Student Research Day
May 3, 2017
Project Abstract Descriptions

Kevin Mertz, BS Biology **Graduating Date:** May 2017
Mentor: Christopher Born **Co-Mentor:** Dioscaris Garcia
Future Plans: Gap year before medical school as a researcher at Stanford University studying value-based healthcare.
Project Title: A Silver-based Titanium Dioxide-Polymer Hybrid Coating as a Preventative of *Candida albicans* and *Aspergillus fumigatus* Biofilms

Antibiotic resistance has become prevalent in today's society. Although bacterial antibiotic resistance is the subject of an increasing number of news stories, resistance is a growing concern in pathogenic fungi as well. The formation of a biofilm by a fungal pathogen plays a large role in its ability to resist treatment with antifungals and survive the host immune response. Resistant fungal infections are particularly worrisome because they are already difficult to diagnose and have high mortality rates in immunocompromised individuals (a growing a population because of high incidence of HIV and cancer treatments). There exists a need for a non-antibiotic alternative for treating and preventing fungal infection and biofilm formation. A silver-doped titanium-dioxide/PDMS hybrid coating for medical devices and orthopaedic implants is a proposed alternative. The effectiveness of this coating has already been shown with multiple strains of bacteria, both resistant and sensitive to antibiotics. This project demonstrates its effectiveness on a current fungal agent of medical device infection, *Candida albicans*, and a concern of the future, *Aspergillus fumigatus*. Our results show that both pathogens are capable of adhering to catheter materials through an extensive Scanning Electron Microscopy (SEM) survey of the exterior surface of urinary catheters. Kirby Bauer zone of inhibition assay and additional Scanning Electron Microscopy of the catheters showed that the silver-based coating is capable of preventing adherence of both pathogens. The dose response results show that the coating is effective against planktonic cells, both in the form of a coating, and when applied as a solution. These results suggest that the antibiotic-independent silver coating is a promising tool for the prevention of fungal biofilms on urinary catheters. Additionally, our data suggests that the ubiquitous fungal pathogen, *Aspergillus fumigatus* may represent a potential emerging threat to the successful use of medical devices in the near future. The data obtained in this study suggests that the coating may be an effective tool against both pathogens.

Briana Warschun, MS Biomedical Engineering **Graduating Date:** May 2017
Mentor: Joseph Crisco
Future Plans: Looking for a job in industry.
Project Title: Changes in Bone Morphology with Carpometacarpal Osteoarthritis

Characterizing the morphology of the carpometacarpal (CMC) joint and how it changes with osteoarthritis progression is important for understanding the pathoetiology of this multifactorial disease. The purpose of this longitudinal in vivo study was to quantitatively describe shape and morphology changes associated with OA progression from enrollment to 3.0 yr follow-up and to determine if and how these changes may differ with sex. Computed tomography scans from 74 subjects with early CMC OA and 24 healthy (control) age-matched subjects were used to obtain 3-D bone models. The trapezial and metacarpal articular surfaces were manually described and compared among sex and health groups using a scalar measure of average differences across facet surface shape as well as a novel scalar measure of the relative position of the facet surface with respect to bone inertia to describe facet orientation. We found that with OA progression bone volume will increase and CMC articulation becomes more curved in the dorsal-volar direction in both men and women. These findings support a modest effect of OA progression on the shape and morphology of the CMC articulation and these changes are likely to continue to increase over time as OA progresses. Likewise, we found sex-related differences among the trapezia facet orientation, which may play a factor in the higher CMC OA prevalence in women than in men.

Travis Spangler, Medical Student **Graduating Date:** May 2018
Mentor: Yupeng Chen **Co-Mentor:** Qian Chen
Future Plans: Applying to Orthopedic Surgery Residency
Project Title: Hybrid Nano-Matrix Enables Growth Plate Cartilage Repair via Promotion of Chondrogenesis

Growth plate fracture remains a significant problem for children in the clinic. Damage of growth plate cartilage may result in chondrocyte hypertrophy, cartilage ossification, angiogenesis, and bony bridge formation, which ultimately leads to growth cessation and/or deformity in patients. Currently, there is no therapeutic approach to promote growth plate cartilage regeneration and inhibit bone bridging after growth plate fracture. To achieve growth plate repair, we designed a novel nano-matrix combining tissue engineering and drug delivery approaches. Such matrix assembles from biomimetic rosette nanotubes (RNTs) serving as scaffolding, and the cartilage specific matrix protein matrilin-3 (MATN3) serving as a hinge of the nanotubes as well as a bioactive protein. We have found that the nano-matrix can inhibit bony bridge formation and thereby correct angular deformity of a long bone after growth plate fracture. However, this treatment did not restore the full elongation of the injured long bone. To promote cartilage regeneration, we proposed to use the nanomatrix to deliver growth factors (such as IGF-1 and TGF-β1), which will further enhance the chondrogenesis-inducing ability of the nano-matrix. As a result, combining with IGF-1, the nanomatrix presented a significant increase in chondrogenesis. Therefore, this biomimetic hybrid matrix may serve as a promising treatment for facilitating growth plate cartilage regeneration and preventing bony bridge formation, which can be administrated in a clinic shortly after growth plate injury occurs.

Srinidhi Bellamkonda, MSc **Graduating Date:** May 2017
Mentor: Joseph Crisco
Future Plans: Working as a Research Engineer at the Bioengineering Lab
Project Title: Head Impact Exposure in Youth Football Players at Practices and Games

Despite over 70 % of all football players in the US being under the age of 14, previous research has focused primarily on high school and collegiate football players. With the goal of learning more about the distribution of head impact exposure in the youth population, this study aimed to compare head impact exposures (frequency and magnitude) between practices and games on football players ages 9 to 14. One hundred thirty-six players from six teams were recruited and equipped with the HIT (Head Impact Telemetry) system enabling impact exposure data collection at every practice and game for a total of 482 sessions. Over a period of two seasons, 49,847 impacts from 345 practices and 137 games were recorded. Individual players sustained a median of 211 impacts with a highest of 1226 impacts per season, with a 50^{th} and 95^{th} percentile peak linear acceleration of 18.3 g and 46.9 g. The 50^{th} and 95^{th} percentile peak rotational acceleration were 1305.4 $rad \cdot s^{-2}$ and 3316.6 $rad \cdot s^{-2}$ respectively and the 50^{th} and 95^{th} peak percentile HITsp, a severity measure, were 13.7 and 24.3, respectively. Overall, players with a higher frequency of head impacts at practice recorded a higher frequency of head impacts at games. Despite the differences in total number head impacts an individual player received per season among each of the six teams, head impact frequencies at practices correlated with those at games. Moreover, players with higher magnitudes of head impacts during practice also recorded higher magnitudes of head impacts during games. In summary, for every individual player, there was a positive significant linear relationship between the head impact exposure values for practices and for games.

Stephany Vasquez, MS **Graduating Date:** May 2017
Mentor: Bahar Bilgen
Future Plans: Design Assurance Engineer at Ximedica
Project Title: High and Low Compressive Loading on Tissue Engineered Cartilage

Osteoarthritis is a debilitating joint disease that affects over 27 million Americans. Tissue engineered cartilage could be a viable option for patients that have lost cartilage due to injury or osteoarthritis. However, reaching optimal mechanical and biochemical properties in tissue engineered cartilage is still a challenge. This study investigates the dose-dependent effects of compressive loading on chondrocyte seeded agarose hydrogels over a 6-week study. Our hypothesis is that early high compressive loading will enhance GAG deposition.
Methods: Primary chondrocytes were isolated from porcine knees and digested in 0.15% Collagenase Type II overnight at 37°C. Cells were suspended in 2% agarose and punched into discs with final dimensions was 4 mm diameter and 1.5 mm thick. After two weeks of static culture in chondrogenic media with TGF-β and Dexamethasone, dynamic loading was applied with a 5% tare strain followed by 10% or 30% dynamic compressive loading, or a combination of the two, at 1 Hz for 3hrs/day and 5 days/week for four weeks. The data was analyzed using ANOVA with Tukey Method with a confidence of 95%.
Results: On day 28, samples loaded with 30% strain showed significantly higher GAG/ dry weight than samples that were cultured in free-swell. On day 42, there was no significant difference between groups and there was a significant decrease in GAG when compared to day 28. Group loaded with 30% strain and then 10% (group 30/10) had significantly higher DNA/ dry weight than any group on day 42. However, there was no significant difference in DNA between groups in the initial 2 weeks of mechanical loading (day 28 of culture). Group 30/10 also had significantly higher collagen per dry weight than the free-swell group, however as seen with GAG, there was also a decrease in collagen for all groups when compared to day 28. There was no significant difference in collagen between groups after 2 weeks of mechanical loading (day 28 of culture). Equilibrium modulus (EM) increased for all groups between day 14 and day 28 of culture but decreased during day 42. On day 28, samples that received no loading and samples that received 10% strain had significantly higher EM than groups loaded with 30% strain. On day 42, group 10/10 had significantly higher EM than the free-swell group.
Discussion: Dynamic loading significantly enhanced GAG production by chondrocytes in the first 28 days of culture. The samples were able to reach EM values consistent with native cartilage (60-250kPa) in the first 28 days. After 28 days, there was a decrease in GAG, collagen, and equilibrium modulus in all the groups. However, DNA production increased after 28 days indicating cell death did not contribute to the decrease in GAG, collagen, and mechanical properties. The results could indicate a decrease in chondrogenic characteristics. Although the data failed to prove the hypothesis, it did suggest that subjecting the samples with a strain of 30% and then 10% could lead to higher DNA and collagen deposition than freeswell.

Peter Lam, MS biomedical engineering **Graduating Date:** May 2017
Mentor: Yupeng Chen
Future Plans: Work in the industry in either a pharmaceutical company or a medical device company
Project Title: Anti-angiogenesis nanomatrix for growth plate fracture repair

Growth plate fractures in children can cause bone deformity and growth arrest due to poor cartilage regeneration and bony bridge formation in the fracture site. Currently, the only treatment for this type of fracture is to perform surgery to remove the bony bridge and insert autologous fat tissue or screws into the site to discourage future bony bridge formation. This procedure is intensive and invasive and have a low success rate. Since the fracture site is in the middle of a long bone, there is a lack of approach to apply bioactive materials or therapeutics to the injured site to repair growth plate. Here, we explore the use of a novel therapy that utilizes rosette nanotubes assembled with a bioactive protein (matrilin-3) to form an injectable nanomatrix. Rosette nanotubes is a biomimetic material that is derived from DNA base pairs and has been found to be served as a scaffold for cell growth and drug delivery. Matrilin-3 is a cartilage specific proteins that have been

found to inhibit angiogenesis and bone formation. The two materials can self-assemble in water solution from simple mixing due to electrostatic forces. Experiments were performed to characterize the nanomatrix and assess its efficacy in anti-angiogenesis property *in vitro* and *in vivo*. As a result, the nanomatrix of matrilin-3 and rosette nanotubes show potential in the treatment of growth plate fractures.

Jonathan R. Franco, BA Biology **Graduating Date:** December 2016
Mentor: Chathuraka Jayasuriya
Future Plans: I intend to matriculate into the Alpert Medical School class of 2021 beginning in August of 2017.
Project Title: Cartilage-derived stem cells for meniscal repair and prevention of posttraumatic osteoarthritis

Background: Meniscal tears are one of the most prevalent knee-related injuries, specifically among young athletes. The clinical treatments of inner meniscal injuries are challenging due to the avascular fibrocartilage tissue matrix, which limits the healing potential. Furthermore, inadequate repair of meniscal injury increases the risk of developing osteoarthritis (OA). A favorable approach to enhance the future of clinical treatment is to utilize effective cell-based therapies to stimulate improved healing responses and restorative function. However, the current applications of cell-based therapy using bone marrow derived mensenchymal stem cells (BM-MSCs) are limited by insufficient repair and cellular hypertrophy (1-3). In an effort to characterize a more effective therapeutic cell source for tissue repair, our lab has targeted mesenchymal stem cells (MSCs) isolated from adult human articular cartilage. Specifically, our lab has recently identified a highly chondrogenic subset of cartilage-derived chondroprogentior cells (CSCs) that are characterized as CD90-/CD105+/CD166+ and tested their potential to integrate and repair partially transected menisci.
Goal: Our initial goal was to determine whether these cells could successfully adhere to and integrate a host meniscus, in addition to identifying the propensity for enhanced chrondrogneic differentiation and meniscal repair. Furthermore, we sought to identify the bioavailability of these CSCs in non load-bearing regions of human articular cartilage and evaluate how effectively they can repair damaged meniscus tissue.
Methods: 1.0 x 106 fluorescently labeled CD90-/CD105+/CD166+ cartilage-derived stem cells were seeded onto decellularized rat menisci and cultured 1 - 4 weeks. The decellularized menisci were then sectioned, stained with DAPI, and imaged to determine the degree of cell adhesion and integration. Sections were separately stained with Safranin-O to evaluate their chondrogenic differentiation potential. Partially transected rat menisci were seeded with 5.0 x 105 fluorescently labeled CSCs and BM-MSCs and evaluated for cellular migration, adhesion patterns, repair response, and chondrogenic potential at 5 weeks.
Results: Analysis of the images indicates the successful integration of these stem cells into the host meniscal tissue and preferential integration in the inner avascular region. The positive Safranin-O staining in the sectioned menisci that were seeded with cartilage-derived stem cells strongly suggests that the integrated stem cells remained chondrogenic. Fluorescent imaging indicates that CSCs and BM-MSCs exhibit similar patterns of adhesion and migration 72 hrs post-seeding. Using an ex-vivo culture system, we demonstrated that these non-autologous CSCs are able to migrate to the site of injury and exhibit significant repair response and partial bridging in rat menisci. Furthermore, compared with BM-MSCs, CSCs exhibit significantly less expression of type X collagen (COLX), a marker of cellular hypertrophy.
Conclusion: These findings are indicative of the potential applications of non-autologous cartilagederived stem cells for cell-based meniscal repair therapies and the prevention of post-traumatic osteoarthritis. It gives preliminary indication that these stem cells can integrate into the host menisci and significantly repair a meniscal defect while retaining their chondrogenic potential.

Kevin Yan, Biology AB **Graduating Date:** May 2017
Mentor: Wentian Yang
Future Plans: Consulting
Project Title: SHP2 regulation of pre-osteoclast development and cytokine production

In the United States, osteoporotic fractures care costs patients and taxpayers over 17 billion dollars annually (Curtis et al., 2017). Even now, hip fractures caused by osteoporosis are expected to affect approximately 50% of women and 25% of men over the age of 50 (Pisani et al., 2016). With an overwhelmingly aging population suffering from osteoporotic diseases, quality of life for the patient suffers dramatically, from mental depression to inability to perform everyday tasks. In the past decade, great strides have been made in the orthopedics field to find ways to combat bone diseases, such as osteoporosis and Paget's disease, through bone repair or transplantation. However, when the body encounters the foreign objects used in these treatments, its natural response is to attack and remove them. Although the responses are aimed towards foreign biomaterials, patient bone mass is often damaged in the process, resulting in worsening patient conditions. Although this temporary solution usually lasts for a few years, it greatly improves the patients' quality of life. A possible solution to this problem is to understand the mechanisms to which bone resorption happens and how to limit bone resorption in the body.

The work presented here focuses the function of a specific enzyme, SHP2, encoded by *PTPN11* in osteoclast development. SHP2 has been previously proven to influence the bone mineral homeostasis (Zhou et al., 2015), primarily by modifying the fusion of preosteoclasts to form giant multinuclear osteoclasts, which are the cells that facilitate bone resorption. By taking a genetic loss-of-function approach and in vitro cell culture system, my goal is to understand how SHP2 deficiency affects osteoclast development and cytokine production. In doing so, we hope to be able to pinpoint key areas of regulation during osteoclastogenesis in hopes of developing a limiting drug to slow down or stop osteoclastogenesis.

In our experiments, we were able to compare the cytokine intensity values of 144 cytokines between SHP2 and SHP2-deficient mice by using Abcam's Mouse Cytokine Array Membrane kit. We were able to quantify the downregulation of important cytokines factors to osteoclastogenesis in SHP2-deficient mice. Furthermore, our data is consistent with past studies that showed SHP2-deficiency regulates osteoclastogenesis (Zhou et al., 2015).

Ishan Sinha, Medical Student **Graduating Date: May 2020**
Mentor: Roy Aaron, MD
Future Plans:
Project Title: Clinical decision support tools for select orthopedic procedures: a qualitative meta-analysis