a novel mechanism that SHH was responsible for stem cell aging and OA pathogenesis through autocrine and paracrine pathway. In conclusion, OA-MSC rather than OAC, is a major initiator and driver of HH signaling that leads to enhanced cellular senescence, SASP release, and OA pathogenesis in human cartilage aging.

Name: Shaun Forbes, MA **Graduating Date: May 2020**
Mentor: Roy Aaron, M.D.
Future Plans: Finishing last year of Ph.D. program.
Project Title: Bounding the Implications of Non-Compliance in Randomized Controlled Trials in Orthopedic Surgery

Randomized controlled trials (RCTs) are the current standard to test causal clinical hypotheses and the intention-to-treat (ITT) method is typically used to assess the outcomes. However, under circumstances of poor protocol fidelity, the ITT method only measures the effect of allocation or assignment to a treatment. In particular, when patients "cross-over" between arms of an RCT, ITT analyses may actually underestimate treatment efficacy. Cross-overs are particularly common in orthopedic trials such as those evaluating arthroscopic partial meniscectomy (APM) vs. physical therapy for osteoarthritis of the knee (OAK). Thus, we introduce a transparent "bounding" approach that estimates the actual effect of treatment while explicitly accounting for cross-overs. Applying our approach to three clinically-influential studies assessing APM for OAK, we found that given significant cross-overs there remains considerable ambiguity in the treatment effect. However, the bounds include a potential substantial positive treatment effect for APM which is underestimated when using the ITT approach. Thus, clinical decisions (and decision-analyses) based on these bounds may differ from those based on the ITT effect.

Name: **Jillian Glasser, BA** **Graduating Date: May 2019**
Mentor: Christopher Born, M.D. **Co-Mentor:** Dioscaris Garcia, Ph.D.
Future Plans: Brown University and University Orthopedics, Division of Adult Reconstruction, Research Fellowship
Project Title: A Rapid Visualization Assay for the Detection of Orthopedic Related Infection on Surgical Hardware, Tissue, and Synovial Fluid

One of the most significant problems in the field of orthopedic surgery today is bacterial infection. Each year, nearly 40,000 of the two million fracture fixation devices implanted to treat closed fractures become infected. The infection rate can rise to nearly 30% for fracture fixation devices used to treat open fractures (1). Moreover, the number of total hip and knee arthroplasty infections is projected to reach almost 60,000 in 2019 (2). Currently, some of the methods used to diagnose these infections are PCR, culturing, and gram staining. While regularly used in the hospital setting, these methods can have issues with accuracy, efficiency, contamination, and the time it takes to report necessary results (3,4,5). To combat these problems, we have developed a rapid visualization assay using fluorescently conjugated antibodies and Confocal Laser Scanning Microscopy (CLSM) to detect gram positive and gram negative bacterial cells with high selectivity and contrast in synovial fluid, tissue, and surgical explants in 30 minutes.

Name: Jacob Jamison, BA **Graduating Date: May 2019**
Mentor: Qian Chen, Ph.D.
Future Plans: Teaching English in Hamburg, Germany on a Fulbright award.
Project Title: Effect of Nucleoside Reverse Transcriptase Inhibitors (NRTIs) on Bone Health in Young Adult and Aged Mice

HIV patients undergoing antiretroviral therapy (ART) have been shown to manifest decreased bone mineral density (BMD) compared to control cohort at hip and spine, putting HIV patients undergoing ART at a higher risk of bone fracture due to osteopenia or osteoporosis. In this study we examined whether knee joints of mice are similarly affected. We observed the effects of Lamivudine (3TC), a Nucleoside Reverse Transcriptase Inhibitor (NRTI) which is used often in ART, and its effect on male mice knee joints in young adult and aged groups. Trabecular bone health of treatment groups was determined through measurement of Micro-CT (uCT) bone parameters.

Name: Brian McHugh, Sc.M **Graduating Date: May 2019**
Mentor: Joseph J. Crisco, Ph.D.
Future Plans: Undecided
Project Title: Accuracy of Measurement Systems for Quantifying In Vivo Wrist Kinematics

The wrist is capable of six degrees of freedom and is involved in the completion of functional tasks known as activities of daily living. Understanding wrist kinematics is important to evaluate normal wrist function, progression of pathologies and success of therapies. The first aim of this study was to investigate the agreement between an electrogoniometer and optical motion capture, two commonly used methods to quantify in vivo joint kinematics. One limitation of this study is both systems are placed on the skin and are subject to a source of error known as soft tissue artifact, where the skin does not necessary reflect the underlying bone movement. The second aim of this study was to investigate the agreement between optical motion capture and biplanar videoradiography. Biplanar videoradiography measures true skeletal motion with two high speed x-ray systems.

Name: Jake Newberry, Sc.M. **Graduating Date: May 2019**
Mentor: Chathuraka Jayasuriya, Ph.D.
Future Plans: Pursuing a career in the biomedical industry/MCAT
Project Title: Cartilage Progenitors Stimulate Meniscus Tear Reintegration and Fibrochondrocyte Proliferation to Facilitate Healing in Two Independent Ex-Vivo Models of Meniscus Injury Repair

Meniscus tears are among the most frequent orthopaedic injuries that commonly affect active and young populations. These tears ultimately increase the risk of developing post-traumatic osteoarthritis (PTOA). Tears located in the inner two-thirds of the meniscus present a significant clinical challenge because these regions lack vascularity, resulting in poor capacity for healing. Mesenchymal stem/progenitor cell (MSC) -mediated meniscus tissue repair is an emerging and promising strategy that is expected to one day revolutionize the way we treat meniscus injuries in patients. Currently, pre-clinical studies utilize MSCs from several tissue sources to investigate the healing response stimulated by these cells. These sources include bone marrow, synovium, adipose, meniscus, and cartilage tissues. Considering that meniscus is a cartilaginous tissue, we investigated in the present study using cartilage-derived progenitor cells (CPCs) to try and stimulate healing of meniscus injuries. Unlike MSCs from other sources of adult tissues (i.e. bone-marrow, synovium), CPCs are resistant to hypertrophic terminal differentiation – a biological process that is a hallmark of osteoarthritis and cartilage erosion. Furthermore, CPCs exhibit increased expression of chondrogenesis marker SOX9 – a key regulator of cartilage formation. This study demonstrates that human CPCs mediate superior defect filling, tear bridging, and reintegration compared to BMMSCs in an ex-vivo rat meniscus injury model. Evidence revealed for the first time that CPCs possess a paracrine effect that enhances the rate of meniscal fibrochondrocyte proliferation while maintaining the ability to induce trophic and endogenous effects on neighboring cells. Additionally, further analysis revealed that in order to observe similar healing effects in a larger ex-vivo animal model requires combination therapy of cells with the chemotactic factor SDF-1 to enhance cell mobilization and cell infiltration to the site of injury for scaled applications. This study also demonstrates that utilization of a highly porous biocompatible scaffold in the presence of SDF-1 provided a sufficient platform for facilitating the required expansion and cellular distribution needed to treat larger meniscus tears and resulted in a degree of integration. Overall, this investigation offers a translational perspective on the use of hypertrophy-resistant stem/progenitor cells as a therapeutic catalyst for accelerating meniscal repair, which may potentially act as a preventative measure to decelerate the progression of PTOA in the younger population.

Name: Jeffrey Okewunmi, Sc.B. **Graduating Date: May 2019**
Mentor: Brett Owens, M.D.
Future Plans: Attending medical school at Mount Sinai
Project Title: The Effect of Vitamin D3 on Osteochondral Graft Viability Ex Vivo

Osteochondral (OC) allografts repair a wide range of large articular cartilage defects by restoring mature, hyaline cartilage. Fresh OC grafts have a limited window for transplantation, as chondrocyte viability significantly decreases after 15 days. Accepted graft storage practices have been challenged to preserve chondrocytes over a

longer period of time, which is an indicator of healthy cartilage and allograft survival. Research has found that that the active form of vitamin D elicit changes chondrocytes. Vitamin D has been demonstrated to influence articular cartilage turnover and cartilage thickness, as well as modulate other chondrocyte functions. We hypothesize that 25-hydroxyvitamin D3 treatment may extend preservation of fresh cartilage grafts, prolonging fresh OC graft chondrocyte viability ex vivo. This study opens the door for consideration of viability of fresh cartilage grafts as a bridge in those patients who wait for transplantation of a viable specimen.

Name: Grace Plassche, Sc.B. **Graduating Date: May 2019**
Mentor: Christopher Born, M.D. **Co-Mentor:** Dioscaris Garcia, Ph.D.
Future Plans: I will be working at the Orthopaedic Foundation for Active Lifestyles, a non-profit organization that improves quality of life through cutting edge research and education for the prevention and treatment of musculoskeletal diseases with a focus on orthopaedic and sports injuries.
Project Title: Novel Enviromimetic Culturing Prototype for the Elucidation of Soil Derived Marine Bacteria

The pre-existing standard for cultivation and isolation of bacteria is the agar plate which has been proven largely ineffective at culturing most marine bacteria by RTQ-PCR analysis, which showed that only 2% of all bacteria are discoverable with these methods. In order to address these issues, efforts must be made to simulate a bacterial environment in the lab. We have postulated that mimicking the endogenous environment of saprophytic bacteria can be accomplished by integrating calcium carbonate and calcium phosphate into structures that simulate structures present in the marine environments. This project is aimed at developing a novel culturing prototype that can be used to grow adherent saprophytic bacteria. Stainless steel scaffolds were utilized to construct structures through passivation and adhesive coating and immersed in normal growth medium. *Streptomycete sp*. bacteria cultured from the shores of the Providence and Seekonk Rivers were grown in the presence of these structures and analyzed by scanning electron microscope (SEM) for positive adherence. The results of these experiments show that the enviromimetic structures allow for the adherence and propagation of these bacteria in a faster manner than typically observed from media growth alone.

Name: Casey Tierney, Sc.M. **Graduating Date: May 2019**
Mentor: Joseph J. Crisco, Ph.D.
Future Plans: Moving to Los Angeles and working at Intuitive Surgical as a field service engineer
Project Title: Effects of Experience Level on Head Impact Exposure in Youth Football

The goal of this project was to analyze the effects of experience level on head impact exposure in youth football. Head impact exposure is defined as the frequency of impacts, magnitude of impacts, and location of impacts on the head. It was found that magnitude of impacts and the amount of impacts increased with experience level. Age and weight were also analyzed with respect to experience level. It was found that Age had a significant change on head impact exposure but Weight could be considered irrelevant.

Name: Brian Vuong, Sc.B. **Graduating Date: May 2019**
Mentor: Brett Owens, M.D.
Future Plans: Clinical research in Orthopaedics
Project Title: Extending the Viability of Fresh Cartilage with a Novel High-Subzero Cryopreservation Approach

We sought to develop an improved method of cartilage preservation that is advantageous over current hypothermic storage practices, classical cryopreservation, and vitrification for extending graft viability. Eight different cryoprotectant solutions with different formulations of nature-inspired cryoprotective agents (CPAs), conventional CPAs, and a non-toxic ice nucleating agent were tested for short-term storage of bovine cartilage at -6°C. From 14-day preliminary cell viability testing, we determined the best-performing cryoprotectant solution. We then proceeded to a 56-day long-term storage experiment under three storage conditions: 37°C DMEM, 4°C DMEM, and -6°C with the chosen cryoprotectant cocktail. Viability testing

demonstrated that storage at 37°C and -6°C were similarly effective in maintaining chondrocyte viability, while mechanical testing demonstrated that storage at 4°C and -6°C were effective in maintaining compressive strength of cartilage. In light of these findings, storage of cartilage under high-subzero, controlled partial freezing conditions is a promising alternative over both current standard hypothermic storage and body-temperature storage.

Name: Jiayu Wei, Ph.D. '20 **Graduating Date: May 2020**
Mentor: Wentian Yang, M.D., Ph.D.
Future Plans: Continuing Ph.D. program
Project Title: SHP2 Regulation of Osteoblastogenesis and Bone Mineral Homeostasis

Osteoblasts (OBs) play a central role in the development and maintenance of the vertebrate skeleton. Osteoblast dysregulation is the cause of several metabolic, genetic, and oncogenic skeletal disorders. Osteoblasts arise from multipotent mesenchymal stem cells through a complex multistep process that is regulated in heavily by cellular signaling evoked by protein tyrosyl phosphorylation. To date, substantial work in osteoblasts has focused on protein tyrosine kinases (PTKs), with minimal efforts on protein tyrosine phosphatases (PTPs); which, however, can have equally profound functional consequences. I am studying how the development and function of OBs are regulated by the PTP SHP2. By using genetic loss-of-function approaches and other biological and biochemical means, I report here that SHP2 is essential for osteoblastogenesis and has a developmental stage-specific effect; SHP2 deficiency causes an osteomalacia-like disease. This work is ongoing.