2021 Student Research Day
March 31st, 2021
Abstracts

Department of Orthopaedics
Warren Alpert Medical School of Brown University and Rhode Island Hospital

Name: Adam Fuller, AB Biology and AB Economy
Mentor: Christopher Born, M.D. & Dioscaris Garcia, Ph.D.
Project Title: *Development of Enviromimetic Culturing Cassettes for the Elucidation of Saprophytic Secondary Bioactive Metabolite-Producing Marine Bacteria*

Antibiotic resistance is a major concern for modern healthcare. Due to an extended drought in the antibiotic production pipeline and to a lack of novel secondary bioactive metabolite-producing bacteria, this problem will only get worse. New antibiotics must be found utilizing innovative methods for cultivating antibiotic-producing bacteria. Marine saprophytic bacteria are decomposers feeding on wood debris and crab shells. Conventional agar-based media lack the correct nutrients for these bacteria to thrive. The goal of this project is to create novel cellulose and chitin-based media in an appropriate three-dimensional shape to provide optimal growth conditions for saprophytic bacteria allowing previously uncultivable bacteria to be grown and assessed for antibiotic production.

Name: Angela Zhu, Biology AB
Mentor: Brett Owens, M.D. & Li Yue, Ph.D.
Project Title: *The Effect of Relaxin-2 in the Human ACL*

Approximately 175,000 to 300,000 anterior cruciate ligament (ACL) reconstructions are estimated to be conducted each year. Studies have shown that female athletes, in particular, are 2 to 8 times more likely to injure their ACL than are males. Relaxin is a peptide hormone that plays an important role in the musculoskeletal system, where it activates an enzyme, called collagenase, that breaks down collagen in damaged tissue to generate new, healthy tissue. Studies have shown Relaxin-2 may make female athletes more susceptible to ACL injury. A recent study has demonstrated that relaxin-2 markedly up-regulated intracellular processes in human female ACL cells, but no effect is detected in male cells. It is possible that the high incidence of female ACL rupture may be due to the changes in relaxin-2 levels. Therefore, we hypothesized that relaxin-2 exhibits an effect on male-derived ACL cells and relaxin-2 receptors are present in both male and female ACL. The objectives of this study were to determine the presence and quantity of relaxin-2 receptors in the male and female ACL from patients with ACL reconstructions, and to investigate the intracellular effect of relaxin-2 on male and female ACLs. Immunohistochemical staining was then performed on twenty samples, ten male and ten females, to detect the expression of relaxin-2 receptors to evaluate objective one. To evaluate objective two, part of every ACL was used for cell culture to determine gene expressions.

Name: Bardiya Akhbari, Ph.D., Biomedical Engineering, Ph.D.
Mentor: Joseph 'Trey' Crisco, Ph.D.
Project Title: *Biomechanics of Total Wrist Arthroplasty*

Total wrist arthroplasty (TWA) is a surgical solution that provides pain relief and preserves some wrist motion for patients with severe wrist pathology. However, TWA designs suffer from high complication rates, and to date, the in vivo biomechanics have not been assessed. Therefore, this study aimed to evaluate the biomechanics of a TWA design and compare it to the biomechanics of healthy wrists with the hope of improving future designs. To do so, we compared four primary outcome measures (range-of-motion, the center of rotation, the alignment of the components, and the articular contact pattern) between TWA and healthy subjects.

Name: **Caitlin Barrett,** Cell and Molecular Biology ScB
Mentor: Christopher Born, M.D. & Dioscaris Garcia, Ph.D.

Project Title: *Usage of a Rapid Visualization Assay in the Detection of Bacterial Presence and Delineation of Orthopaedic Infection on Surgical Explants, Tissue, Synovium, and Allograft Materials*

In the United States there are roughly 500,000 arthroplasties performed yearly. Approximately 40,000 of these patients undergo revisionary surgery due to aseptic loosening. As the number of arthroplasties performed increases, rapid diagnosis of periprosthetic joint infection (PJI) and distinguishing infection from aseptic complications will be increasingly relevant. Current methods rely upon elevated levels of inflammatory biomarkers or culturing to indicate clinical infection at the surgical site, but these tests have been shown to be less specific than desired or requiring extended time before clinically useful. This study assessed the sensitivity and specificity of a rapid visualization assay which uses fluorescently conjugated antibodies and Confocal Laser Scanning Microscopy (CLSM) to detect bacterial presence on surgical explants, tissue, and synovial fluid.

Name: David Edgar, Master's in Biomedical Engineering
Mentor: Braden Fleming, Ph.D.
Project Title: *Defining the Laws' Texture Signature of Magnetic Resonance CISS and T2* Sequenced Images of the Anterior Cruciate Ligament*

This project uses Laws' texture mapping to analyze texture features found in MR images of the ACL. k-means clustering is used to group voxels with a similar texture signature together for the analysis. The ACL is then divided into three ROI (the origin, insertion, and midsubstance) and the different texture signatures of each region are defined. The goal of this research is to relate the differences found in each ROI back to the collagen organization of the ligament. Second harmonic generation imaging was performed in these three ROI to help correlate between the texture features and the collagen organization.

Name: David Olaleye, Master's in Biomedical Engineering
Mentor: Joseph 'Trey" Crisco, Ph.D.
Project Title: *Trailfinder Wheelchair*

The space of wheelchairs is full of designs that address the situations that their users are in, but the most thought of image of wheelchairs have not changed. Part of the reason why is the existence of depot wheelchairs that are used by hospitals and similar other institutions. The Trailfinder wheelchair is a design of wheelchair geared towards being all-terrain and suitable for use indoors. This design fits within the constraints of ADA guidelines while also providing users with improved stability for outdoor activities when compared to standard depot wheelchairs.

Name: Kathleen Turajane, Master's in Biotechnology
Mentor: Wentian Yang, M.D, Ph.D.
Project Title: *The Establishment of BioID2 System to Identify SHP2's Targets in the Regulation of Osteoclastogenesis*

Src-homology-2 domain containing protein tyrosine phosphatase 2 (SHP2), a widely expressed protein tyrosine phosphatase, plays a critical role in osteoclast (OC) development and skeletal remodeling. Conventional SHP2 knockout mice are embryonic lethal. To investigate the role of SHP2 in OCs, our lab generated OC-specific SHP2 deficient mice using the "Cre-loxP" system and Ctsk-Cre as a driver. Phenotypic characterization demonstrates that these SHP2 mutants are severely osteopetrotic, manifesting a marked increase in bone density. Additional analyses revealed that SHP2 is required for OC development by regulating the fusion of pre-OCs during osteoclastogenesis [1]. However, the mechanism by which SHP2 regulates the osteoclastogenic program is not fully understood. To identify the protein substrates of SHP2, we adopted the BioID2 technology and have designed and built our unique plasmid constructs by fusing SHP2 (full length) or its SH2 domains (N-SH2 + C-SH2) to a promiscuous biotin ligase BirA (BirA*) [2]. The aim of this study was to establish the system and explore the application of BioID2 to study SHP2's interacting proteins during the osteoclastic differentiation of macrophage BAC1.2F5 cells induced by RANKL and/or M-CSF.

Name: Ryan Bain, ScB in Chemical Engineering
Mentor: Christopher Born, M.D. **&** Dioscaris Garcia, Ph.D.

Project Title: *An Analysis of the Chemical and Physical Properties of a Silver Carboxylate Titanium-Dioxide Polydimethylsiloxane Antimicrobial Matrix on Implant Materials*

Antibiotics are no longer sufficient to combat bacterial infections. The wide variety of prokaryotic species, in addition to the quick rate of bacterial evolution, has resulted in a large group of pathogens that can no longer be treated by traditional methods. Due to the increase in antibiotic resistance, post-operational surgical site infections have been on the rise. Healthcare acquired infections cause an increased burden and excess lengths of hospital stays, which accounts for up to 90% of total costs. Moreover, infection rates in biomedical implants can be as high as 4% for some devices and can cost up to $50,000 to remediate. These infections may be comprised of biofilms, which are communities of microorganisms attached to an implant surface and are resistant to antibiotics, resulting in recurrent surgical site infections (SSIs). Once formed, biofilms are difficult to remove. The current treatments for SSIs often require months of antibiotics and reopening of the surgical wound in order to mechanically debride the implant surface. However, in recent years the use of silver as an antimicrobial agent has gained attention for its ability to combat biofilm formation by its broad antibacterial spectrum, long-lasting release, and low incidence of antibiotic resistance. In response to the looming threat of antibiotic resistance, our lab has validated an antibiotic-independent, antimicrobial coating for orthopedic implants. It is comprised of a titanium dioxide (TiO_2) and polydimethylsiloxane (PDMS) matrix embedded with silver carboxylate. Upon implantation, the silver carboxylate is free to elute into the surrounding environment and protect the coated surface. Previous studies in our lab have demonstrated the antimicrobial efficacy on a large collection of both gram positive and negative bacteria. With the biological behavior of the coating being well understood, it is now necessary to examine the physical properties of the coating, as well as how the coating changes the properties of an implant when applied. Physical parameters observed were hydrophilicity, roughness average, silver carboxylate elution rate using the graphite furnace atomic absorption spectroscopy (GFAAS), and coating durability.

Name: Sai Allu, AB Biology and AB Economy
Mentor: Christopher Born, M.D. **&** Dioscaris Garcia, Ph.D.
Project Title: *Synergistic Effects of Silver-Carboxylate on Chlorhexidine Gluconate in the Context of Preventatives of Surgical Site Infections by Cutibacterium acnes (C. acnes) and MRSA*

Surgical site infections (SSIs) are one of the primary contributors to surgical morbidity and mortality with a substantial financial impact on the healthcare system. Two primary causes of these infections are *Cutibacterium acnes* (*C. acnes*) (formerly known as *Propionibacterium acnes*) and methicillin-resistant *Staphylococcus aureus* (MRSA). They occur frequently in upper body orthopedic surgeries despite preoperative application of antiseptics, specifically chlorhexidine gluconate (CHG), to the site. [57] Research has shown there to be persistence of C. acnes and MRSA due to CHG's inability to penetrate pilosebaceous pores. However, previous efforts in our lab have indicated that silver carboxylate was able to penetrate into the pilosebaceous glands. While neither CHG nor silver carboxylate individually are fully effective, there is a possibility that using them together might bridge the gap for efficacy. Combining silver's numerous antimicrobial mechanisms with the broad-spectrum nature of CHG could be more efficacious than current methods at inhibiting bacterial growth. Prevention of SSIs is crucial to improve patient outcomes and reduce costs, especially in the field of orthopaedic surgery.

Name: Timothy Keeley, AB Biology
Mentor: Qian Chen, Ph.D.
Project Title: *Nucleic acid delivery vehicle with glucan shielding*

The goal of this study was to formulate and transfect a nanopiece small inhibitory RNA (siRNA) delivery vehicle into cartilage cells for the treatment of post-traumatic osteoarthritis. A major challenge of siRNA delivery is the ability to deliver into tissues effectively and safely, particularly into dense avascular tissue like joint cartilage. Our JBAK nanopiece was developed to overcome the challenges of nucleic acid delivery. JBAK nanopieces are novel delivery vehicles that are capable of delivering nucleic acid cargo into dense avascular tissues. The JBAK nanopiece is formed through a self-assembly mechanism that consists of hydrogen bonding interactions and hydrophobic effects as well as the encapsulation of a negatively charged siRNA through base

stacking and electric attractions by the positively charged JBAK nanopiece. The aim of my project was to determine the transfection efficiency of in vivo chondrocyte delivery of the siRNA encapsulated JBAK nanopiece. The transfection efficiency (TE) is the ratio of the number of cells with effective transfection of the siRNA divided by the total amount of cells quantitatively and qualitatively measure through Fluorescence microscopy and flow cytometry. The results of this study will also serve as preliminary data for the construction of an oral intestinal macrophage targeting delivery vehicle with nucleic acid payloads and 1,3 D beta-glucan shells to inhibit PTOA progression.

Name: Umar Masood, Master's in Biotechnology
Mentor: Wentian Yang, M.D., Ph.D.
Project Title: *In Vitro SHP2 and SOX9 Expression in Murine Prg4+ Articular Cartilage Chondrocytes*

Previous study in our lab demonstrates that SHP2 deletion drives the chondrogenic differentiation of Prrx1+ osteochondroprogenitors both in vitro and in vivo. The goal of this study is to determine SHP2's role in regulating the proliferation and anabolic gene expression of PRG4+ articular chondrocytes. SHP2 deletion in PRG4+ articular chondrocytes promote cell proliferation and chondrogenic gene expression (Acan and Col2a1). Western Blot and qRT-PCR analysis demonstrate that SHP2 deficiency increased the protein abundance of SOX9 and the transcript abundance of Acan and Col2a1. SHP2 negatively regulates the abundance and transcriptional activity of SOX9, the expression of cartilage anabolic genes, and proliferation of PRG4+ articular chondrocytes. This is of importance in both understanding cartilage biology and developing novel therapeutics for cartilage diseases like osteoarthritis.