The University of Pennsylvania Orthopaedic Journal

Volume 26

June 2016

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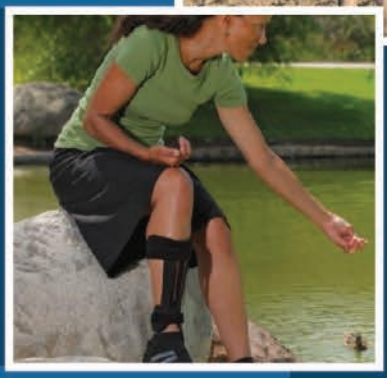

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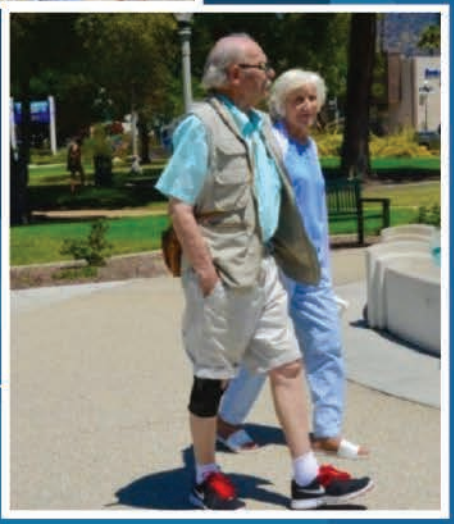

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Letter from the Editors-in-Chief

Cody Hillin, MD MS and James Friedman, MD

Welcome to the 26th edition of the University of Pennsylvania Orthopaedic Journal (UPOJ). The UPOJ began in 1985 under the leadership of Dr. Carl T. Brighton as the nation's first fully resident-run journal of orthopaedic surgery. Since its inception, the UPOJ has been a source of pride for the department, which we strive to continue with this 26th edition.

We have organized this edition of the UPOJ into two major sections. The first section describes original research projects completed over the last year, and is divided into clinical and translational topics as well as basic science subjects. Clinical topics are sectioned by department and many sections include operative techniques specific to that department that may prove helpful for the budding physician. The format of all research articles continues in the "extended abstract" format which has allowed concise yet complete presentation of work from both clinical and basic science research.

The second major section of the journal covers the clinical and educational progress of each orthopaedic department over the previous year. This includes updates from each clinical and research division. Additionally, it details the visits of several distinguished faculty to the University of Pennsylvania over the last year to both lecture and lead cadaveric dissections. The UPOJ concludes with an overview of the current residents, including the chief residents and their post-graduation plans.

As a resident organized journal, the UPOJ would not be possible without the vision of our chairman Dr. L. Scott Levin and program director Dr. Craig Israelite, or the guidance of our faculty advisors Drs. Samir Mehta and Jaimo Ahn. Additionally, the assistance of our section editors to review research articles has been invaluable: Drs. Joshua Rozell (Arthroplasty), Matthew Sloan (Epidemiology), Jenna Bernstein (Hand), Daniel Gittings (Pediatrics), Zachary Zimmer (Shoulder & Elbow), Blair Ashley (Spine), Daniel Lim (Sports), Tyler Morris (Trauma), Russell Stitzlein (Tumor), Nicki Zelenski (Cartilage), and Jonathan Slaughter (Tendon & Ligament). We would like to also thank our sponsors, which have allowed the UPOJ to be financially independent from the Department of Orthopaedic Surgery since 1997.

On behalf of all the contributors to the UPOJ this year, we hope you find the 26th edition an educational highlight of all the efforts of a department continually striving for excellence.

Sincerely,

Cody D. Hillin, MD, MS and James M. Friedman, MD
Editors-in-Chief
The University of Pennsylvania Orthopaedic Journal
Volume 26

Dedication to Gerald R. Williams, MD

Cody Hillin, MD MS and James Friedman, MD

We are pleased to dedicate the 26th volume of The University of Pennsylvania Orthopaedic Journal to Dr. Gerald R. Williams as a scientist and clinician who has provided invaluable research and mentorship to the Penn Orthopaedic program.

Dr. Williams is an orthopaedic surgeon who specializes in the shoulder. He received his undergraduate education with a degree in Chemistry from Ursinus College, in Collegeville Pennsylvania. He went on to graduate from Temple University School of Medicine with honors. He completed his orthopedic residency as well as a one year fellowship in shoulder and elbow at the University of Texas Health Science Center in San Antonio in 1990.

Dr. Gerald Williams is an exemplary surgeon scientist who along with Joseph Ianotti M.D. PhD established excellence in clinical shoulder surgery in the Department of orthopedics of the University of Pennsylvania. A disciple of the legendary Charles Rockwood, throughout his career has interfaced with the best surgical minds in shoulder surgery throughout the world. In addition to his textbook on shoulder surgery, Dr. Williams has served as the president of the American shoulder and elbow society and despite his transition to another practice in Philadelphiahas remained a strong supporter of Penn orthopedics in the missions which we embrace. Furthermore his dedication to his family is an example we all should emulate and we are proud to call him one of own as he deservedly ascended. to the presidency of the AAOS.

Currently Dr. Williams is the John M. Fenlin Jr., Professor of Shoulder and Elbow Surgery at the Rothman Institute, Jefferson Medical College. In 2014 Dr. Williams was elected to be the second vice-president of AAOS. In 2016 he will become the president of AAOS.

Honored and Humbled

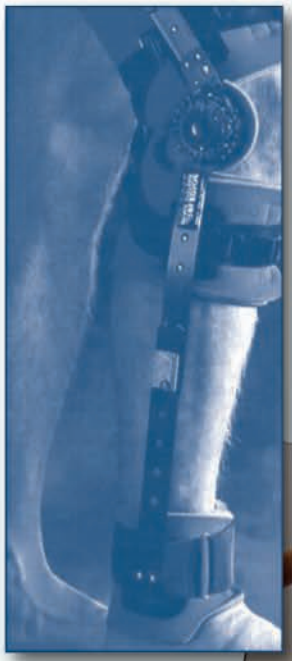
Dr. Gerald R. Williams

I remember exactly where I was when Dr. Levin informed me of the decision to dedicate this year's University of Pennsylvania Department of Orthopedics Journal to me. I was sitting in Jay Parvizi's research office having just finished a Board conference call for the American Academy of Orthopedic Surgeons. It was the end of a long day and what Dr. Levin had just told me hadn't quite registered. As I thanked him for the honor and hung up the phone, the importance of the moment suddenly hit me and I thought: "why me?" There are so many others who are more deserving than I. In fact, I began thinking of all the famous surgeons and researchers who have graced the halls of your storied department—past and present—and felt incredibly insignificant.

Brighton, Steinberg(s), Lotke, Heppenstall, Torg, Sennett, Cuckler, Glasgow(s), Esterhai, Iannotti, Soslowsky, Kaplan, DeLong, Born, Lackman, Friedenberg, Bora, Osterman, Bednar, Ramsey, Getz, Glaser, Israelite, Nelson, Bozentka, Okereke—and these are just the names of the people I actually worked with. I began thinking that I should know more about the department's history so I googled it. I shouldn't have been surprised that I found an article entitled The History of Orthopaedic Surgery at the University of Pennsylvania written by Dr. Zachary Friedenberg and published in your journal in 2002. I suspect you have all read it but if you haven't, you should. Here is the link: upoj.org/wp-content/uploads/v15/v15_16.pdf. I am sure you know that your department was the first in the United States—started by DeForest Willard in 1889. You may not know that, in part, it was funded by a $4,000,000—in 1889!—endowment from Peter (P.A.B.) Widener, who was a wealthy Philadelphia businessman who made his money in, among other things, streetcars. As a side note, two of his sons perished on the Titanic. The first resident started at the end of the tenure of Arthur Bruce Gill (1920-1942). As we all know, the residency has flourished under the influence of many.

I came to Penn in August of 1991. Officially, I was recruited by Dr. Joseph Iannotti. However, I know there were others who supported me—Dr. Heppenstall, Dr. Torg. Dr. Brighton was the chair who hired me and I am forever grateful. My career flourished during my time at Penn. I was fortunate to have worked with everyone there, especially Dr. Iannotti, Dr. Soslowsky, Dr. Ramsey, Dr. Getz, and Dr. Glaser. As I left in January of 2007, your specactular current chairman, Dr. L. Scott Levin was just starting. He is one of the most outstanding leaders I have had the pleasure of knowing. He is a dear friend and I am very proud of him

Finally, when James Friedman, MD, a current third year resident, asked me to write something in response to this dedication, I really struggled with what I should say. Truly, I feel like a sapling among redwoods. After some thought, I came to the conclusion that maybe I should just say thank you. You must know that I received from my relationship with your department much more than I gave. I am truly honored and humbled.



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Techniques in Cementation for Hip Hemiarthroplasty

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Summary

Hip fractures in the elderly population remain a significant public health concern. With a rise in the incidence of osteoporosis, orthopaedic surgeons must be familiar with different fixation options in both trauma and arthroplasty to minimize complications and implant failure following treatment of femoral neck fractures. Cemented hip hemiarthroplasty is a reliable method for restoring functional mobility and early rehabilitation after femoral neck fractures, but the technical aspects of the procedure are challenging. If used effectively, however, cementation decreases implant loosening and the risk of periprosthetic fracture, thereby decreasing the potential for reoperation in this vulnerable population. We describe a technique for ideal cementation of the femoral component of a hip hemiarthroplasty and discuss the indications and implications in specific patient populations.

Introduction

By the year 2050, there will be an estimated 3.9 million hip fractures worldwide and 700,000 in the United States.[1] The impact of this phenomenon on the community is tremendous, in that there remains a 30% risk of mortality in elderly patients who sustain a hip fracture within one year.[2] Patients must be immediately mobilized to limit short-term complications such as urinary tract infections, pneumonia, and deep venous thrombosis, but also to mitigate the decline in functional independence.[3]

The use of cementation in hip hemiarthroplasty has several advantages. With focus on decreasing the incidence of revision surgery and post-operative complications, cemented fixation in osteoporotic bone has been shown to have up to a 10-fold decrease in the rate of periprosthetic fracture.[4-7] According to a study of over 347,000 patients in the Norwegian Hip Arthroplasty Registry, 10-year implant survival was lower in the uncemented group compared with the cemented group for patients over the age of 65.[8] In one recent randomized clinical trial of 160 elderly patients with a femoral neck fracture at minimum two year follow-up, increased peri-operative fracture and subsidence, and decreased Oxford hip scores were reported in the uncemented group compared with the cemented group. However, there was no difference noted in mortality rates at any timepoint.[9]

There are also many practical advantages of cementation. Prior to implantation, the version of the stem can be adjusted in small increments. In addition, some surgeons may choose to add antibiotics to the cement as an added method for infection prophylaxis. Cement interdigitates with the larger trabeculae of osteoporotic bone thus allowing for immediate post-operative weightbearing. In elderly patients at risk of delirium, dementia, and falls, the reduced immediate osseous integration of an uncemented stem may put these patients at higher risk of periprosthetic fracture.[10]

Disadvantages of cemented hemiarthroplasty include a slightly longer operative time,[11] a steep learning curve with a heightened focus on avoiding femoral stem varus, an increased risk of fat embolism during cement pressurization, and the potential difficulty of stem extraction during revision surgery.

Procedure

The patient with a femoral neck fracture is positioned in the lateral decubitus position or the supine position depending on the approach performed. The authors advocate for use of the posterior approach. During the surgical approach, attention must be given to avoiding inadvertent injury to the labrum. Since the final prosthesis will rely on the natural negative pressure created by the acetabular labrum, it is important to preserve this suction seal when possible; in the setting of capsular injury from the femoral neck fracture, the capsulo-labral junction may be disrupted and the seal may not be maintained. The posterior capsulotomy commences distally, at the level of the quadratus femoris, with non-absorbable tagging sutures applied to maintain tension on the capsule while progressing proximally. A number 15 scalpel blade can be used to "feather" the labrum from the capsule at the level of the capsulo-labral junction.

Once the fracture site has been exposed and the femoral head is visible, a cobb elevator may be used to rotate the head such that the articular side is visible, taking care not to lever the head out of the acetabulum as this may cause an

iatrogenic fracture of the posterior wall. Using the corkscrew extractor (Figure 1) through the denser subchondral bone will facilitate head extraction. Once the corkscrew engages the femoral head, the head is rotated out of the acetabulum inferiorly, again avoiding iatrogenic injury to the acetabulum.

Acetabular exposure may be achieved using a variety of retractors. To aid in the ease of exposure, placing the leg in slight abduction, extension, and internal rotation allows for better visualization. A Shnitt is placed over the anterior lip of the acetabulum to create a small rent in the anterior capsule at the level of the anterosuperior column. A #2 hip retractor is then inserted into the defect and seated on the anterior column. Next, a #7 retractor is placed distal to the transverse acetabular ligament and over the posterior acetabular wall (Figures 2 and 3). The two retractors should be orthogonal to each other. Using a combination of the suction tip and the electrocautery, the pulvinar tissue is removed from the cotyloid fossa. The extracted femoral head is measured using the sizing templates to estimate the component size. Hemiarthroplasty trials can be used to ensure the correct size femoral head has been selected. The ideal fit allows for the femoral head to engage the native acetabulum. Femoral head-acetabular mismatch may result in either femoral head uncoverage if the head is too large, or a high incidence of instability and "rattle" if the femoral head is too small.

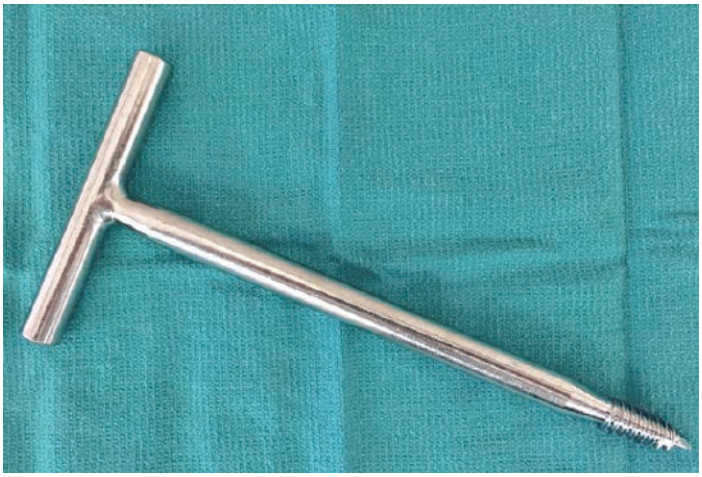

Figure 1. Corkscrew used for femoral head extraction.

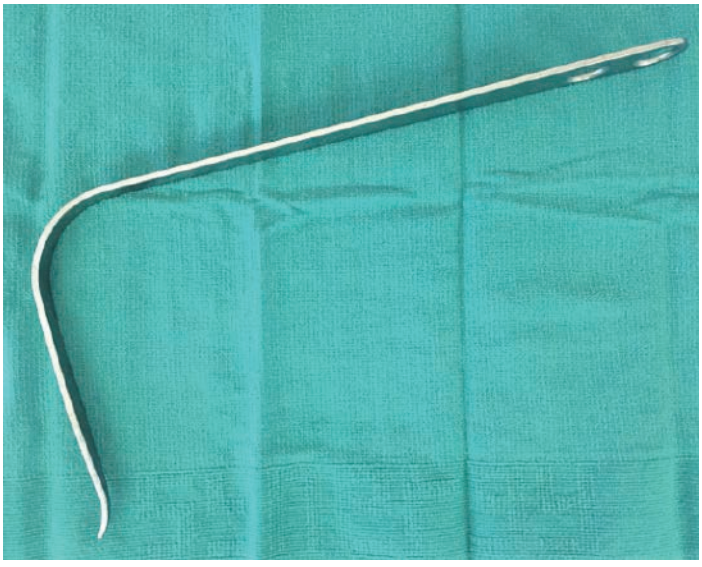

Figure 2. #2 acetabular retractor.

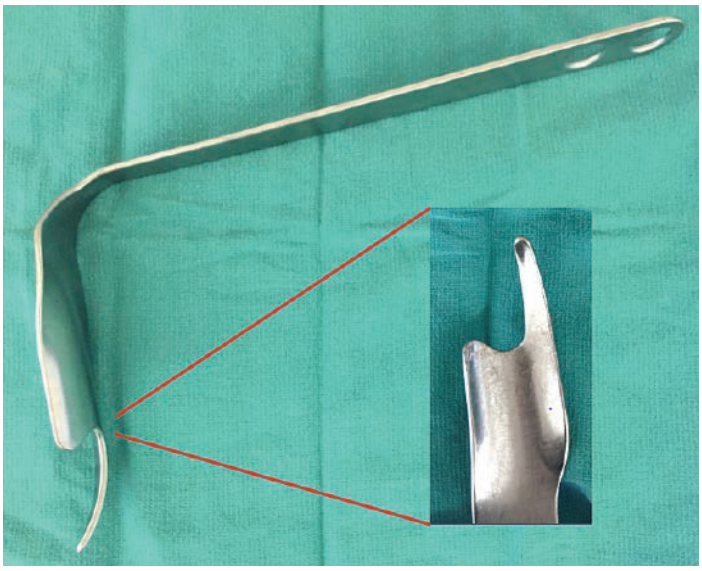

Figure 3. #7 acetabular retractor.

Following femoral head selection, the proximal femur is then exposed. The femoral canal is broached in standard fashion, making sure to achieve a lateral position within the canal to avoid varus implantation. Following trial reduction, the final neck length is chosen. A centralizer approximately 1-2 mm smaller than the final implant diameter is selected to be placed at the distal tip of the stem. If the final stem size is less than 14mm, two bags of polymethylmethacrylate cement are used, while three bags of cement are typically needed for stems greater than 14 mm. A cement restrictor of the appropriate size is placed within the canal 1 cm distal to the distal extent of the intended stem. Prior to placement, the flanges of the cement restrictor are cut in each quadrant, thus decreasing the stiffness during insertion to minimize femoral cortical perforation in osteoporotic bone.

The canal should now be thoroughly irrigated and dried. A femoral lavage brush is used to debride the canal of any excess fat and debris. While the cement is being vacuum mixed on the back table, hydrogen peroxide from a new bottle is dripped into the femoral canal allowing for further desiccation and optimal cement interdigitation. Once the canal is adequately prepared, inform the anesthesia team that cementation will take place. This will alert them to any potential changes in blood pressure, oxygenation, or heart rate that may be associated with the process.

Next, assess that the cement has the appropriate consistency. While holding the cement gun vertically, extrude a small amount of cement. If the cement is moldable yet able to maintain the tubular shape acquired from the cement gun nozzle, proceed with cementation. The cement gun is inserted

into the canal to the level of the cement restrictor. Cement is then introduced into the canal, allowing the building pressure in the canal to push the cement gun out of the femoral canal; do not prematurely retract the cement gun. Once the cement adequately fills the canal, a pressurizing device is placed over the proximal femoral canal and additional cement is introduced under pressure (Figure 4). It is critical to re-notify the Anesthesia team of cement pressurization, as this is the time period of highest risk for fat embolism. Finally, implant the prosthesis in the correct version until it is adequately seated. The goal is to create a 2 mm circumferential, uniform cement mantle. Once the version is set and the implant is properly seated, maintain firm pressure on the stem until the cement hardens but do not change the version (Figure 5). Any subtle rotation of the stem during implantation will result in cement mantle imperfections and increase the chances of early component loosening.

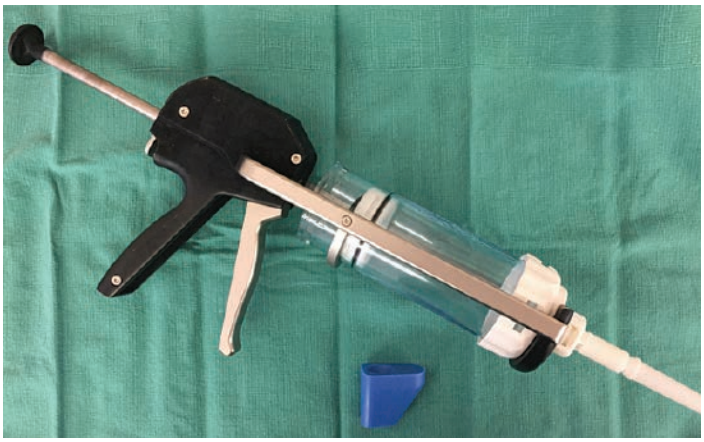

Figure 4. Cement gun with pressurizing rubber attachment to insert into the proximal femoral canal.

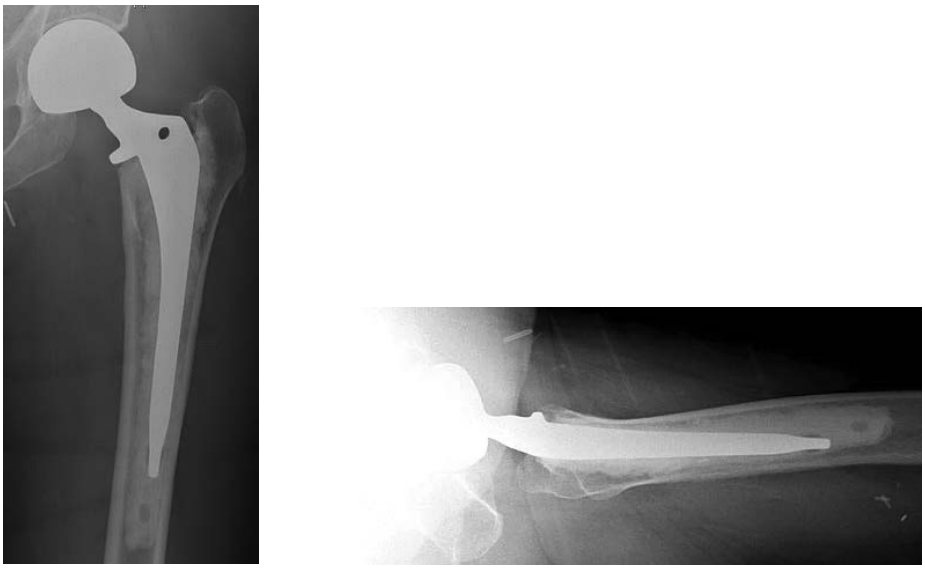

Figure 5. Anteroposterior and lateral radiographs of a cemented left hip hemiarthroplasty in an 88 year-old female who sustained a left subcapital femoral neck fracture.

Discussion

Cemented hip hemiarthroplasty is a technically challenging but useful technique for the treatment of osteoporotic intracapsular hip fractures. In this subset of patients with increased medical comorbidities, there are several implications of this technique. In patients with cardiac disease, cementation may be a risky option. In vitro models have shown that the monomer utilized in the cement can cause vasodilatory-induced hypotension via a direct relaxation of the vascular smooth muscle,[12,13] while in vivo models more clearly correlate with the development of pulmonary emboli. However, in one study of twenty patients monitored via transesophageal echocardiography, cementation produced a transient but significant reduction in cardiac output of 33% and a reduction in stroke volume of 44% so there may be some association between the cement monomer and hypotension.[14]

In patients with pulmonary disease, the increased risk of fat embolism is a deterrent to cementation. When implanted, the cement undergoes an exothermic reaction and expands in the spaces between the prosthesis and bone. The increased pressure forces trapped air and medullary contents (i.e. fat) into the systemic circulation under pressure. In older patients with a higher fat to marrow ratio, there is higher risk of fat embolism. Thus, during the cementation process, it is critical to continuously assess the patient for changes in oxygen saturation and blood pressure.

In contradistinction, patients with renal disease and renal osteodystrophy are excellent candidates for cemented fixation. Decreased excretion of phosphate by the kidneys combined with the inability of the diseased kidneys to utilize vitamin D allow for a high calcium-phosphate product and weaker bone, thus putting these patients at higher risk for implant loosening if cement is not used.[15]

When the cementation technique is performed properly, there may be a lower long-term reoperation rate. At one year follow up, Deangelis and colleagues found no difference in functional outcomes or acute complications when comparing uncemented and cemented cohorts.[16] Comparing reoperation rates among elderly patients undergoing cemented vs uncemented hemiarthroplasty, Viberg et al. found that the cemented cohort had a decreased hazard ratio and a superior long-term implant survival rate after three years compared with the uncemented group.[17] The Norwegian Registry evaluated 11,116 hemiarthroplasties in a prospective observational study demonstrating that at five year follow-up, uncemented hemiarthroplasty had a 2.1 times increased risk of revision, most commonly for periprosthetic fracture. While there was a higher risk of intra-operative mortality in the cemented group, longer term mortality risk was not significantly different.[18] In a

study by Taylor et al, hemiathroplasty with a cemented implant provided a comparable outcome to the uncemented group in patients without severe cardiac disease, though there was a trend toward better function and mobility in the cemented group6.

Regardless of the technique of femoral component implantation selected, cemented and cementless hemiarthroplasty patients displayed an approximately 18-24% decrease in independence when compared to their pre-operative level of functioning.[6] Thus it is important to evaluate the patient in terms of functional capacity but also medical stability prior to deciding whether a cemented prosthesis is the best option. In otherwise healthy, elderly patients with osteoporosis, cemented hemiarthroplasty using the described techniques is a good option in terms of post-operative pain and reoperation rates.

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Novel Classification System for Bone Loss in the Setting of Revision Total Knee Arthroplasty

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Introduction

Management of bone loss is important in achieving long-term implant survivorship during revision total knee arthroplasty (TKA). With an incidence approaching 80%, bone loss is exceedingly common in this setting.[1] A quantitative bone loss classification system and associated algorithm would be helpful to guide surgeons regarding strategies to manage of bone loss during revision TKA. Currently, the two most popular classification systems in use, the Anderson Orthopaedic Research Institute classification[2,3] and the University of Pennsylvania classification,[4] have been identified as being deficient in guiding treatment and have failed to be universally accepted.[5,6] A quantitative classification system is needed that is objective, reproducible, user-friendly, and able to accurately guide surgical strategy as well as allow for valid comparisons between various bone loss management options.[1,6] The aims of our study were to evaluate the intra- and inter-observer reliability of a newly developed quantitative radiographic classification system and to assess whether the radiographic classification of bone loss could be combined with a treatment algorithm to predict the revision prostheses and strategy utilized to manage the bone loss.

Materials & Methods

We gathered anteroposterior (AP) and lateral preoperative radiographs from all patients who had a revision TKA performed by the senior author (CLN) between April 2006 and December 2009. From this cohort, 54 knees were eligible for inclusion in the inter- and intra-observer reliability portion of this study, with 17 procedures excluded. Exclusion criteria were as follows: (1) prior total femur or proximal tibia replacement, and (2) lack of appropriate preoperative radiographs. The radiographs were then evaluated, using our classification system, by three attending surgeons and one PGY-3 orthopaedic resident. The evaluators were each provided with a description of the classification system (Table 1). All 54 radiographs were de-identified and evaluated by each physician on two separate occasions, at least three weeks apart, using a secure online survey distributed and managed with REDCap, an electronic data capturing tool.[7]

On the femoral side, our classification is based upon anatomic principles. The goal of femoral component revision is to achieve long-term fixation of an axially, rotationally, coronally and sagittally stable femoral implant of appropriate size and at an appropriate joint line in proper rotation, alignment and position. The normal femoral joint line is approximately 2.5–2.8cm below the medial femoral epicondyle, and the normal length of the posterior flange of the femoral component is approximately 2cm8. Therefore, uncontained condylar bone loss of up to 1.5cm would allow near joint-line restoration with distal metal augments of

TABLE 1. Classification of Femoral and Tibial Bone Loss

Rating	Parameters
M0 or L0	Compartment never violated by prosthesis
M1 or L1	Femur: < 1.5 cm
	Tibia: Above tip of the fibular head
M2 or L2	Femur: 1.5 – 2.5 cm
	Tibia: Between fibular head and tibial tubercle
M3 or L3	Femur: Compromised collateral ligament insertion (>2.5cm)
	Tibia: Distal to tibial tubercle
C0	Canal never violated
C1	Stemmed implant with intact cortical tube
C2	Stemmed component with cortical thinning of the canal
C3	Stemmed implant with significant remodeling or canal ectasia

approximately 10-12mm and still provide sufficient posterior condylar bone to establish prosthetic rotational stability with the posterior flange or posterior metal augments of appropriate thickness. Bone loss of more than 1.5cm results in a decreased ability to establish rotational stability at the normal joint line against metaphyseal bone with distal and posterior augments. Therefore, consideration for use of bulk allografts or metaphyseal porous metal sleeves or cones may be necessary to ensure stability. Bone loss that does not compromise the femoral epicondyles allows maintenance of the collateral ligament attachments, and therefore allows use of non-constrained or non-linked varus-valgus constrained knee designs. Bone loss proximal to the femoral epicondyles is associated with loss of collateral ligament stabilizers and typically requires use of a rotating hinge or segmental megaprosthetic device.

On the tibial side, our classification system is based on the anatomic relationship of the joint line to the fibular head and tibial tubercle. The relationship between the tip of the fibular head and the normal joint line varies. Nevertheless, the normal joint line has been estimated to be about 1.5cm proximal to the tip of the fibular head.[8,9] Additionally, tibial size and metaphyseal strength diminish as tibial bone loss extends further distally.[10] The insertion of the lateral collateral ligament is into the fibular head, while the superficial medial collateral ligament inserts further distally along the medial tibia, well below the level of the fibular head. Moreover, when tibial bone loss extends below the tibial tuberosity, there is normally a loss of extensor mechanism function requiring repair or reconstruction at the time of the revision procedure.

When evaluating bone loss on either the femur or tibia one must also consider the canal. The presence of a prior stem, particularly with loosening, cortical thinning and femoral ectasia may lead to greater bone loss after removal and may compromise metaphyseal or diaphyseal fixation with standard stem implants.

The associated treatment algorithm (Tables 2 and 3) we developed is largely based on these same principles. In order to assess the validity of the classification system and the associated algorithm, we compared the treatment predicted by the first survey attempt of the senior author with the actual management strategy utilized for each case, based on operative notes and a record of implanted devices. There was sufficient information from 48 femurs and 47 tibias for this assessment.

When calculating intra-observer agreement, inter-observer agreement and validity, each of the 6 sub-classifications (compartments) for each knee was used as a point of potential agreement or disagreement. Observed agreement (%) and Fleiss' kappa[11,12] were used to quantify the level of agreement.

Results

The average kappa value for intra-observer agreement was 0.78, which qualifies as substantial agreement according to the Kappa Interpretation Scale developed by Landis and Koch.[13] The intra-observer agreement and observed agreement for each physician ranged from 0.69–0.89 and 79%–93%, respectively.

The inter-observer kappa score comparing all four raters' evaluations were 0.70 (95% CI 0.67-0.73) and 0.71 (95% CI 0.68-0.73), for the first and second attempts respectively. The observed agreement among all four evaluators was 64% for both the first and second attempts.

The predictive algorithm had near perfect agreement with the ultimate treatment utilized, with a kappa value of 0.94 (95% CI 0.86-1.02) and an observed agreement of 96%. There were six procedures, including eleven compartments, that were manage differently than would have been predicted by the treatment algorithm and the preoperative bone loss

TABLE 2. Treatment Options for Femoral Bone Loss

Rating	Recommended Treatment
M0 or L0	Metal augments generally not needed
M1 or L1	Distal and/or posterior metal augments
M2 or L2	1) - Porous metal sleeve or cone
	- Or bulk allograft
	2) Impaction grafting with wire mesh
	3) May add metal augments as necessary
M3 or L3	1) Rotating hinge or distal femoral replacement
	2) Allograft prosthesis composite with fixation of host epicondyles
C0	Short cemented or diaphyseal engaging press-fit stem
C1	Short cemented or longer press-fit stem
C2	Longer cemented or press-fit stem
C3	1) Cemented stem favored over press-fit
	2) Megaprosthesis or distal femoral replacment
	3) Femoral osteotomy, in cases of marked deformity

TABLE 3. Treatment Options for Tibial Bone Loss

Rating	Recommended Treatment	
M0 or L0	1) Standard stemmed implant	
	2) Short cemented stem with metal augmentin opposite compartment	
M1 or L1	1) - Short cemented or diaphyseal engaging press-fit stem	
	- May also require cement or particulate bone graft	
	2) Porous metal cone or sleeve	
M2 or L2	1) Metal augments	
	2) Porous metal cones or sleeve	
	3) Impaction grafting with wire mesh	
M3 or L3	*First, confirm whether or not extensor mechanism is intact	
	*Intact extensor mechanism	1) Porous metal cone/sleeve or bulk allograft
		2) Addition of metal augments to the above as needed
		3) Also, CCK or rotating hinge required
	*Intact, but tenuous	1) Porous metal cone to support biologic fixation to tubercle
		2) Proximal tibial allograft with fixation of host tubercle to cancellous allograft bone
	*Disrupted extensor mechanism	1) Proximal tibial allograft with attached extensor mechanism
		2) Porous metal cone with extensor mechanism repair or reconstruction with tendon autograft/allograft or Marlex mesh
C0	Short cemented or diaphyseal engaging press-fit stem	
C1	Short cemented or longer press-fit stem	
C2	Longer cemented or press-fit stem	
C3	1) Cemented stem favored over press-fit	
	2) Megaprosthesis or proximal tibial replacment	
	3) Tibial osteotomy, in cases of marked deformity	

classification. Three of the compartments were managed more aggressively than predicted and eight were managed less aggressively.

Discussion

Comparing different strategies for management of bone loss during revision TKA requires a rational, valid and reliable classification system to quantify the degree of bone loss. We have proposed such a system that quantifies and classifies bone loss using important anatomical landmarks that are relevant when considering management.

We designed this study to evaluate the inter- and intra-observer reliability of this new classification system as well as demonstrate its ability to predict the intraoperative management when paired with the treatment recommendations we presented.

Certain limitations were identified when reviewing the cases where our system failed at predicting actual treatment. The importance of assessing every compartment for bone loss regardless of whether it has been violated by an implant was highlighted by one case that featured significant central osteolysis in the setting of an appropriately oriented stemless component. In another case, the knee was flexed for the AP radiograph, causing many of our evaluators to interpret what was an isolated posterior femoral defect as extending across the condyles more proximally. When evaluating radiographs preoperatively, it is important to evaluate their quality, and repeat radiographs as necessary.

We recognize that two-dimensional radiographs may under- or over-predict the degree of bone loss encountered during revision TKA, and believe in the future this study will be supplemented with prospective intraoperative evaluations of bone loss in order to better assess its preoperative accuracy and also its utility as an intraoperative classification system. Nevertheless, we believe this is a good initial step which will be useful during preoperative planning and determination of management strategies. The future direction of this classification system will be to demonstrate the validity of the radiographic classification and treatment algorithm in other surgeons' hands to demonstrate its utility among surgeons with a variety of training backgrounds and practice settings.

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U·P·O·J

Diagnosing Infection in Patients Undergoing Conversion of Prior Internal Fixation to Total Hip Arthroplasty

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Introduction

Hip fractures accounted for over 340,000 hospital admissions in 2008 and are expected to rise to over 580,000 by 2040 due to an aging population in the United States.[1,2] Patients initially managed with open reduction and internal fixation that result in nonunion, early fixation failure, and post traumatic arthritis can be effectively treated with either revision internal fixation (with or without bone grafting) or conversion to total hip arthroplasty (THA).[1-11] Although conversion THA for failed hip internal fixation has good results, previous reports demonstrate that conversion THA has an increased incidence of superficial and deep infection compared to primary THA.[19] Periprosthetic joint infection is a devastating complication in THA, resulting in a substantial morbidity to the patient and cost burden to the health care system, and the diagnosis is often unclear.[20]

Currently, there are no recommendations for the diagnosis and management of infection prior to conversion of prior internal fixation to THA. The purpose of this study is to identify the incidence of infection in patients undergoing conversion of prior internal fixation to THA. We investigated several preoperative risk factors for infection and evaluated the utility of preoperative erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) as screening tools to identify patients with occult infection.

Materials & Methods

This study is an Institutional Review Board approved retrospective review of patients at a single institution who underwent conversion of prior internal fixation to THA from 2009-2014. We searched the hospital's patient database and identified 33 patients that underwent conversion of prior internal fixation to THA, were greater than 18 years of age, and had laboratory data for ESR and CRP. The study's primary outcome variable was the presence of infection diagnosed by positive culture results at the time of conversion THA or at short- term follow up. Patients diagnosed with infections preoperatively underwent either removal of hardware with antibiotic cement spacer implantation, staged conversion THA, and 6 weeks of intravenous antibiotics, or single-staged conversion THA with an antibiotic cement -impregnated implant and 6 weeks of intravenous antibiotics. Patients with positive intraoperative cultures that were diagnosed as infected postoperatively were treated with a debridement and/or 6-week course of intravenous antibiotics. After conversion THA or infection treatment, patients were followed for at least 60 days postoperatively (mean follow up of 1 year, range 2-30 months).

An a priori power analysis indicated the need to enroll a minimum of 31 patients to detect a standard large effect size $w = 0.5$, assuming a type-I error rate of 0.05 and a power of 0.80. Medical co-morbidities, smoking history, body mass index (BMI), prior hip surgery, and preoperative inflammatory markers (ESR and CRP) were documented and analyzed with univariate and multivariate logistic regression analysis (Table 2). Because we could not assume our small sample size was normally distributed, continuous variables were compared using the non-parametric Mann-Whitney U test. Categorical variables were compared using the Chi-square test; when the observed or expected values were less than 5, the Fisher Exact Test was used (Table 1). Receiver operating characteristic (ROC) curves were then generated to determine test performance of traditional inflammatory markers, ESR and CRP. Statistical significance was set at $p = 0.05$.

Results

The 33 patients in this study included 9 (26%) with a previous intramedullary nail, 8 (23%) with acetabular internal fixation, 2 (6%) with slipped capital femoral epiphysis internal fixation, and 10 (28%) with femoral neck percutaneous screws. There were 16 males and 17 females included in the study with a mean age of 56 years (range 19-88 years). This study included 6 infected patients (18%) and 27 non-infected patients (82%). Logistic regression analysis showed no significant differences in age, BMI, and co-morbidities including diabetes mellitus, cardiac disease, smoking history, obesity, morbid obesity, and advanced age over 70 years between the two groups (Table 2).

Mean ESR and CRP were significantly higher ($p < 0.05$) in the infected group (41.6 mm/hr and 2.02 mg/dL) compared to the non-

Table 1. Comparison of risk factors of patients undergoing conversion total hip arthroplasty who were both infected and non-infected

Risk Factor	Infected (n = 6)	Non-infected (n = 27)	P value
Mean Age (years)	66.2	52.5	0.191
Mean BMI (kg/m2)	29.2	27.3	0.562
Mean Preoperative ESR (mm/hr)	41.6	19.3	0.003
Mean Preoperative CRP (mg/dL)	2.02	1.27	0.003
Diabetes (%)	1 (16)	2 (7)	0.464
Cardiac disease (%)	1 (16)	5 (19)	1.000
Smoking history (%)	2 (33)	7 (26)	1.000
Obesity (%)	1 (16)	3 (11)	1.000
Morbid Obesity (%)	1 (16)	2 (7)	0.464
Age > 70 years	3 (50)	6 (22)	0.309
ESR > 30 (%)	5 (83)	4 (15)	0.007
CRP > 1 (%)	4 (67)	4 (15)	0.037

Table 2.Univariate and multivariate logistic regression analysis on risk factors for infection at the time of conversion of prior hip surgery to THA

	Univariate Analysis			Multivariate Analysis		
Risk Factor	Odds Ratio	95% Confidence Interval	p value	Odds Ratio	95% Confidence Interval	p value
Age > 70 years	3.50	0.55 – 22.20	0.186	7.77	0.24 – 425.61	0.315
Diabetes	2.50	0.18 – 33.17	0.507	4.60	0.01 – 2041.6	0.623
Cardiac disease	0.88	0.08 – 9.29	0.915	0.30	0.00 – 283.23	
0.733						
Smoking history	1.43	0.21 – 9.58	0.717	1.86	0.05 – 61.79	0.727
Obesity	1.60	0.14 – 18.72	0.716	1.68	0.02 – 132.73	0.816
Morbid Obesity	2.50	0.18 – 33.17	0.507	0.54	0.01 – 32.00	0.774
ESR > 30	28.75	2.62 – 315.42	0.001	27.66	1.08 – 705.88	0.044
CRP > 1	11.50	1.55 – 85.15	0.012	3.45	0.23 – 51.15	0.367

infected group (19.3 mm/hr and 1.27 mg/dL). There was a significant incidence ($p < 0.05$) of elevated ESR > 30mm/hr and elevated CRP > 1mg/dL in the infected group (84% and 67% respectively) when compared with the non-infected group (15% and 15% respectively). Two (33%) of the infected patients had a CRP that was not elevated (CRP < 1mg/dL) but had an elevated ESR (ESR > 30mm/hr). Of the non-infected patients, 5 (18%) had either an elevated ESR or CRP, but these patients did not develop symptoms of prosthetic joint infection (PJI) during the follow-up period. Univariate analysis demonstrated that ESR > 30mm/hr (OR 28.75 (95% CI 2.62-315.42)) and CRP > 1mg/dL (OR 11.5 (95% CI 1.55-85.15)) were risk factors for the diagnosis of infection at the time of conversion THA. When controlling for confounding variables, multivariate analysis also showed that the odds ratio for ESR > 30mm/hr was 27.66 (95% CI 1.08-705.88) and 3.45 (95% CI 0.23-51.15) for CRP > 1mg/dL.

ROC curves assessing the utility of inflammatory markers as a diagnostic tool for infection at the time of conversion THA showed a good fit for both ESR (AUC = 0.894) and CRP (AUC = 0.891) (Figure 1). Using a CRP > 0.7mg/dL had 100% sensitivity, 80.7% specificity, 100% negative predictive value, and 54.5% positive predictive value. Using an ESR > 30mm/hr had 83.3% sensitivity, 84.6% specificity, 95.6% negative predictive value, and 55.5% positive predictive value. Using a CRP > 0.7mg/dL or an ESR > 30mm/hr had 100% sensitivity, 76.9% specificity, 100% negative predictive value, and 50% positive predictive value. Using both CRP > 0.7mg/dL and ESR > 30mm/hr had 83.3% sensitivity, 88.4% specificity, 95.8% negative predictive value, and 62.5% positive predictive value.

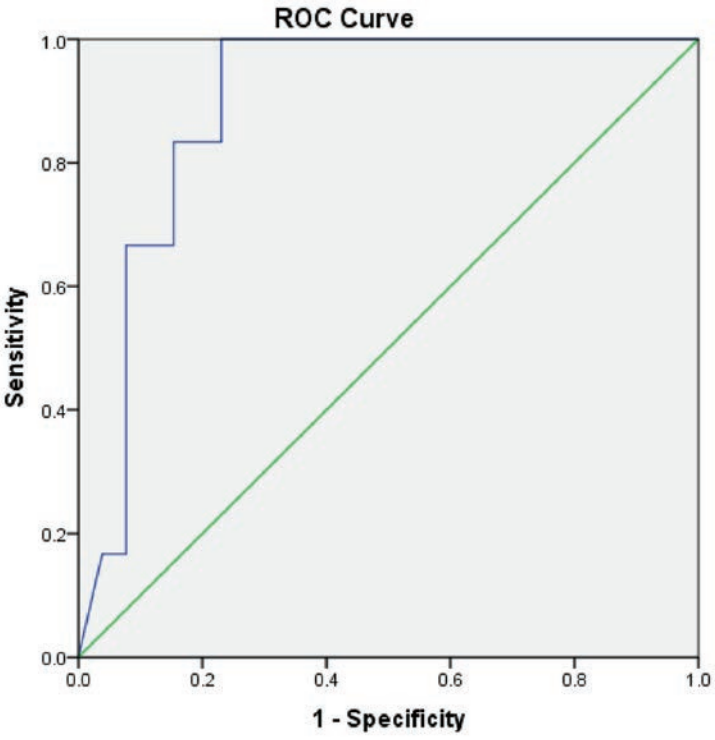

Figure 1a. Receiver operating characteristic curve for preoperative ESR as predictor for infection at the time of conversion THA (AUC 0.894, 95% confidence interval 0.783 – 1.000.

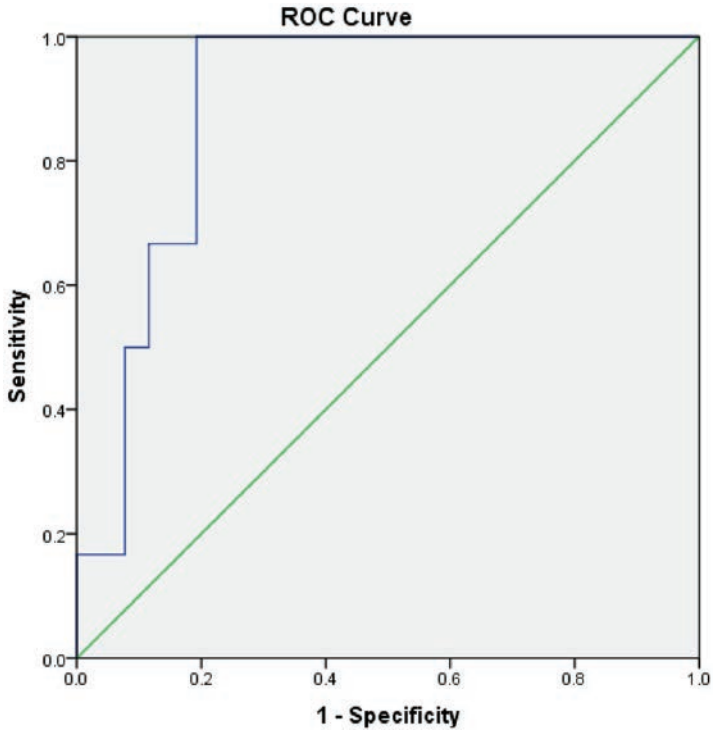

Figure 1b. Receiver operating characteristic curve for preoperative CRP as predictor for infection at the time of conversion THA (AUC 0.891, 95% confidence interval 0.779 – 1.000.

Discussion

This study has several limitations. First, we only included patients with inflammatory markers drawn prior to conversion THA. This results in a strong selection bias, as 97 patients who underwent conversion THA did not have an ESR or CRP. We also recognize the limitations of our retrospective chart review with a relatively small sample size (n = 33), which is only powered to detect large effect sizes. We risk committing type II error, particularly in the case of variables such as age and diabetes which were associated with increased risk of infection in our series, but not statistically significant. Finally, as a surgical procedure, conversion THA can be quite variable with patients having a variety of demographics, co-morbidities, and types of previous internal fixation procedures. These factors can limit our ability to make specific conclusions regarding the true impact of patient risk factors on infection prior to conversion THA. Nevertheless, given that the case mix of procedures in both groups was similar and comparable, certain generalizations about infection risks in patients undergoing conversion THA can be made.

Conclusions

Given the complexity of conversion THA and morbidity of infection constant vigilance for occult infection must be maintained. The assessment for infection prior to conversion THA should begin with a detailed patient history. Symptoms such as a pain following a pain-free interval after ORIF, nighttime pain, or pain at rest should raise suspicion for infection. A physical examination should be performed and include an assessment of prior hip incisions. Following history and physical examination, routine laboratory studies should include CBC, ESR, and CRP. Elevated inflammatory markers should prompt a preoperative hip aspiration. Synovial fluid analysis including white blood cell count with differential as well as aerobic and anaerobic cultures should be performed[24].

Elevated ESR and CRP are associated with infection prior to conversion THA. Although elevated ESR and CRP are useful tools to screen for occult infection prior to conversion THA, given the high incidence of discordance in inflammatory markers in this series, patients with both elevated and borderline inflammatory markers should prompt further evaluation with diagnostic hip aspiration including white blood cell count, differential, and culture prior to conversion THA surgery as the results may affect preoperative planning.

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Epidermal Growth Factor Receptor (EGFR) Signaling in Cartilage Prevents Osteoarthritis Progression

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Introduction

Osteoarthritis (OA) is the most common chronic condition of the joints affecting approximately 27 million adults in the United States alone. OA is a disease of mechanics but the molecular mechanism by which normal, healthy articular cartilage maintains its strength to resist mechanical loading thereby preventing OA progression is poorly understood. We recently demonstrated a pivotal role of epidermal growth factor receptor (EGFR) signaling in growth plate development1 and endochondral ossification2, indicating that this is a critical pathway regulating chondrocyte function. To understand the role of EGFR in maintaining articular cartilage and in OA development, we generated cartilage-specific *Egfr* null mice and characterized their knee joint phenotypes under physiological and pathological conditions.

Methods

All animal work was approved by the Institutional Animal Care and Use Committee at the University of Pennsylvania. *Animals and surgery-* Cartilage-specific *Egfr CKO* (*Col2-Cre* $Egfr^{Wa5/f}$) mice and their *Wa5* ($Egfr^{Wa5/f}$) and *wild-type* (*WT, Col2-Cre* $Egfr^{f/+}$ and $Egfr^{f/+}$) siblings were generated by breeding *Col2a1-Cre,* $Egfr^{Wa5/+}$, and $Egfr^{f/f}$ mice. $Egfr^{Wa5}$ codes for a kinase-dead, dominant negative receptor. To induce OA, male mice at 3 months of age were subjected to destabilization of the medial meniscus (DMM) surgery of the right knees and sham surgery of the left knees. *Histology and immunohistochemistry (IHC)-* Mouse knee joints were harvested at indicated times for a serial of 6 μm-thick sagittal sections across the entire medial compartment of the joint for quantification of cartilage thickness, chondrocyte number, subchondral bone plate thickness, and Mankin score, as well as IHC staining for EGFR, phosphorylated-EGFR (p-EGFR), phosphorylated-Erk (p-Erk), osteocalcin, and sclerostin. *μCT-* The distal femurs and proximal tibias were scanned by μCT 35 (Scanco Medical AG) at a resolution of 6 μm to calculate trabecular bone structural parameters. *Nanoindentation-* Atomic Force Microscopy (AFM)-based nanoindentation was performed on the superficial zone of cartilage using a borosilicate colloidal spherical tip ($R \approx 5$ μm, nominal spring constant $k \approx 7.4$ N/m, AIO-TL tip C) and a Dimension Icon AFM with indentation depth of ~ 1 μm at 1 μm/s and 10 μm/s rates. Effective indentation modulus, E^{ind} (MPa), was calculated from the loading portion of indentation force-depth curves using the Hertz model. *Primary chondrocytes-* Primary epiphyseal chondrocytes were obtained from mouse newborn pups by enzymatic digestions. Cells were cultured in chondrogenic medium (DMEM/F12 medium with 5% fetal bovine serum, 50 μg/ml of L-ascorbic acid, and 1% glutamine). *Statistics-* Data are expressed as means±SEM and analyzed by unpaired, two-tailed Student's t-test.

Results

To delineate the function of cartilage EGFR signaling, qRT-PCR and IHC were first performed with articular cartilage in 2-month-old mice. We observed strong EGFR expression in all mice. p-EGFR and p-Erk were only detected in *WT* and *Wa5* mice but not in *CKO* mice, confirming that *CKO* mice were deficient in EGFR activity in articular cartilage. The most abundant EGFR ligand in cartilage is TGFα followed by epiregulin and betacellulin. While there was no histological changes in articular cartilage among *WT, Wa5*, and *CKO* mice at this developmental stage (Fig. 1A), cartilage surface E^{ind} was significantly decreased in *Wa5* mice (41.3% of *WT*) and further diminished in *CKO* mice (25.3% of *WT*) (Fig. 1B). Four months later, while the nanomechanical property remained low in *Wa5* and *CKO* mice, *CKO* mice had early OA symptoms, including partially depleted uncalcified zone, decreased Safarin O staining, and increased chondrocyte hypertrophy (Fig. 1). Wa5 cartilage had similar alterations but to a much lesser extent. Osteophytes in knee joints were observed in 66.7% of CKO mice but never in WT or Wa5. Primary chondrocyte culture confirmed that activating EGFR stimulates cell proliferation and survival and inhibits their terminal differentiation. DMM was performed in 3-month-old mice to induce OA. Three months later, *WT* developed mild-to-moderate OA (Makin score: 6.0 ± 0.5), *Wa5* exhibited advanced OA (11.5 ± 0.5), and *CKO* had the most severe OA (14.0 ± 0.0) with a complete loss of entire cartilage layer restricted at the medial side (Fig. 2A). Moreover, we observed a local and drastic

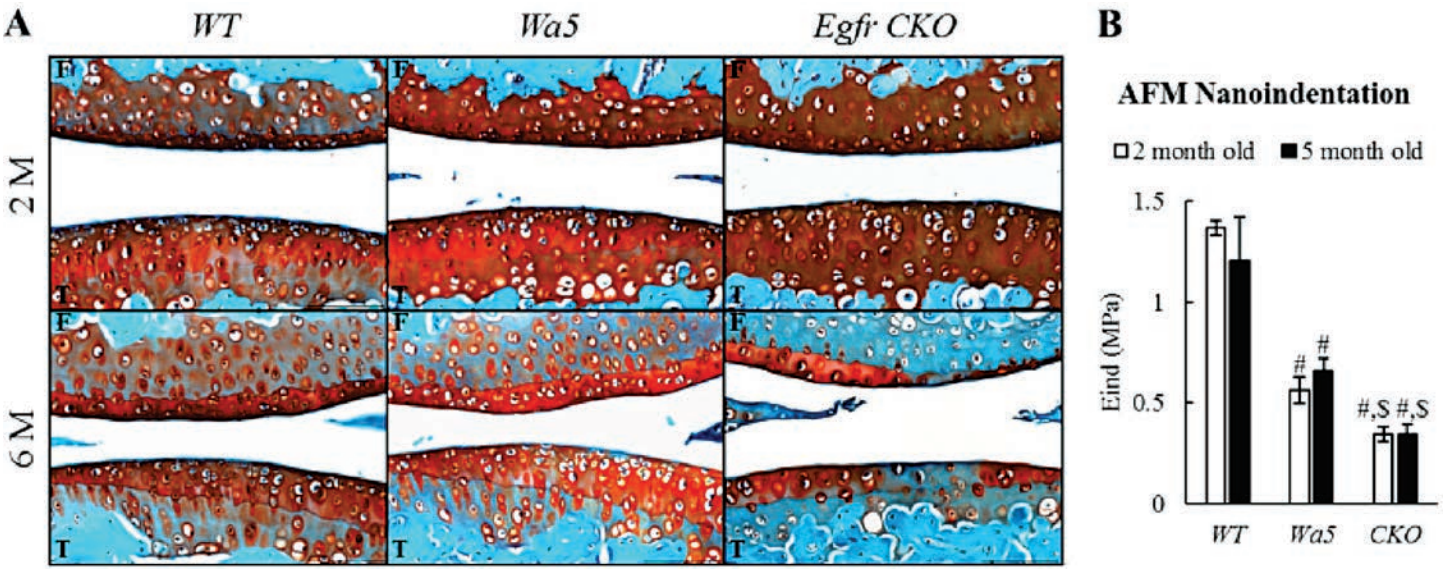

Figure 1. EGFR signaling is important for maintaining articular cartilage structure and mechanical strength. (A) Safranin O staining of knee joints in 2 and 6 month-old mice. F: femur; T: tibia. n = 6/genotype/time point. (B) Nanoindentation shows that EGFR deficiency decreases the stiffness of articular cartilage. n = 5/genotype/time point. #: $p < 0.05$ vs *WT*; $: $p < 0.05$ vs *Wa5*.

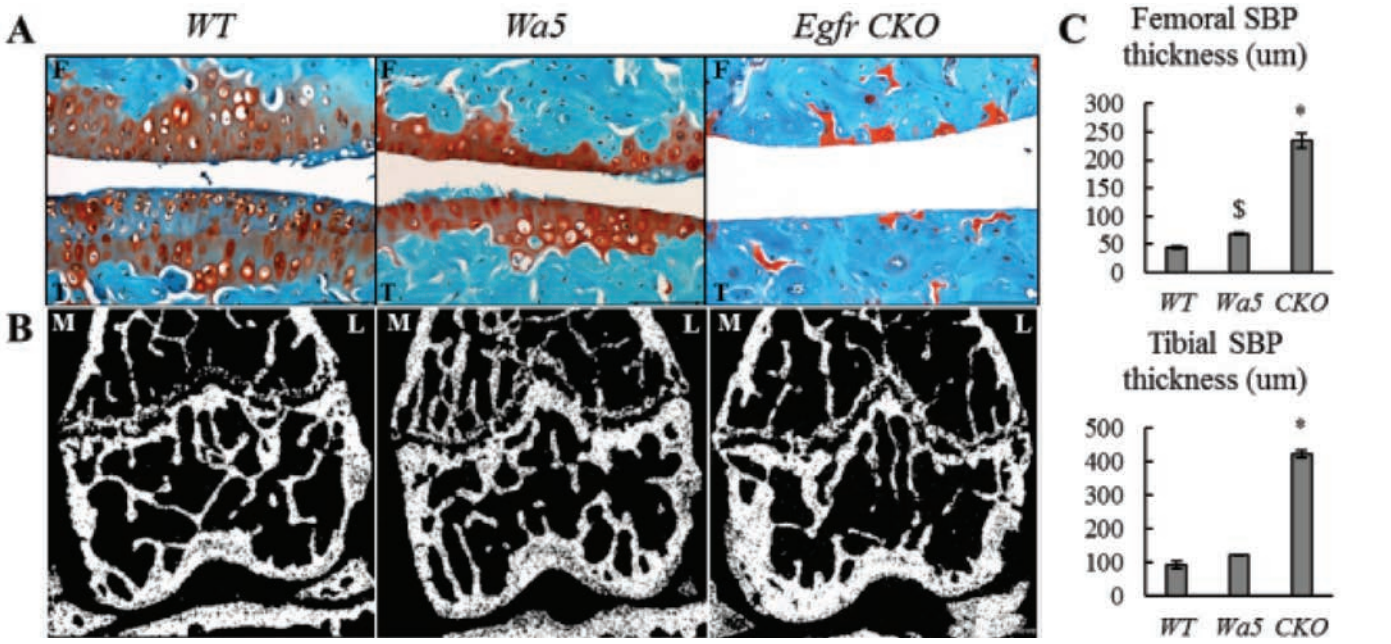

Figure 2. EGFR chondrogenic deficiency causes severe osteoarthritis after DMM. (A) Safranin O staining of knee joints in mice at 3 months post DMM surgery. (B) μCT images reveal local SBP thickening only at the medial site of *CKO* mouse joint. M: Medial; L: Lateral. (C) Quantification of SBP thickness. n = 6/genotype. $: $p < 0.01$; *: $p < 0.001$ vs *WT*.

subchondral bone plate (SBP) thickening (4.5-fold) only under the cartilage damage area (Fig. 2B, C). This was correlated with locally reduced sclerostin amount by osteocytes within SBP (Fig. 3) and increased number of osteoblasts lining SBP surface. Since subchondral and metaphyseal trabecular bone parameters were not altered, we reason that SBP sclerosis was caused by increased mechanical loading and decreased sclerostin amount after cartilage depletion.

Discussion

Our studies demonstrate that chondrogenic EGFR signaling and its cognate ligands, most likely TGFα, are essential for maintaining articular cartilage and are critical for OA development. *Egfr CKO* mice have much weaker articular cartilage and develop spontaneous OA at a much earlier stage compared to *WT*. We also demonstrate that *CKO* mice exhibit much more accelerated OA symptoms in a surgical OA

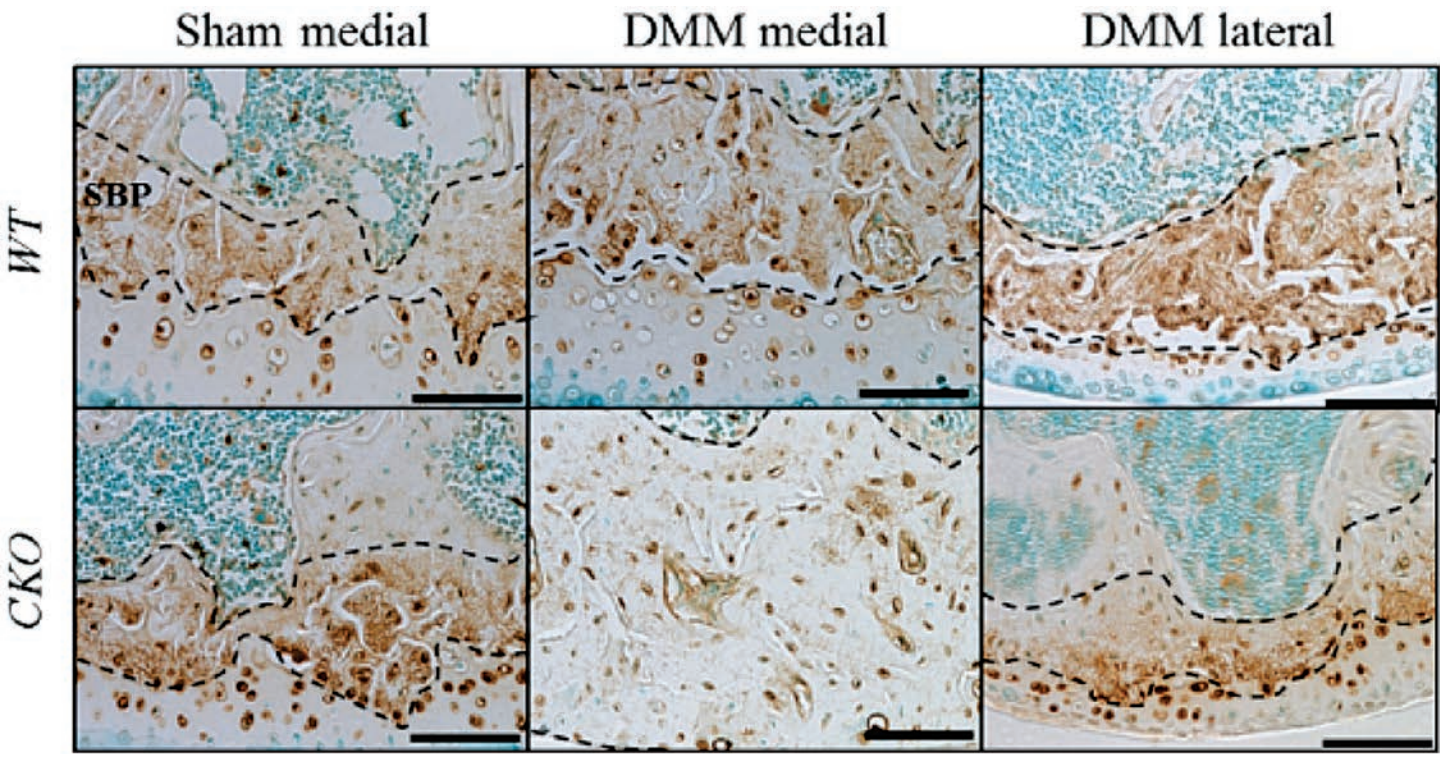

Figure 3. Increased bone formation in SBP in *CKO* mice is accompanied by decreased sclerostin amount. Scale < 100um.

model. The limitation of this study is that articular cartilage of *CKO* mice was already abnormal before surgery. Further investigation using an inducible system (*aggrecan-CreER*) to diminish EGFR activity at the same time of DMM is currently underway to delineate more precisely the role of EGFR in OA development.

Conclusion

We provide the first direct evidence that chondrogenic EGFR signaling is critical for articular cartilage homeostasis and OA development and that local crosstalk between cartilage and SBP plays an important role in accelerating OA progression.

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Hot Topics in Orthopaedic Clinical Research Methodology

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Background

Epidemiologic research has increased in prevalence and importance across all medical specialties. Epidemiology shares historic roots with the development of public health practices. The field is focused on determining the cause of diseases among populations and the effectiveness of strategies to control those diseases. In this way prevention and treatment strategies may be tested in order to minimize the impact of disease.

In the modern era of expanding and unsustainable health care costs, government payers and insurance companies in conjunction with quality improvement advocates have promoted "evidence-based medicine." These are clinical practices that have been proven to effectively and efficiently provide safe treatment for patients. This "evidence" is the result of rigorous epidemiologic study. Baldwin et al. previously described in detail how epidemiologic questions are developed, and outlined the fundamentals of study design and data analysis with a focus on orthopaedic surgery research.

In this review, discussion will focus on the importance of clinical research in orthopaedics, and an evaluation of outcome measurement tools, quality improvement initiatives, and clinical trials. Finally, the principles behind construction of a multivariate regression model will be described, in order to illuminate this centrally important, yet seemingly esoteric epidemiologic tool.

Hot Topics

Passage of the Patient Protection and Affordable Care Act in 2010 mandated a program for public reporting of medical outcomes from the individual physician to the hospital level. The aim of this program is to improve quality in two areas: 1) on the provider side through open comparison and value-based repayment, and 2) on the patient side through enhanced consumer decision-making.

The Orthopaedic Core Measurements are most relevant to orthopaedic surgeons. The first version of these measures focuses on total joint arthroplasty, because these procedures cost Medicare $7 billion annually in hospital expenditures, which makes it the top Medicare expenditure.

The specific measures evaluate complications and all-cause 30-day readmission rates following total knee and total hip arthroplasty, and patient satisfaction with orthopaedic surgical care. Looking forward, it is likely that similar Orthopaedic Core Measurements will be developed across all subspecialties with the intent of improved quality and informed consumer choice.

These initiatives have bolstered interest in orthopaedic clinical research and patient reported outcomes. The publication of comparative outcomes among orthopaedic surgeons necessitates both a means for accurate reporting as well as strategies for improving these outcomes. Both of these topics are epidemiologic in nature. The former requires an understanding of the types of outcomes that are measured and the limitations in these measurements. The latter requires an understanding of Quality Improvement (QI) initiatives and Randomized Controlled Trials (RCT).

Outcome Measures

In the development of an epidemiologic study the fundamental question revolves around the relationship between exposures and outcomes. To draw from the CMS Orthopaedic Core Measurements as an example, a study would evaluate the exposure of total joint replacement and the outcome of 30-day all-cause readmission or patient satisfaction with the procedure. Increasing emphasis has been placed on selecting outcomes that are patient-centered, rather than relying on objective data alone. In this way treatments can be tested for their impact on quality of life, rather than improvement in intermediate endpoints (such as knee range of motion), which may be of little interest to the patient. The science of patient-reported outcomes, though initiated by Ernest Codman in the early 20th century with "The end results of health care" is still very fragmented and difficult for clinicians to agree upon.

Physician collected outcomes are measures that are taken by the physician and include subjective symptoms, but also objective measures such as range of motion and physical findings. Harris Hip score is an example of such a measure. In the last several years CMS and other payers have been less interested in these

types of outcomes, though many outcomes reported in earlier as well as recent papers use these measures.

Patient-reported outcomes (PRO) are those that come directly from the patient, and describe symptoms. PRO instruments are questionnaires that have been validated for assessment of the symptoms of interest. They must be validated for particular pathologies, and for different cultures and languages. These instruments should demonstrate specificity for the outcome of interest, incorporate questions that are clearly understood and equivalent among diverse patient populations, include an optimal number of questions, and be reproducible. Furthermore, PROs can be either joint specific (such as the DASH for the upper extremity, or FADI for the foot and ankle) or they can be general health measures such as the SF-36 or SF-12.

These instruments are examples of static questionnaires, forms with a fixed set of questions. Issues with this type of outcome measure are that they are vulnerable to ceiling or floor effects, and they need to be specifically validated for each pathology and population in which they are used. Ceiling effects are when the outcome measure does not pick up the high end of performance well because of its questions, and floor effects are the opposite.

Computerized adaptive testing (CAT) has shown promise as a shorter and more effective means for collecting PRO scores in patients with arthritis and other pathologies. This instrument draws from a larger pool of validated survey questions, sequentially choosing items based on the previous response. In this response-based algorithm, fewer questions can be used across a wider spectrum of function to gather more information than longer questionnaires.[9] Theoretically, CAT can overcome the challenges of ceiling and floor effects in static instruments and may be able to detect fine detail at the extremes of function. CAT instruments outperform static instruments at these extremes of the function spectrum by selecting survey items most appropriate to the specific patient, with maximal information ascertained per question.[10]

While great importance is placed on the assessment of patient-reported outcomes, other outcome measures can and should be used in conjunction with patient reports for a complete clinical picture. For example, if you would like to compare patients with specific clinical diagnoses, such as osteoarthritis grade, cardiac ejection fraction, or other clinical indicators, it may be necessary to have physician-reported outcomes. Functional status comparison between physician and patient-reported outcome can also provide important feedback for providers whose opinions may differ dramatically from their patients. Further, predictive models, those that attempt to calculate risk for certain outcomes require a model based on all predictive variables, including physician reported outcomes such as BMI or severity of disease and PRO such as preoperative and postoperative functional status.

Clinical Trials

The different types of epidemiologic study designs, from retrospective to prospective to meta-analysis, has been previously described[1]. The randomized controlled clinical trial is the pinnacle of research methodology, and the only true "experimental" clinical research design. This study design compares at least two randomly assigned groups, one assigned to a control arm and one assigned to an intervention arm. This randomization, if done properly, should sort known and unknown variables equally amongst the intervention and control groups. In this way, the comparison between these groups minimizes bias in a way that non-randomized studies cannot.

Randomized controlled trials (RCT's) in orthopaedic surgery have become more prevalent only in the last decade. In many ways orthopaedics is beginning to follow other medical specialties in terms of research design and methodology; however, orthopaedic RCT design and execution still lags behind other disciplines.

There are shortcomings possible even with the gold standard design. A recent bibliometric analysis showed that among orthopaedic surgery clinical trials that were published, over one-third were underpowered or did not report a power analysis. Of published orthopaedic trials that described significant difference between treatment groups, one-seventh were underpowered. Among the published orthopaedic trials that found no significant difference between treatment groups, over three-quarters were underpowered. The importance of this for the consumer of orthopaedic research is to view outcomes critically, even when the study design is one classically described as a gold standard.

Quality Improvement Initiatives

Quality Improvement (QI) initiatives are clinical projects that attempt to directly improve an identified health care shortcoming. Compared with traditional research, the goal of QI is to elicit rapid change in a complex system, improve outcomes, or improve the patient experience. This has been an area of intense interest over the last several years. Variance in healthcare has been thought to represent inferior quality. Therefore, clinical pathways have been developed to increase value, which is defined as quality relative to cost. Several examples of QI have been initiated in the orthopaedic community.

In adolescent idiopathic spine surgery, researchers at the Children's Hospital of Philadelphia initiated a rapid recovery pathway of adolescent idiopathic scoliosis. This pathway involved a standardized set of medications, OR procedures, and postoperative rehabilitation protocols that resulted in lower patient pain and an average of almost two fewer days of hospitalization.

At Penn Presbyterian Hospital, a multivariate logistic regression analysis model was used to ascertain factors that result in unplanned admission to the intensive care unit (ICU). Following development of this model, patients were risk-stratified into elective admission to the ICU following joint replacement based on those criteria. This pathway led to fewer unplanned admissions to the ICU, and safer postoperative care for these patients.

On the University of Pennsylvania orthopaedic trauma service, a pathway for hip fractures has been generated. It is known that delays to the operating room and longer hospital stays result in increased patient morbidity and mortality, lower quality of care and decreased patient and caregiver satisfaction. The trauma service in conjunction with anesthesiology, geriatric medicine and rehabilitation, generated a protocol to decrease variation and increase expediency of surgery and quality of care. The results are under current investigation.

Multivariate Regression Models

Complex prospective study designs with randomization and blinding require only simple statistics, because confounders have been equally distributed between groups by virtue of randomization. However, non-randomized studies must stratify by significant confounders (in which case the confounders cannot be directly studied) or perform a regression analysis, which eliminates the effect of one variable on another and estimates accurate effect size. If univariate statistics are used alone, there is no evidence that the effect observed is because of the variable of interest or a result of some confounder, either identified or not identified.

Multivariate regression modeling is the solution to this dilemma. Logistic regression is available when the outcome is dichotomous, and linear regression is used when the outcome is linear. Some modeling such as Poisson regression for count variables and multinomial regression for categorical variables is beyond the scope of this discussion. The math behind these models is complex, but the process for developing a model requires only a dataset, a literature review, and a statistical software program. The strength of the multivariate model comes from its ability to provide an association between an outcome and multiple exposures that eliminates the redundant variability that results from each individual factor. Multivariate models are often reported as an association between exposure, x, and outcome, y, that is "controlled for" all other factors studied. For example, a factor, such as age, is entered into the model, and through the regression calculation, the variability between x and y that is due to differences in age among subjects is removed from the association between x and y. This result could be reported as "controlled for age" or "age-adjusted".

The first place to start building a multivariate regression model is with the known or expected variables of interest. These are identified from the literature, from common variables of known effect (such as age or a socioeconomic indicator), and investigatory variables from the study hypothesis. Once a list of intended variables has been compiled it can be helpful to visually assess the relationship between each variable with the outcome of interest by building scatterplots for each variable with the outcome of interest.

With variables of interest identified, model development begins (Figure 1). The ideal model will include the fewest number of variables that explain most of the variability in the outcome. Each variable included in the final model should be highly predictive of the outcome without being highly correlated with another variable in the model. This avoids the issue of multicollinearity, which can make a model cumbersome and difficult to interpret.

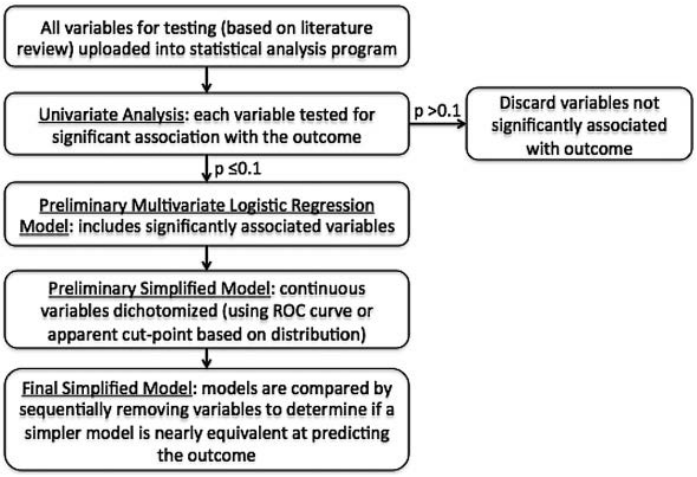

Figure 1. Backwards Model Building Strategy for Multivariate Logistic Regression Model.

Next, a univariate analysis, or a simple linear regression is performed, which by analyzes the association between each variable and the outcome of interest. This will provide a t-statistic and a p-value for significance for each variable with the outcome. Once completed, the model can be developed using a forward or a backwards model building strategy. The forward strategy chooses variables sequentially to add to the model, while the backwards model begins with all variables included in the model and sequentially removes insignificant variables.

Next, continuous variables are dichotomized. This involves choosing cut- points in continuous variables, so that groups such as "high" or "low" within a value range may be compared to one another. This strategy may decrease the precision of the model, but makes the findings easier to understand. For example, imagine the risk of postoperative infection increases 1% with each point in BMI compared to an index value. This may intuitively make more sense if modeled in groups of regular weight, overweight, and obese, where the result could be described as obese patients having a 10% greater risk of postoperative infection compared with regular weight patients. Different cut points may be obtained by simply looking at the frequency of outcome in each group or by more complex methods such as receiver operator characteristics analysis.

Models can be compared using the log-likelihood estimation, which sequentially simplifies the model and determines whether the simpler model differs significantly in predictive value for the outcome compared with the original model. A model that includes many variables is likely not the most parsimonious, or simplest model to predict the outcome.

Summary

The practice of medicine continues to move toward evidence-based practice and payment structures force

physicians to improve outcomes and efficiency, Orthopaedic surgeons must adapt to these changes through improved epidemiologic research and enactment of the results of these studies. It is imperative that the modern orthopaedic surgeon understands the results of QI initiatives and clinical trials in order to appropriately incorporate best practices into their patient care.

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Operative Technique: Use of a Volar Plate for Restoration of Volar Tilt Intra-operatively

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Introduction

Use of volar locking plates has improved surgical fixation of distal radius fractures, allowing for anatomic reduction and early rehabilitations. AAOS recommends surgical fixation of distal radius fractures when post reduction radial shortening is > 3mm, dorsal tilt > 10 degrees, and intra-articular displacement/step off is > 2mm, particularly in patients > 55 years of age.[1] One of the more difficult aspects of anatomic alignment to achieve is volar tilt. This technique involves utilization of initial distal fixation of the volar plate to achieve an intraoperative correction of volar tilt and improve anatomic alignment.

Background

It is well accepted that the restoration of joint anatomy in distal radius fractures is important for better outcomes, and optimal kinematics through the wrist joints.[1,2] With newer implants such as volar locking plates, we have an ability to achieve better reduction of distal radius fractures intraoperatively, and secure the reduction with increased stability across the wrist joint.[3] We also have the ability to use bridging to overcome comminution.[4] Correction to a volar tilt of 11 +/− 5 degrees has been shown to restore biomechanical function of the wrist.[5] Prior to the use of volar locking plates, in order to restore volar tilt it would be necessary to use a dorsal approach, bone graft, and a dorsal plate. In the case discussed in this paper osteotomy and bone graft was used due to delay in presentation, but this technique can be extrapolated to fractures in which osteotomies are not needed but volar tilt needs to be restored.

Preoperative Evaluation

This operative technique is useful in patients who present with distal radius fractures in which restoration of volar tilt is necessary. In this case, a 36 year old male presents to clinic with left wrist pain and deformity 5 weeks after falling off a skateboard. He had initially been seen at an outside hospital, but was lost to orthopaedic follow up in the interim. Preoperative X-rays at the time of presentation showed a hyperextension deformity of the wrist (Figure 1). Decision was made to take the patient for open reduction and internal fixation of the left distal radius.

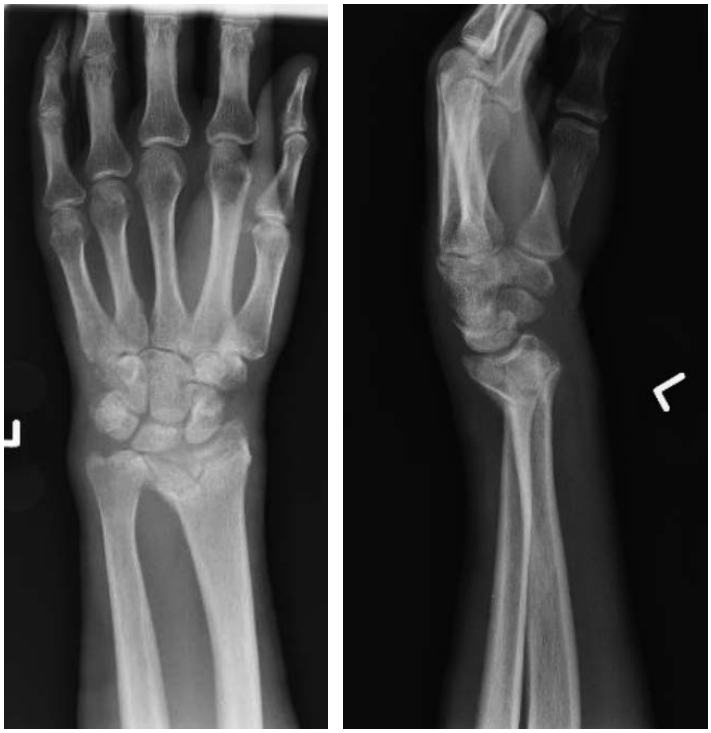

Figure 1. Preoperative radiographs showing loss of radial height, and loss of volar tilt.

Procedure

The patient was placed supine with the arm on a radiolucent hand table. A standard volar approach to the distal radius was performed through the FCR sheath, retracting the FPL and exposing pronator quadratus, which was then released from its radial and distal attachments. Subperiosteal dissection was performed allowing for exposure of the fracture site.

The fracture site was probed, and was healed with no micromotion. Because of the unacceptable alignment, it was decided that an osteotomy would be performed. A guidewire was placed as a provisional guide for the osteotomy and then confirmed via fluoroscopy (Figure 2). The osteotomy was then performed using a sagittal saw and completed with an osteotome. The distal fragment was mobilized with a laminar spreader to ensure that the dorsal callous was freed.

Reduction was then attempted by hyperflexing the wrist, but this only achieved neutral volar tilt and was deemed unsatisfactory (Figure 3). Thus, it was elected to attempt reduction using the volar plate. An appropriately sized volar locking plate was fixed to the distal fragment, and adjusted using fluoroscopy. The plate was first fixed provisionally with K wires, and then with locking screws. (Figure 4) To account for the correction desired, the plate was fixed distally while protruding out of the wound. Then, using the volar locking plate as leverage, the distal fragment was reduced to the shaft using a lobster claw. Reduction was checked via fluoroscopy (Figure 5). This reduction maneuver recreated near anatomic volar tilt. At this point, the reduction was found to be appropriate and the sliding hole in the volar locking plate was

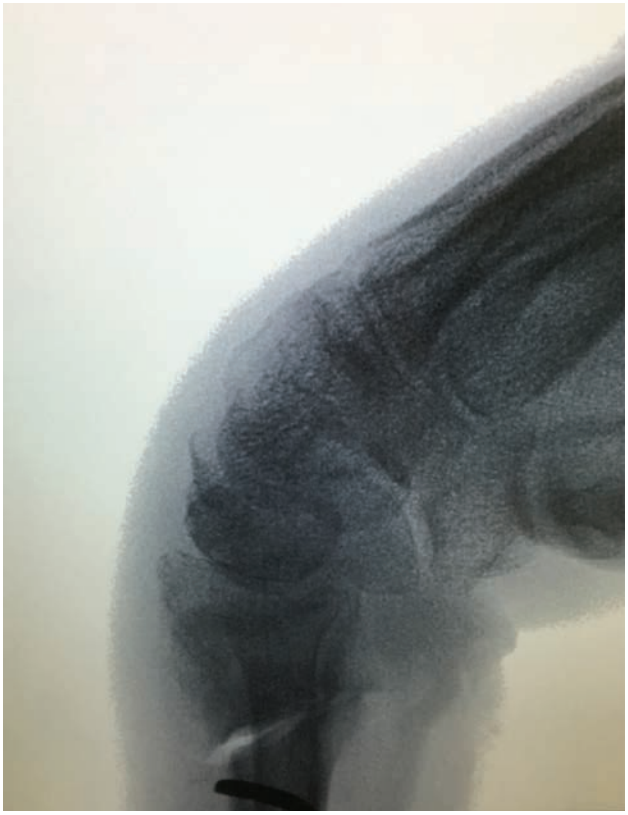

Figure 3. Hyperflexion of the wrist during initial attempted reduction of the fracture only produced neutral volar tilt.

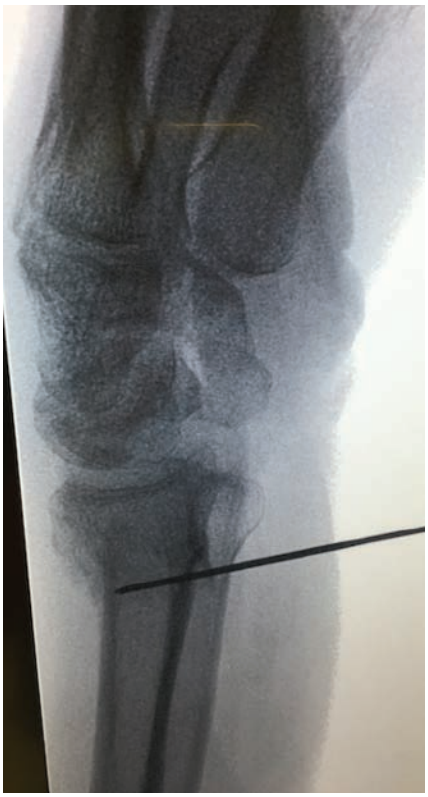

Figure 2. Guidewire placed to assess location for osteotomy.

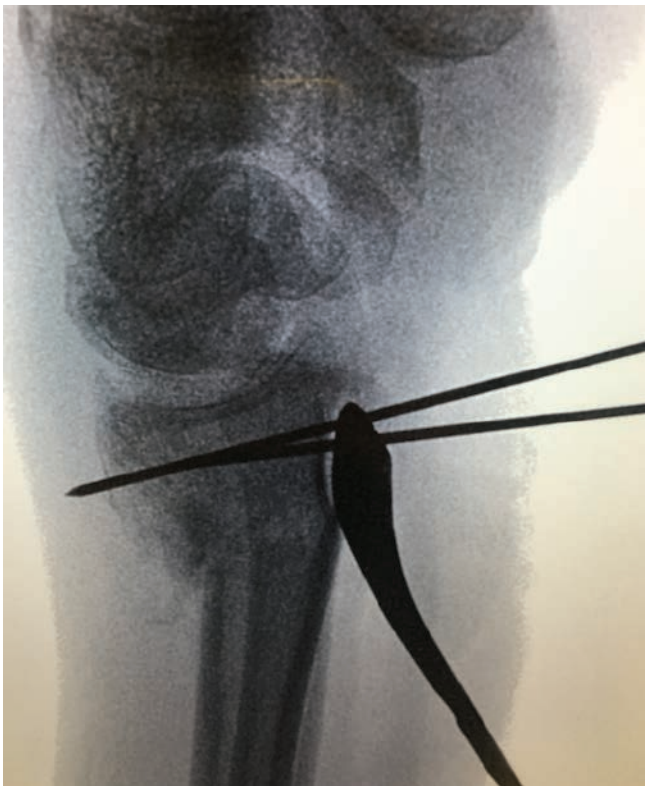

Figure 4.

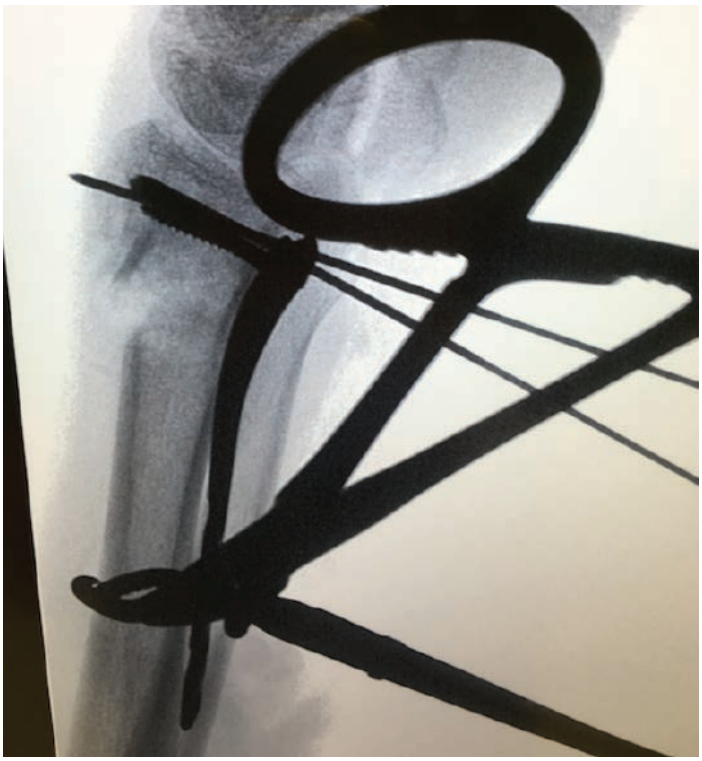

Figure 5. Lobster claw used to reduce the proximal aspect of the plate to the radial shaft. Volar tilt is restored.

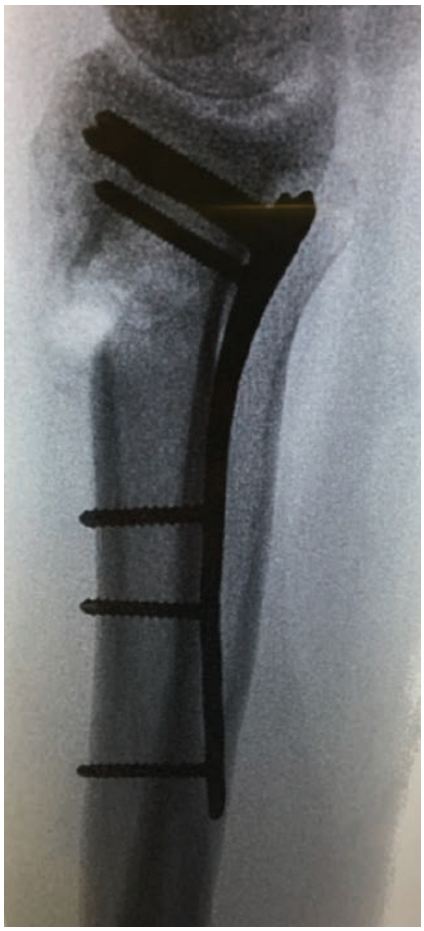

Figure 6. Volar tilt is restored, articular surface is reduced, plate affixed to bone.

filled with a non-locking screw. The fracture was then further reduced to the plate to ensure appropriate alignment of articular components, and distal locking screws were placed subchondrally to support the articular surface. Additional screws were also placed in the shaft of the plate. (Figure 6) The defect from the osteotomy was then filled with allograft.

Postoperative Protocol

Immediately following surgery, the patient was placed into a short arm cast, allowed to start digit range of motion exercises, and was seen 1 week postoperatively for cast change. Two weeks postoperatively, the patient was given a 2 pound lifting restriction, sutures were removed, and the cast was changed. 6 weeks postoperatively, the patient was seen in clinic, cast was removed, and he was placed into a cock up wrist splint. Patient will remain nonweightbearing until the osteotomy site shows full healing on imaging.

Discussion

Restoration of volar tilt has been shown to restore biomechanical function of the wrist. Although this has not been shown to lead to better long term outcomes,[6,7] anecdotally we believe that restoration of near anatomical alignment can only lead to better outcomes. This paper discusses a technique used to restore volar tilt using distal fixation of the locking plate to leaver the reduction. This is a technique that can be used to achieve reduction when an osteotomy is performed on a malunion, as well as in hyperextension type distal radius fractures.

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U·P·O·J

Treatment of Hip Flexion Contracture with Psoas Lengthening through the Middle Window of the Ilioinguinal Approach

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Abstract

Hip flexion contractures may affect both children and adults with neuromuscular disorders. The iliopsoas is the major deforming force. Iliopsoas lengthening can be accomplished over the pelvic brim to treat hip flexion contractures. The traditional interval for this procedure occurs between the iliopsoas and sartorius. This technique however, puts the femoral nerve at risk through indirect exposure. The ilioinguinal approach is a well established technique that accesses the iliopsoas tendon and also allows for direct visualization of the femoral neurovascular structures. The purpose of this article is to describe a technique to lengthen the iliopsoas tendon using the middle window of the ilioinguinal approach in order to protect the femoral nerve. Iliopsoas lengthening may be safely performed through this approach.

Introduction

Hip flexion contracture is a common problem in patients with spastic paresis such as cerebral palsy (CP) and patients with trauma about the hip. These contractures may lead to impairment in gait and activities of daily living. The iliopsoas muscle is the main deforming force in these patients[1]. One effective treatment for hip flexion contractures is intramuscular psoas lengthening over the pelvic brim.[2-7]

Traditionally, intramuscular psoas lengthening is approached through the intermuscular interval between the iliopsoas and sartorius. First, an oblique incision is made along the inguinal ligament from the anterior iliac spine extending distal and medial. The external oblique fascia is identified and divided. The internal oblique and transversus abdominis are bluntly dissected to access the ilium. The iliacus and psoas are then identified medially and the tendon is divided from lateral to medial. The femoral nerve is contained within the same fascial compartment as the iliacus and psoas muscle and tendon. Medial to the iliopsoas lies the femoral neurovascular bundle in a distinct fascial compartment. Prior anatomic studies in children show that the distance between the neurovascular bundle and tendon is as close as 4mm. Internally rotating and flexing the hip can help differentiate the psoas tendon from the femoral nerve.[8] Indirect exposure in this technique puts the femoral neurovascular bundle at risk of iatrogenic injury.

The ilioinguinal approach has been previously described to perform intramuscular psoas lengthening for the treatment of snapping hip with good results.[9] The purpose of this article is to describe the use of the middle window of the ilioinguinal approach to perform intramuscular psoas lengthening for the treatment of hip contractures.

Surgical Technique

The procedure is conducted under general anesthesia. Preoperatively, a Thomas test is performed and hip range of motion is examined. Preoperative antibiotics are administered, typically a 1st generation cephalosporin. The patient is placed in a supine position on a regular table with a bump under the ipsilateral hip. The patient's hip is then prepped and draped in typical sterile fashion (Figure 1).

Bony landmarks include the iliac wing, anterior superior iliac spine, and pubic tubercle. The femoral artery is palpated and marked on the skin as an additional landmark. A 5-6cm incision is made medial to the anterior superior iliac spine along the inguinal crease through subcutaneous fat. The external oblique aponeurosis is incised parallel to the skin incision cranial to the inguinal ligament to expose the inguinal ring. The ilioinguinal nerve is identified and protected. The spermatic cord in men or round ligament in women is retracted to the medial aspect of the wound with a vessel loop. The transversus abdominis is released with a 1-2mm cuff of the inguinal ligament to free the muscular attachments from the inguinal

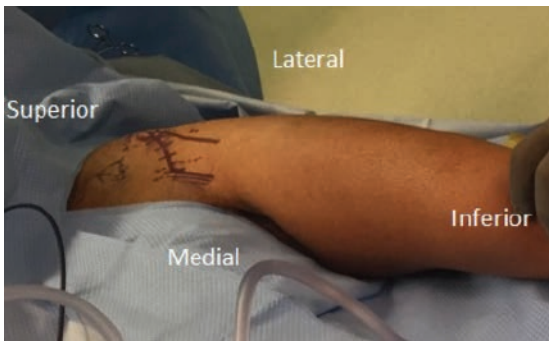

Figure 1. Iliac wing, anterior superior iliac spine, pubic tubercle and inguinal ligament marked.

ligament. This release begins at the anterior superior iliac spine and progresses medially to the conjoint tendon of the internal oblique and the pubic tubercle. The lateral femoral cutaneous nerve is encountered just deep to the conjoint tendon approximately 1-2cm medial to the anterior superior iliac spine. At this point, the iliopsoas muscle is exposed in the lateral portion of the wound with the femoral nerve lying on its anteromedial surface. The femoral artery and vein may be palpated medial to the iliopectineal fascia. The iliopectineal fascia is left intact to protect the vessels. The femoral nerve is dissected free from the iliopsoas and protected. The iliopsoas tendon may be lifted off the pelvic brim and transected safely with the hip in flexion (Figure 2). Hip range of motion is then reassessed. Note that the muscle is uninjured by this procedure and the insertion on to the lesser trochanter is undisturbed. The wound is then copiously irrigated and closed in a layered fashion.

Postoperatively, the patient is allowed to weight bear as tolerated. Perioperative antibiotics are continued for 24 hours after surgery. Aspirin is administered twice a day for six weeks for deep vein thrombosis prophylaxis. Physical therapy is initiated immediately after surgery. Therapy includes stretching of the iliopsoas tendon and progressive concentric resistive exercises of all the muscles around the hip.

Discussion

Hip flexion contracture is a debilitating condition that affects many patients with spastic paresis and prior hip trauma. Intramuscular psoas lengthening over the pelvic brim has been a well established technique to treat this condition.[2-7] The traditional approach for psoas lengthening has been described using the interval between iliopsoas and sartorius. This technique however, does not provide direct visualization and protection of the surrounding neurovascular structures such as the femoral nerve, artery and vein. The ilioinguinal approach has been previously described to perform intramuscular psoas lengthening for the treatment of snapping hip with good results.[9] This article is the first to describe the ilioinguinal approach for intramuscular psoas lengthening for the treatment of hip flexion contracture.

The ilioinguinal approach allows the surgeon to directly identify the femoral nerve in the same fascial compartment as the psoas. It also allows for direct visualization and protection of the femoral artery and vein. This technique is particularly important when treating patients that are able to ambulate in order to protect their function. In our experience all patients who have undergone this procedure have had improvements in their hip range of motion post operatively.

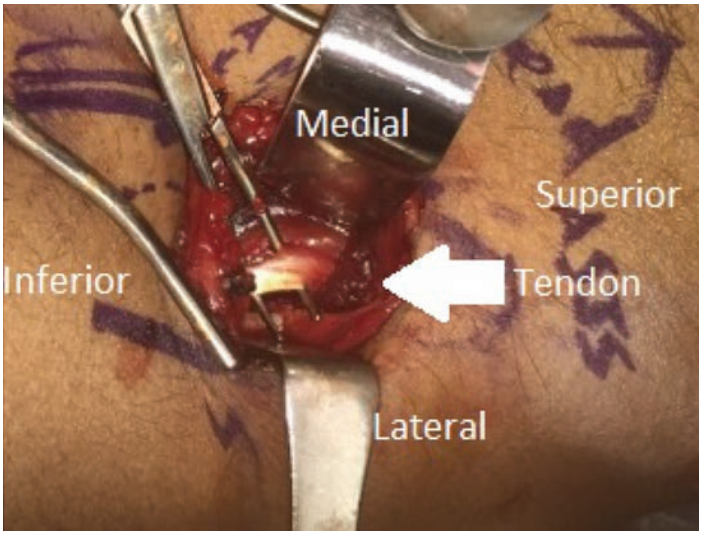

Figure 2.Psoas tendon identified with surrounding neurovascular structures protected.

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Pediatric Shoulder Instability: Current Trends in Management

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Introduction

Pediatric shoulder instability commonly results from traumatic anterior dislocation of the humeral head.[1,2] Male adolescents aged 15-17 years participating in contact or collision sports have the highest risk of primary and recurrent dislocation.[3] In contrast to adolescents, children younger than 10 years seldom develop shoulder instability.[4] Adolescents are generally at a higher risk than their younger counterparts due to the dramatic increase in collision sports participation that occurs when children begin middle school at the onset of adolescence. Depending on the severity of instability and the patient's activity level, patients can range from being relatively asymptomatic to being unable to participate in sports or even engage in regular activities of daily living. Children and adolescents are at increased risk of developing recurrent shoulder instability as they are typically eager to return to sport and less likely to adhere to an appropriate course of physical therapy.[5,6] Limiting recurrences decreases the risk of future negative sequelae such as traumatic labral and cartilage injury.[7] Since non-operative treatments often fail in this highly active population, a number of surgical techniques are used to address specific defects of soft and bony tissue. In this article, we discuss our approach to shoulder instability in the high-risk pediatric patient.

Operative vs. Non-Operative Management

For the orthopaedic surgeon, the decision to surgically address shoulder instability in the child or adolescent ultimately depends on the rate of recurrence and the extent to which shoulder instability sufficiently impairs quality of life. Non-operative strategies generally rely on avoidance of strenuous shoulder activity, bracing and physical therapy to increase stability of the glenohumeral joint. These strategies have a particularly high failure rate in young athletes who often remain highly engaged in sports in spite of shoulder injury.[5] The reported rate of recurrence after primary dislocation in patients younger than 25 years of age is 40-95%; the greatest risk factor being age of primary dislocation under 20 years of age.[8,9,10,11] The risk of recurrent instability correlates inversely with the age at first dislocation, however, preadolescent children have been found to have good or excellent results with low recurrence rates after conservative treatment.[12,13]

Although some of the most competitive adolescent athletes will undergo surgical repair after primary dislocation, pressure to finish a season, as well as peer and scholarship pressures can influence the decision to continue participating in sports despite recurrences of shoulder instability. The clinician should bear these influences in mind when recommending a particular treatment, as the risk of recurrent instability when returning to play untreated is significant.

Several studies highlight the inefficacy of the non-operative approach in the adolescent population. In a retrospective cohort study of 65 pediatric patients aged 15-18 years, 19/27 (70%) of patients managed non-operatively developed recurrent instability, while only 5/38 (13%) of those treated arthroscopically developed recurrent instability.[14] A previously published review by the senior author of 32 patients with Bankart lesions aged 11-18 years followed over an average of 25.2 months sought to determine the potential benefit of arthroscopic repair following primary dislocation. The study compared 16 patients with Bankart lesions undergoing arthroscopic repair after primary dislocation to 16 patients undergoing arthroscopic repair after an average of 10.5 months of non-operative management. The authors concluded immediate Bankart repair limits multiple recurring shoulder dislocations that hinder quality of life and potentially lead to future negative sequelae.[15] Similar conclusions have been drawn when comparing the efficacy of non-operative treatment to the Latarjet procedure among skeletally immature patients. Khan et al retrospectively compared 23 non-operative patients with 26 patients undergoing the Latarjet procedure and found no significant differences between groups regarding functional scores and pain levels yet 92% of the post-surgical group returned to the same level of pre-injury activity compared to only 52% of the non-operative group.[16]

Non-operative management (physical therapy or activity modification) is most appropriate for a younger child with low activity demands and a single dislocation of the non-dominant shoulder with no symptoms. Operative intervention

is most appropriate for a high activity demand adolescent collision sport athlete with recurrent instability and evidence of damage to soft tissues, bony structures or both on MRI.

Operative Techniques for Restoring Shoulder Stability in the Pediatric Patient

The goals of operative treatment are to restore shoulder stability in an attempt to decrease the rate of dislocation and the risk of future sequelae such as axillary nerve damage, post-traumatic arthritis and glenoid bone loss. For an athlete with functional impairment of the unstable shoulder, the potential to restore quality of life is immense. Multiple techniques for operative management of shoulder instability have been reported by various authors.[24,25] In addition to patient lifestyle and wishes, the specific type of technique used is a multifactorial consideration of the severity of recurrent instability, the extent of glenoid labrum avulsion, the extent of capsular stretch as well as pre-existing laxity, the presence and severity of a Hill-Sachs deformity, and the extent of glenoid bone loss.

Open vs. Arthroscopic Repair Of Glenoid Labrum Tears

Arthroscopic repair of soft-tissue damage (glenoid labrum avulsion, SLAP tears, capsular laxity, rotator cuff tendinopathy, etc.) has been the mainstay of shoulder instability treatment for over two decades. While open techniques have traditionally been considered the gold standard repair, recent studies have found they result in similar rates of recurrent instability to arthroscopic techniques but have significantly longer recovery time.[17,18] A retrospective review of 99 children with Bankart lesions compared 28 children undergoing open repair with 71 children undergoing arthroscopic repair found no significant difference in redislocation rates (21%) or functional outcomes.[18] Open procedures remain a viable alternative in cases of severe glenoid fracture or subscapularis tendon avulsion fracture.[19]

It is crucial to address any and all bony defects contributing to instability. Studies suggest both open and arthroscopic repairs of isolated soft tissue defects are likely to result in recurrent shoulder instability if bony defects, such as glenoid bone loss or engaging Hill-Sachs lesions, are not addressed. A study found that for both open and arthroscopic techniques, the adolescent shoulder undergoing Bankart repair had a two-year survival rate of 86% and a five-year survival rate of 49%.[6] A prospective study found 15% of 131 children and adults followed a minimum of two years after arthroscopic Bankart repair developed recurrent anterior shoulder instability. This relatively high failure rate was mostly attributed to several risk factors: age of primary dislocation less than 20 years, involvement in competitive/contact sports or those with overhead activity, shoulder hyperlaxity, a Hill-Sachs lesion (HSL) present on AP radiograph of the shoulder in external rotation, and loss of sclerotic inferior glenoid contour.[20] While this study only addressed the arthroscopic failure rate, it highlights the importance of addressing bony defects. HSLs and glenoid bone loss in particular are common causes of instability that are typically repaired arthroscopically and open, respectively. While arthroscopic soft-tissue repair is acceptable for the vast majority of children and adolescents with soft-tissue lesions, those with significant bony defects likely require additional procedures best performed either open or arthroscopically. The severity of both soft-tissue and bony defects must be considered together before the decision to proceed with open or arthroscopic repair is made.

Addressing Hill-Sachs Lesions: The Remplissage Technique

HSLs are posterolateral humeral head compression fractures that typically result secondary to anterior dislocations of the shoulder, whereby the posterolateral aspect of the soft, cancellous humeral head is compressed against the anteroinferior aspect of the dense, cortical glenoid. The resulting impression left in the humeral head can then engage on the anterior glenoid rim during abduction and external rotation, causing shoulder dislocation. The presence of HSLs is critical to determine as they can be key causes of recurrent instability and can worsen in severity (depth and width) with each dislocation.

The location, diameter and depth of HSLs vary depending on the type and number of traumas sustained. The most clinically relevant consideration is simply whether HSLs engage on the anterior glenoid rim. In this consideration, the concept of the glenoid track has become an important new paradigm.[22] To engage on the anterior glenoid rim and cause shoulder dislocation, an HSL must be "off track," i.e. it extends over the anterior margin of the glenoid and engages the glenoid rim. Conversely, a lesion is "on-track" and non-engaging if it lies completely within the glenoid track (Figure 1). This concept of glenoid track was developed as a means to quantitatively

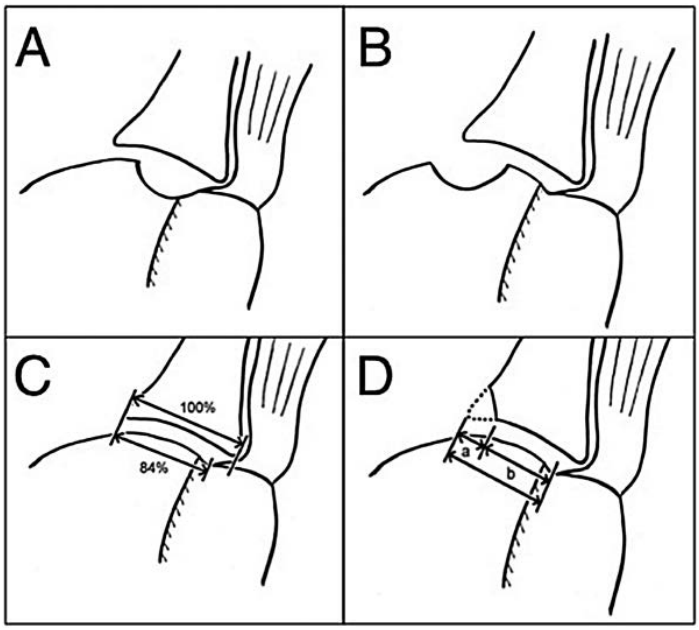

Figure 1. A: On-track, non-engaging Hill-Sachs lesion. B: Off-track, engaging Hill-Sachs Lesion. C: Full glenoid width maximizes chance of Hill-Sachs lesion being on-track. D: Glenoid bone loss increases the likelihood of engagement as Hill-Sachs lesions more likely to be off-track.[22]

assess the Hill-Sachs lesion in relation to the size of anterior bone loss, then integrate that quantification into a treatment algorithm.[21,22] Standard stabilization techniques such as Bankart repair are unlikely to restore shoulder stability when large, engaging, "on-track" HSLs are present.[21,22] A study found 21/194 (10.8%) of children who underwent Bankart repair developed recurrent instability. Of those 21 failures, 67% were found to have engaging HSLs, while children with no bony defects of the shoulder developed recurrent instability only 4% of the time. Inverted pear configuration of the glenoid, as well as engaging HSLs were deemed contraindications to arthroscopic Bankart repair.[23]

From a management standpoint, shoulder arthrograms can help assess the size and depth of HSLs as well as their relationship to the glenoid track.[24] By measuring the widths of the glenoid track and the Hill-Sachs lesion, one can then compare these to classify the lesion as "on-track" or "off-track." For the difficult subgroup of instability patients with high potential for failure after a standard arthroscopic Bankart repair, HSLs are surgically addressed via the remplissage technique, an arthroscopic capsulotenodesis of the posterior capsule and infraspinatus tendon to fill the HSL. Remplissage, which in French means "fill in," effectively makes the Hill-Sachs defect extra-articular, preventing it from engaging the glenoid, and ultimately improving stability. Although this technique has only recently become popular, research has shown that it is effective for addressing HSLs. In a study evaluating the efficacy of the technique, only 2/24 patients treated with the Remplissage technique had recurrent instability, both of which occurred after significant trauma. The procedure produced no significant loss of external rotation.[25] A systematic literature review by Buza et al to evaluate the outcomes of arthroscopic Hill-Sachs Remplissage showed similar results, as only 9/167 shoulders (5%) experienced episodes of recurrent glenohumeral instability. These rates of instability were comparable to patients without HSLs.[26]

For the vast majority of children and adolescents with engaging HSLs sustained from recurrent anterior dislocations and no significant other bone defects, the arthroscopic remplissage technique achieves excellent results. However, in the rare event that an HSL reaches a critical size, the Remplissage technique is unlikely to be effective and other humeral head resurfacing approaches must be considered.

Addressing Glenoid Bone Loss: The Bristow-Latarjet Procedure

The significance of glenoid bone loss must be considered since the combined surgical approach of arthroscopic Bankart repair and Hill-Sachs Remplissage are successful when there is an intact glenoid or minimum bone loss. In those rare cases where glenoid bone loss is significant, a more robust approach is required. Given the long-term instability associated with failure to address bony deficits of the glenoid, there has been renewed interest in the Bristow-Latarjet procedure. First described in 1954, the Bristow-Latarjet procedure restores congruity of the shoulder joint using the coracoid process as an augmentation of the anteroinferior glenoid rim (Figure 2).[27] The procedure is recommended in cases where glenoid bony defect is >25% of glenoid width of if risk of instability is higher, as it is with collision-sport athletes.[28] An instability severity index score has been developed to guide surgical decision making for patients with recurrent instability.[29]

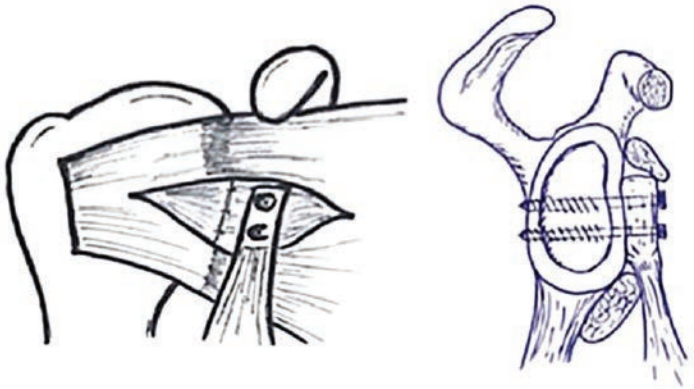

Figure 2. Transfer of the coracoid process to the anterior glenoid with two-screw fixation restores the congruity of the glenoid, stabilizing the glenohumeral joint. The downward displacement of the inferior belly of the transected subscapularis muscle is also thought to provide a stabilizing "sling effect" to the humeral head.[32]

Several studies have demonstrated that the Bristow-Latarjet procedure is highly effective for recurrent stability secondary to significant glenoid bone loss. In a prospective study of 79 patients with recurrent anterior instability and bone loss of more than 20% of the glenoid, 98% of patients had stable shoulders and 83% returned to sports at preinjury level.[27] A retrospective study of 63 shoulders undergoing the Bristow-Latarjet procedure found only 1/63 (1.6%) developed recurrent instability at 5-year follow-up.[30] Another study found a 5% dislocation rate at 20-year follow-up.[31] While the dislocation rate is lower (0-8% rate), the procedure is associated with a higher incidence of complications, such as screw breakage, nonunion, and stiffness.[32] The Bristow-Latarjet procedure remains one of the best surgical techniques to address significant glenoid bone loss.

Treatment Algorithm

Determinants of treatment options include symptoms of recurrent instability and laxity, associated pathology such as soft-tissue and bony defects, and adherence by the patient and family. The case vignettes shown in Figure 3 highlight key management strategies. Primary dislocators with low activity demands who lack symptoms of recurrent instability may be managed conservatively. For athletes with high activity demands and recurrent instability, operative intervention is recommended. Arthroscopic soft-tissue repairs suffice in cases where bony defects are minimal to non-existent, but large, engaging Hill-Sachs lesions or significant glenoid bone loss (> 25% of glenoid width) require alternate approaches.

Conclusion

Shoulder instability is an increasingly common problem in the pediatric population as participation in youth sports continues to rise. Since a relatively high activity level predisposes one to recurrent instability, non-operative

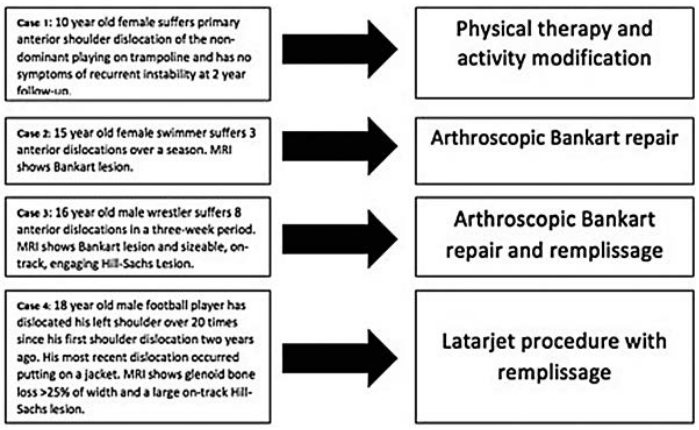

Figure 3. Case vignettes highlighting key scenarios in the management of shoulder stability in children and adolescents.

treatment has a higher risk of failure among pediatric patients. In addition to patient activity level and potential collegiate athletic aspirations, the management approach requires a multifactorial consideration of the severity of recurrent instability, patient-specific pathoanatomy, and recovery time. While several surgical techniques exist to restore shoulder stability, pediatric patients' pathological and functional risk factors can help guide a surgeon's decision.

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Prone Positioning for Open Reduction Internal Fixation of Pediatric Medial Epicondyle Fractures

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Introduction

Medial epicondyle fractures account for up to 20% of fractures about the elbow in children and adolescents.[1] The medial epicondyle serves as the anatomic origin of the flexor-pronator mass, which provides dynamic stability to valgus stress of the elbow.[2] It also serves as the proximal attachment site to the ulnar collateral ligament, which serves as the primary static stabilizer to valgus stress.[3] Operative indications remain unclear as displacement is not always easily established on plain radiographs and even athletes can have good outcomes treated with and without surgery.[4, 5] When operative treatment is required, reduction of the fracture fragment can often be challenging with standard supine positioning because the forces required to expose the medial elbow in this position tend to dislocate the elbow and pull the fracture fragment away from the fracture bed. Here, we present a case of a displaced medial epicondyle fracture in an adolescent athlete and offer an alternative technique utilizing the prone position for operative treatment.

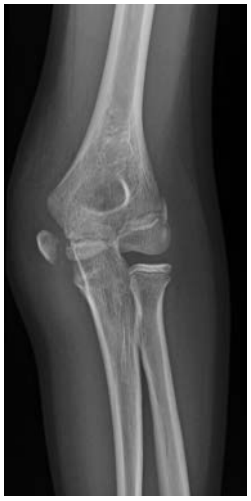

Figure 1. Radiographs showing a displaced medial epicondyle fracture.

Background

The patient is an 11-year old male who fell onto an outstretched left upper extremity during a wrestling match and radiographs revealed a displaced medial epicondyle fracture (Figure 1). Open reduction and internal fixation of the medial epicondyle fracture was recommended based on the degree of displacement and the elbow stability.

Procedure

Positioning and Exposure

The patient is placed prone on the operative table with gel rolls placed at the level of the sternum and ASIS (Figure 2). The abdomen is sufficiently free and a radiolucent hand table is used. The shoulder is fully internally rotated and the dorsal aspect of the wrist placed on the hand table. Occasionally, especially in overhead athletes, the patients lack sufficient shoulder internal rotation to be placed in this position. If this is the case, additional bolsters can be placed under the chest to elevate the shoulder or a sloppy lateral position can be used. The

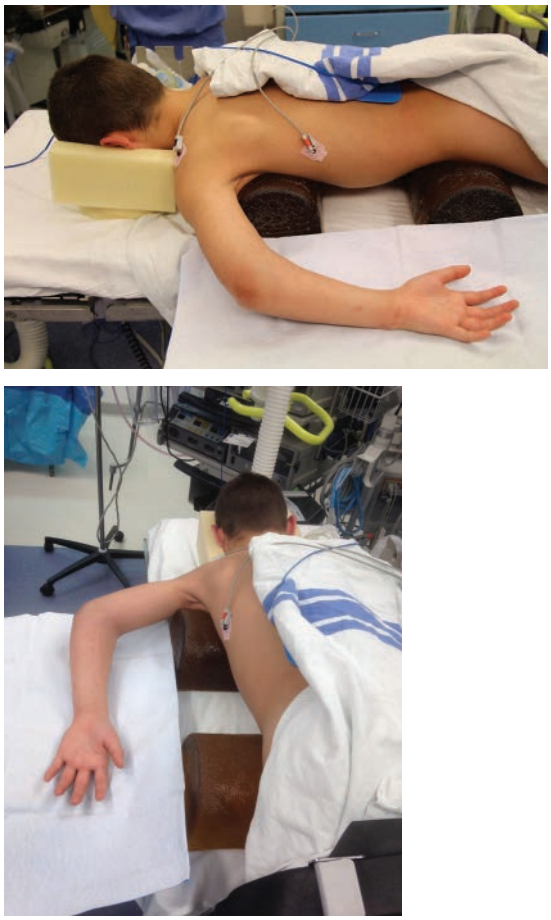

Figure 2. Positioning on the operative table with the patient placed prone and gel rolls placed at the level of the sternum and ASIS.

fluoroscopy unit is positioned, either coming from the head of the bed (if there is no assistant) or from the direction of the hand table (which allows assistant positioning directly opposite to the surgeon). A longitudinal incision is made, centered either over or just anterior to the medial epicondyle. Dissection is carried through the skin and subcutaneous tissue. Care should be taken to identify and protect crossing sensory nerves in this region. In the acute setting, there is usually significant soft tissue and capsular disruption that gentle blunt dissection will often lead directly to the fracture bed. Next, the ulnar nerve is identified posterior to the fracture bed. A formal neurolysis or transposition is not performed unless there are extenuating circumstances such as ulnar nerve subluxation. The nerve is protected during the entire case. The medial epicondyle fracture fragment is then identified. It is often displaced anteriorly and distally, in line with the pull of the attached flexor-pronator mass. The fracture fragment is grasped with a towel clip or pointed reduction clamp taking care not to fragment the piece. A suture can be also placed at the attachment point of the flexor-pronator mass to help facilitate control of the fracture fragment. In older patients, the authors debride any remaining apophyseal cartilage on both the fracture bed and the undersurface of the medial epicondyle. This aids assessment of bony union during subsequent follow-up radiographs and helps guide rehabilitation and return to activity recommendations.

Reduction and Fixation

Varus and internal rotation forces at the elbow facilitate reduction of the elbow joint. Wrist flexion and forearm pronation relax the flexor-pronator mass and facilitate fracture reduction. With the patient in the prone position and the shoulder internally rotated, the upper extremity has a natural tendency to lie with a varus/internal rotation force on the elbow. Pronating the forearm in this position by placing the dorsal wrist on the table causes the wrist to flex (Figure 3). It is the authors' experience that the medial epicondyle fracture fragment is almost always easily reduced in this position because the joint remains stable and reduced and there is little muscular resistance from the attached muscular origins. Once reduced, the medial epicondyle can be held with a pointed reduction clamp, towel clip, K-wires, reduction sutures or a combination thereof. The authors prefer to fix the fracture with a single 4.5 mm partially threaded cannulated screw but a variety of fixation options are appropriate. A washer can be used to enhance compression and reduce the risk of fracture fragmentation and is usually used if there is comminution or if a significant portion of the fracture fragment is cartilaginous. Prior to drilling for the screw, another K-wire can be placed into the fragment (being careful not to worsen any comminution) in order to resist rotation. Because the medial epicondyle lies just posterior to the mid-sagittal plane of the distal humerus, screws placed perpendicular to the fracture line usually have a slightly posterior to anterior trajectory. Prone positioning also facilitates drilling as the surgeon views the medial side of the elbow directly and can drill in a "downhill" trajectory. The screw head often appears prominent on radiographs, however,

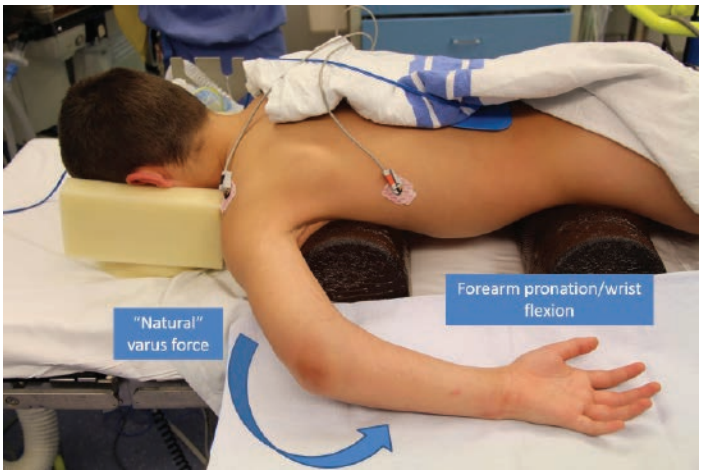

Figure 3. Placing the patient in the prone position with the shoulder internally rotatedcauses the upper extremity to liewith a varus/internal rotation force on the elbowwhileplacement of the patient's dorsal wrist on the table pronates the forearm and causesthe wrist to flex.

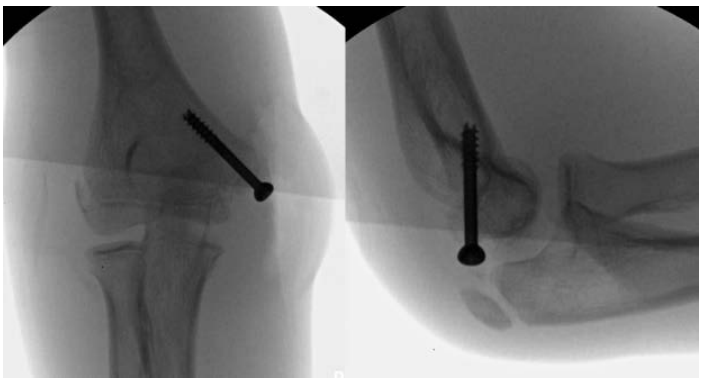

Figure 4. Use of fluoroscopy to confirm reduction and fixation.

this is accounted for by the cartilaginous nature of the medial epicondyle as well as the overlying flexor-pronator mass soft tissue. Compression and fragment congruity usually confers rotational stability but this can be reinforced by performing a periosteal repair. Tying the reduction stitch placed in the flexor-pronator mass around the screw can also help with construct stability. Fluoroscopy is used to confirm reduction and fixation and the wound is closed in a routine manner and the patient is placed in a posterior splint (Figure 4).

Post-operatively, a short period of immobilization is used to protect the surgical wound followed by early elbow, forearm, and wrist range of motion. The patient is progressed through rehabilitation and is allowed to gradually return to play once pain, range of motion, and strength have returned to baseline and bony union is evident on radiographs. This typically ranges from 4-6 months after treatment.

Conclusion

In contrast to supine positioning, where the maneuvers required to visualize the fracture bed tend to place more tension on the fracture fragments and subluxate the elbow

joint, prone positioning for open reduction and internal fixation of medial epicondyle fractures facilitates fracture reduction and fixation because it counteracts the deforming forces at the elbow and on the medial epicondyle fracture fragment. While there may be additional time required to position the patient for this procedure, the ease with which fracture exposure, reduction and fixation can be achieved often offsets this additional setup time. The authors propose this surgical technique as an option for treating this injury.

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New Approach to Characterize Juvenile Osteochondritis Dissecans

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Background

Juvenile osteochondritis dissecans (JOCD) is an idiopathic condition affecting the subchondral bone and the articular cartilage in children with open growth plates. If left untreated, patients with OCD lesions can develop early onset osteoarthritis.[1,2] The incidence of OCD ranges from 18-30 cases per 100,000 in the population; however, over the past decade this number has increased particularly in young athletes due to rising awareness, more frequent use of advanced imaging, and competitive sports participation at an earlier age.[3–5] Current non-surgical treatment, including activity restriction and/or immobilization for 6 to 18 months, has been reported to be effective in only 50-67% of children with stable OCD.[3] Currently no systematic approach exists to identify those patients who can benefit from non-surgical treatment. Thus, non-operative approaches may fail after up to 18 months of activity restriction resulting in an unstable lesion that requires surgical intervention.[6–10] Since the underlying pathomechanism of this condition is unknown, effective and early intervention for successful treatment is difficult. The American Academy of Orthopedic Surgeons published a guideline to improve the diagnosis and treatment of OCD of the knee in immature and young mature patients; however,due to the nature,unknown mechanism, multifactorial etiology of the condition, and treatment variability among practicing surgeons, most of these suggestions were inconclusive.[11–13] This suggests a compelling need to further investigate the possible risk factors associated with development of OCD in young patients.

Over 50% of studies that investigated the etiology of this condition suggested trauma or repetitive movement associated with increased stress in the dominant knee.[13] However, no studies have considered if specific 3D femoro-tibia alignment and 3D range of motion are associated with development of OCD in athletes. A previously published study looked at the association between medial condyle OCD and varus axis and lateral condyle OCD and valgus axis using AP X-ray images.[14] Although this study strongly suggested an association between femoro-tibial alignment and the development of an OCD lesion, only varus/valgus 2D angles were included and 3D alignmentof the femur and tibia were not considered. Despite the years of clinical evaluation of OCD and the collective knowledge on the etiology, diagnosis, and treatment of this condition, a 3D biomechanical analysis associating 3D skeletal parameters to abnormal retropatellar or tibial spine loading and development of OCD in adolescents and young adults has not yet been performed.[13]

The emerging technology of low-dose stereoradiography system can be used to acquire biplanar X-rays and generate 3D reconstruction of the bones in an upright position.The weight-bearing position allows for analysis of the 3D alignment of the bones as patients stand. We aimed to explore the applicability of this new technology in exploring the relationship between the geometrical parameters of the lower extremities and the location of the unilateral or bilateral lesion of the knee in juvenile OCD.

The objective of this study was to characterize the 3D femoro-tibial geometrical parameters using sterEOS 2D/3D X-ray post-processing platform in a group of adolescents with stable or unstable unilateral or bilateral OCD of the knee. We hypothesized that femoral mechanical axis alignment and femoro-tibial torsion are significantly associated with the location of the OCD lesion.

Methods

Subjects

A total of three patients who presented with unilateral or bilateral OCD of the knee on their MRI were recruited for this pilot analysis. The exclusion criteria were previous surgical intervention on the knee or hip and any other neuromuscular condition. Patients had to be able to stand for 30 seconds without any external support. Patient ages ranged from 8 to 18 years. One asymptomatic age, sex-matched control was included. This pilot analysis was approved by the Institutional Review Board at the Children's Hospital of Philadelphia and consent was obtained from all participating subjects.

Clinical data collection and 3D imaging

Patients' charts and MRI were consulted to determine the location of the OCD lesion. Full biplanar X-ray images (AP and lateral) of

the lower extremities, with the pelvis included in weight-bearing standing position, were taken by the EOS stereo-radiography imaging system (EOS imaging, Paris, France). 3D reconstruction of the lower extremities was generated in SterEOS2D/3D, a validated and FDA-approved software for 3D reconstruction of the spine and lower extremities images (Figure 1A). The center of the femoral heads was determined by fitting a circle to the femoral heads in AP and lateral X-rays. Femoral and tibia condyle notches were digitized manually. A tangent line to femoral and tibial condyles was digitally traced to calculate the femur (FMA) and tibia mechanical angles (TMA) (Figure 1B). A total number of 21 2D /3D alignment and morphological parameters of the lower extremities and pelvis were measured in the cohort, including pelvic incidence, sacral slope, sagittal pelvic tilt, lateral pelvic tilt, pelvic rotation, femoral heads diameter, femoral offset, neck shaft angle, neck length, mechanical femoro-tibial angle (MFT), valgus/varus angles, flexion/extension angles, femur length, tibia length, mechanical and anatomical axes lengths, valgus/varus angles, knee flexion/extension, femoral and tibial mechanical angle, HSK angle, and axial plane parameters (tibial and femoral torsion, and femoro-tibial rotation).

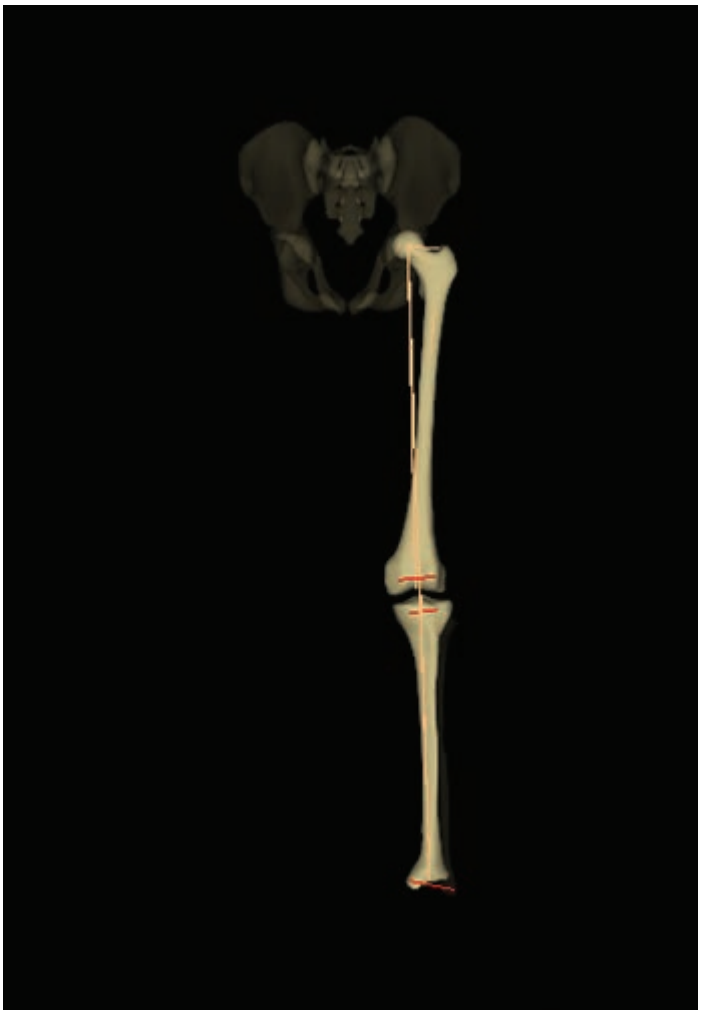

Figure 1A. EOS 3D reconstruction of a case patient's lower extremity.

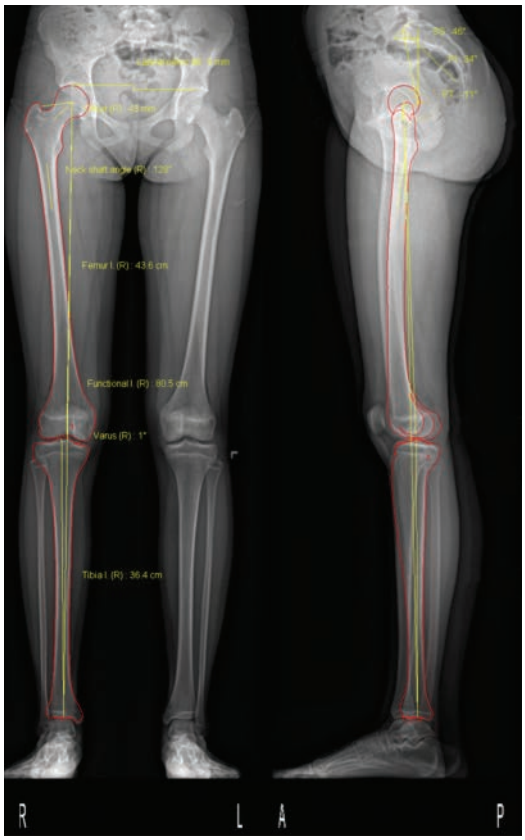

Figure 1B. Alignment and morphological parameters of the lower extremities in both AP and lateral views.

Results

One patient had left knee OCD while the other two had a lesion in their right knee. Patients' clinical data, including the demographics and the location of the lesions, are summarized in Table 1 for the three patients. The 2D and 3D measurements of the legs for the patients and control are summarized in Table 2. Higher femorotibial rotation was observed in OCD patients (10.7 versus 2.8 degrees).

Discussion

OCD is a rare disease with an unknown pathogenesis. Although multiple factors have been associated with OCD development in adolescents, the impact of the 3D alignment of the femur and tibia on the mechanical loading of the knee and development of OCD has not been investigated. We evaluated the clinical application of stereoradiography of the lower extremities in clinical assessment of OCD of the knee.

The association between varus/valgus knee angles measured on 2D X-rays and the OCD lesion has been investigated before; however, the relationship between 3D alignment of the femur and tibia, femoro-tibia torsion, and the OCD lesion has not previously been investigated.[14] Our results suggest SterEOS 2D/3D software can accurately produce the 3D geometry of the bone using EOS x-ray images.[15,16] Understanding the 3D alignment of the femur and tibia and its impact on the transferred force between the two bones is essential in

Table 1. Demographics and clinical data of case patients.

	Case 1	Case 2	Case 3
Age	9	14	14
Gender	Female	Female	Male
Laterality of OCD	Left	Right	Right
Location of lesion	Medial femoral condyle (lateral aspect)	Medial femoral condyle (lateral aspect)	Lateral femoral condyle
Treatment type	Non-surgical	Surgical	Surgical
History of OCD	No	Yes (Left)	No

Table 2. 2D and 3D leg measurements for the case patients and age-matched control.

Femur		**Case 1**	**Case 2**	**Case 3**	**Control 1**
Femoral head diameter (mm)	3D	29	44	47	34
	2D	--	--	--	--
Femoral offset (mm)	3D	31	43	34	33
	2D	--	--	--	--
Neck length (mm)	3D	38	54	51	40
	2D	39	53	44	43
Neck shaft angle (deg)	3D	127.5	127.5	139.6	125.7
	2D	125.1	128.2	137.8	122.7
Lengths					
Femur length (cm)	3D	31.9	43.6	41.0	31.8
	2D	31.9	43.5	38.7	31.6
Tibia length (cm)	3D	26.5	36.4	34.6	26.5
	2D	26.3	36.4	34.3	26.2
Functional length (cm)	3D	59.1	80.5	75.6	59.1
	2D	58.9	80.5	73.4	58.4
Anatomical length (cm)	3D	58.5	79.9	75.6	58.3
	2D	58.2	79.9	73.0	57.8
Knee					
Valgus/Varus (deg)	3D	0.4	-1.2	2.8	0.6
	2D	0.9	-1.3	3.6	0.3
Knee flexion/knee extension	3D	5.3	-2.2	11.6	0.6
(deg)	2D	5.8	-1.8	9.8	0.2
Femoral mechanical angle (deg)	3D	91.6	92.5	92.4	90.5
	2D	91.1	92.7	94.8	90.9
Tibial mechanical angle (deg)	3D	87.7	86.4	91.9	89.8
	2D	88.0	86.4	88.7	89.1
HKS (deg)	3D	4.6	4.1	3.9	3.7
	2D	4.3	3.5	3.7	4.1
Torsions					
Femoral torsion (deg)	3D	-5.9	1.1	-16.4	-4.8
	2D	--	--	--	--
Tibial torsion (deg)	3D	31.2	53.1	40.6	41.3
	2D	--	--	--	--
Femorotibial rotation (deg)	3D	12.5	-10.9	-17.0	-2.8
	2D	--	--	--	--

characterizing the underlying biomechanical parameters associated with OCD progression and development.

The stereoradiography system, EOS™ imaging, also reduces the radiation dose 3 to 4 times compared to the scanograms performed with computed radiography (CR) while conserving X-ray images' quality. The reliability of this imaging modality has been tested in identifying the lower limb torsion and length discrepancy.[15,16] While application of the stereoradiography imaging in OCD clinical evaluation reduces the radiation exposure and examination time significantly, the reliability of this new technology in clinically evaluating OCD of the knee has not been investigated.

This study demonstrates the applicability of low-dose stereoradiography of OCD of the knee. The 3D parameters can improve the classification of OCD of the knee and highlight the mechanical factors associated with OCD development. This novel approach has potential to improve understanding of the underlying pathomechanism associated with OCD of the knee.

Conclusion

The applicability of the stereoradiography imaging in 3D evaluation of OCD of the knee was investigated. This technique can reduce the radiation dose while providing quantitative information regarding the mechanical factors associated with OCD development.

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U·P·O·J

The Ponseti Method for Clubfoot Treatment in Low and Middle-Income Countries: A Systematic Review of Barriers and Solutions to Service Delivery

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Background

Use of the minimally invasive Ponseti method has been increasing in low and middle-income countries, where most of the world's children with clubfoot are born. This method requires a system of service delivery involving screening, serial casting with or without a tenotomy to achieve correction, and long-term use of an orthosis to maintain correction. The goal of this systematic review is to evaluate the barriers to service delivery and the solutions that have been proposed or implemented to address these barriers.

Methods

A literature search of Medline, Embase, and SCOPUS produced 3251 results. Twenty-four papers were selected for final review. Barriers and their attempted solutions were organized into a previously described health barrier model. We reported on high-impact, sustainable solutions that are feasible for organizations to implement, as opposed to solutions that require major policy or country-wide infrastructure changes.

Results

Common barriers found to have the most impact on patient care included financial constraints, transportation, difficulties with brace and cast care, self-perceived health status, lack of physical resources, and provider's lack of knowledge and skill. The most common solutions detailed were education of the provider or patient and financial assistance for patients.

Conclusions

Recognizing that contextually relevant solutions to the challenges of setting up a system for clubfoot service delivery are required, several common barriers have emerged within this systematic review of papers from multiple countries, including spatial accessibility, affordability, and availability. Programs can best prepare for challenges by placing clinics close to population centers and/or allocating funds to subsidize transportation, ensuring that an adequate supply of materials are available for the casting and tenotomy, and enhancing the education of families and health providers. Strengthening communication and establishing partnerships between individuals and organizations promoting the Ponseti method will improve systems for service delivery.

Doxycycline Improves Sedentary, but not Exercised, Supraspinatus Tendon and Muscle in a Rat Model

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Introduction

Matrix metalloproteinases (MMPs) have been implicated in the progression of tendon degeneration, but are also altered following non-injurious exercise. Potentially, one distinguishing feature between maladaptations and beneficial adaptations to exercise is the role of MMPs. MMP inhibition, through drugs like doxycycline, has been proposed to improve muscle and tendon healing.[1] Previous studies investigated doxycycline in acute injury models;[2-5] however, these studies were not designed to replicate chronic tissue adaptations. Therefore, the objective of this study was to investigate doxycycline on sedentary and exercised supraspinatus tendon and muscle using our rat model of non-injurious exercise.[6] With this model, we demonstrated distinct acute and chronic effects of exercise to the muscle and tendon and identified several altered genes associated with matrix turnover.[7] We hypothesized that doxycycline would abolish the beneficial adaptations present with exercise but have no effect on sedentary muscle and tendon properties.

Methods

One hundred seventy one male, Sprague-Dawley rats (400-450g, IACUC approved) were divided into 1 acute or 2 chronic time points and exercise (EX) or cage activity (CA) groups, as previously described.[6,7] Rats in the doxycycline (DOX) groups were administered an oil suspension of doxycycline hyclate (Wedgewood Pharmacy) orally (10mg/kg) every 24 hours.[3] Rats in the acute EX group were sacrificed 24 hours after a single bout of exercise (EX24). Rats in the acute DOX group began receiving DOX 24 hours prior to their exercise bout (EX24DOX). A separate group of rats maintained normal CA and received 3 doses of DOX until sacrifice 1.5 hours after their last dose (CA24DOX) and were compared to a non-drug treated CA group (CA24). For chronic time points, EX animals walked on a flat treadmill (10 m/min, 1 hr/day, 5 days/wk) for 2 or 8 weeks (EX2, EX8). Control animals maintained normal CA (CA2, CA8). DOX groups received DOX 7 days/wk for 2 or 8 weeks (EX2DOX, EX8DOX, CA2DOX, CA8DOX). Assays performed include supraspinatus tendon mechanical testing, tendon histology, and muscle histology. *Tendon Mechanics:* Mechanical testing protocol: 1) preconditioning, 2) stress-relaxation at 4% strain, 3) frequency sweep (0.1, 1, 2, 10 Hz) of 10 sine cycles, 0.125% strain amplitude, 4) return to gauge length, 5) stress-relaxation at 8% strain, 6) recovery to 4% strain, 7) return to 8% strain, 8) frequency sweep at 8% strain, 9) return to gauge length, 10) ramp to failure at 0.3%/s. Due to slip in the fixture that occurred near 4% strain, only the 8% data were analyzed (steps 5, 8, 10). *Tendon Histology:* 7μm paraffin sections of tendon were H&E stained and imaged with polarized light (chronic only) to determine the collagen alignment; cell density and shape were quantified with software (Bioquant). *Muscle Histology:* 10μm cryosections transverse to fibers were stained with anti-laminin and DAPI and analyzed[8] for centrally nucleated fibers and average fiber size. For fiber type analysis, sections were stained with anti-MyHC-I, MyHC-IIa, and MyHC-IIb and anti-laminin. Deep and superficial muscle regions[9] were analyzed for fiber type distribution and fiber type cross-sectional area. *Statistics:* To determine the effects of DOX, t-tests were used to compare DOX and non-drug treated groups separately for CA and EX at each time point. Significance: $p \leq 0.05$, Trends: $p \leq 0.1$. All data is presented as mean + standard deviation.

Results

Tendon Mechanics: Combined with a single bout of EX, DOX decreased tendon CSA and increased tendon modulus and max stress (Figure 1), bringing properties to within 0.2-10% of previously measured baseline levels. DOX also increased dynamic modulus (not shown). Viscoelastic parameters were not altered by DOX combined with acute EX. In chronic groups, DOX decreased tendon CSA and increased modulus for all groups (Figure 1) and increased dynamic modulus at all frequencies for the 2 week time point (EX and CA) and 8 weeks CA (not shown). DOX increased stiffness and max load in chronic CA groups but decreased stiffness and max load in chronic EX groups (Figure 1). Percent relaxation decreased with 2 weeks of CA but increased with 2 weeks of EX and 8 weeks of CA when combined with DOX (Figure 1). At 2 and 10 Hz, DOX reduced tan(δ) with 2 weeks of CA but increased tan(δ) with 2 and 8 weeks of

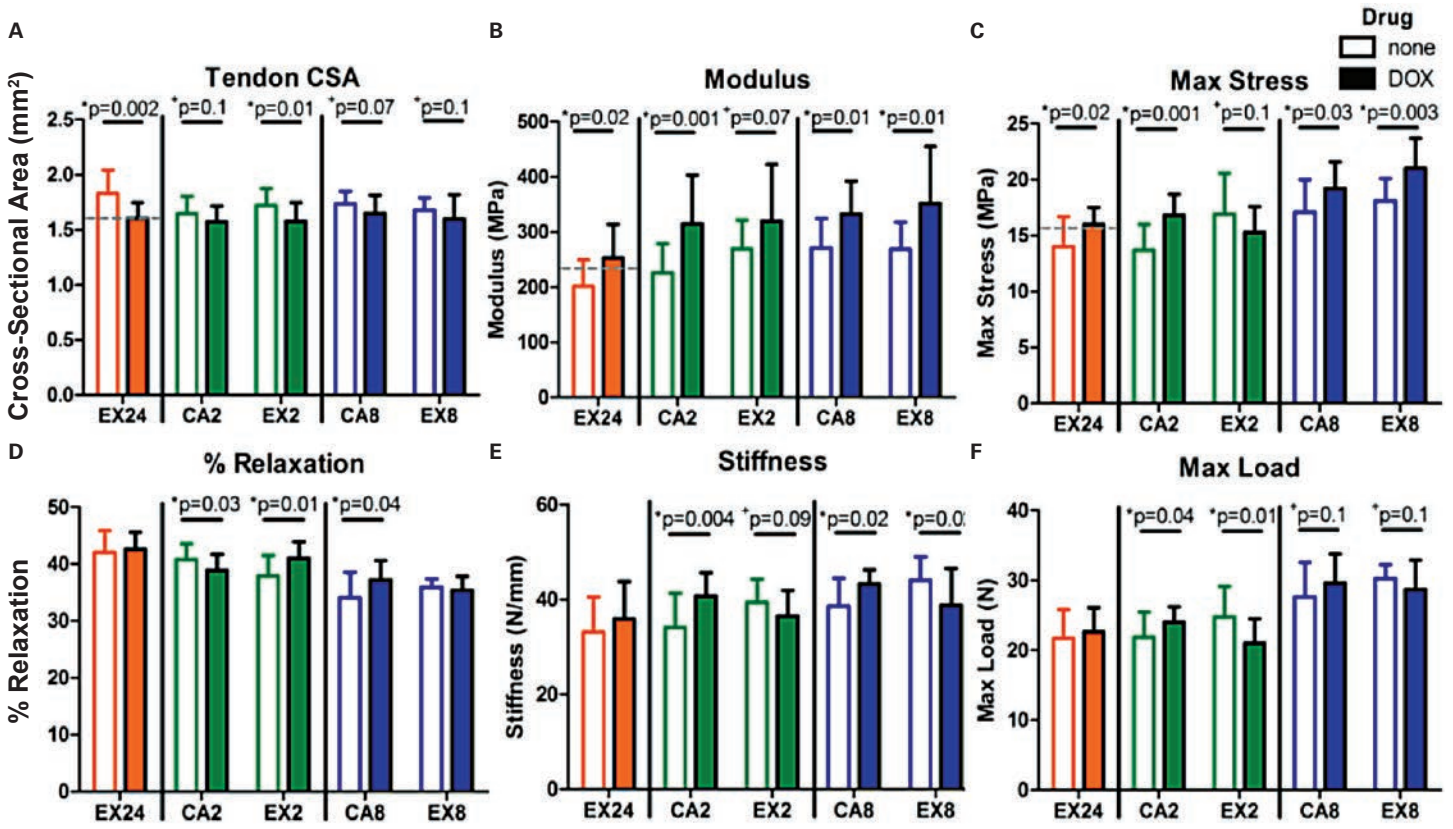

Figure 1. Tendon Mechanics. DOX combined with a single exercise bout brought tendon CSA, modulus, and max stress closer to baseline (gray dashes) by 24 hours. DOX reduced tendon CSA and increased modulus chronically. DOX had differential effects on % relaxation, stiffness, and max load in chronic CA and EX groups. n=10-15 acute, n=12-17 at 2 wks, n=10-14 at 8 wks.

EX (not shown). *Tendon Histology:* Acute administration of DOX had no effect on cell density or shape and only trended toward decreasing cell density when combined with a single bout of exercise (not shown). At 8 weeks, DOX decreased cell density with CA and EX. DOX resulted in rounder cells in the CA groups at 2 and 8 weeks and the EX group at 8 weeks (not shown). Tendon collagen organization increased with 8 weeks of DOX in the CA group (not shown). *Muscle Histology:* For all groups, the average percent of centrally nucleated fibers was below 1%, and DOX had no effect (not shown). DOX decreased the average muscle fiber CSA in all the EX groups but did not affect CA groups (Figure 2). Some muscle fiber type-specific changes were evident with DOX, including trends toward increased percent MyHC-IIa fibers, specific to CA groups (not shown).

Discussion

In contrast to our hypothesis, doxycycline significantly increased tendon mechanics and organization in sedentary groups. These increases were not always present with exercise. In fact, DOX combined with chronic exercise decreased stiffness and max load. We previously found MMP activity is higher in sedentary than exercised tendons, which helps explain these results. Our findings support previous studies that showed MMP inhibition of stress-deprived tendons prevented loss of mechanical properties.[10,11] When combined with exercise (but not CA), DOX decreased muscle fiber CSA, further suggesting that DOX combined with increased activity is not beneficial. In conclusion, results suggest that doxycycline at pharmaceutical doses induces beneficial supraspinatus tendon adaptations without negatively affecting the muscle in sedentary animals, supporting the use of doxycycline to combat degenerative processes; however,

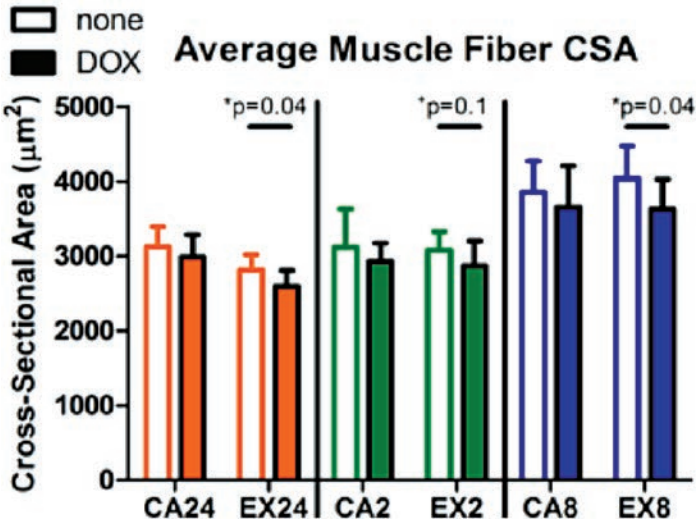

Figure 2. Muscle Fiber CSA. DOX decreased average muscle fiber CSA in EX, but not CA, groups. n=7-8 acute, n=7-9 at 2 wks, n=6-8 at 8 wks.

when combined with exercise, doxycycline does not produce the same beneficial adaptations in rat supraspinatus tendons and reduces muscle fiber cross-sectional area, suggesting that the drug is not advantageous when combined with activity.

Significance

Doxycycline, a commonly used drug, may be successful in preventing degeneration in tendon and muscle due to a sedentary state, but is not beneficial when combined with non-injurious exercise.

Acknowledgements

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Effects of Autologous Tenocyte-Seeded Nanofibrous Scaffolds in Rotator Cuff Repair are Age-Dependent

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Introduction

Rotator cuff tears affect millions of individuals each year, with an increased prevalence in the elderly population. Although advancements in surgical methods and rehabilitation protocols have improved clinical results, rotator cuff repair failure remains common.[1] To further improve surgical outcomes, rotator cuff repair augmentation has been studied, wherein scaffolds are used to aid in mechanical support at the time of surgery, and/or to deliver cells and/or biologic factors to the repair site. For example, local delivery of mesenchymal stem cells or tenocytes can increase collagen content and decrease inflammation at the repair site.[2-4] However, whether the success of such therapies is age-dependent is unknown. Therefore, the objective of this study was to determine the effects and mechanisms of action of autologous juvenile, adult, and aged tenocytes delivered using aligned nanofibrous scaffolds on healing tissue properties in our novel rat model of augmented rotator cuff repair.[5] Our hypotheses were: 1) Tenocyte-seeded scaffolds will increase collagen and cell organization at the repair site compared to scaffold only controls, resulting in enhanced tendon-bone healing with improved mechanical properties, and 2) tenocytes from juvenile rats will result in greater improvement of functional outcomes in cuff healing compared to adult or aged rats.

Methods

A total of 57 Fisher (F-344) rats were divided into three age groups: juvenile (4 weeks), adult (8 months) and aged animals (16 months). Animals underwent bilateral transection of their supraspinatus tendons and simultaneous harvest of the intra-articular biceps tendons. Biceps tendon cells were harvested by morselizing explant tissue and allowing cell migration onto tissue culture surfaces over 1 week. Cells were expanded in culture and split at confluence. At P2, cells from each donor were seeded onto an electrospun poly(δcaprolactone) (PCL) nanofibrous scaffold (3x5x0.5 mm) at $2x10^5$ cells/scaffold. Three weeks later, augmented supraspinatus repair was performed in which the right shoulder received a tenocyte-seeded scaffold while the left shoulder received an acellular scaffold as a control. Animals were sacrificed 8 weeks after the second surgery and frozen (for mechanical analysis, n = 12) or fixed in formalin (for histologic analysis, n = 6). One additional animal from each age group received Qtracker labeled cells bilaterally for cell tracking and was sacrificed at 1 week. Tissues were cryosectioned and processed for fluorescent imaging. *Tendon Mechanical Testing:* For testing, animals were thawed and the humerus was dissected with the supraspinatus intact. For local optical strain measurement, stain lines were placed on the tendon. Cross sectional area was measured using a custom laser device. Tensile testing was performed as: preload, preconditioning, stress relaxation, and ramp to failure. Stress was calculated as force divided by initial area and 2D Lagrangian strain was determined. *Histology:* Tendons were processed using paraffin procedures. Sagittal sections (7 um) were collected and stained with Hematoxylin–Eosin (H&E) or Safranin O-Fast Green (SafO). Cell density and cell shape were graded by three blinded investigators, using a scale of 1-3 (1 = low, 2 = moderate, 3 = high) for cellularity and 1-3 (1 = spindle shaped, 2 = mixed, 3 = rounded) for cell shape. SafO staining was quantified using ImageJ. Polarized light images were used to quantitate tendon organization as described.[6] Tissue mechanics, SafO quantification, and polarized light analysis were assessed using t-tests, comparing scaffold control and cell-seeded scaffold treatment within age groups. Histology scores were evaluated using a Mann-Whitney test. Significance was set at $p < 0.05$ (*) and trends at $p < 0.1$ (†); ** denotes $p < 0.01$.

Results

The presence of delivered tenocytes in the shoulder one week after surgery was confirmed via fluorescent imaging of Qtracker-labeled tenocytes. Concurrent in vitro culture of labeled cells demonstrated that tenocytes readily adhered to and colonized the PCL scaffolds (data not shown). Elastic and viscoelastic mechanical properties improved with cell-seeded scaffolds in both juvenile and aged animals when compared to scaffold-only controls. Specifically, in cell-seeded groups, stiffness and midsubstance modulus increased in juvenile animals, and midsubstance modulus increased in aged

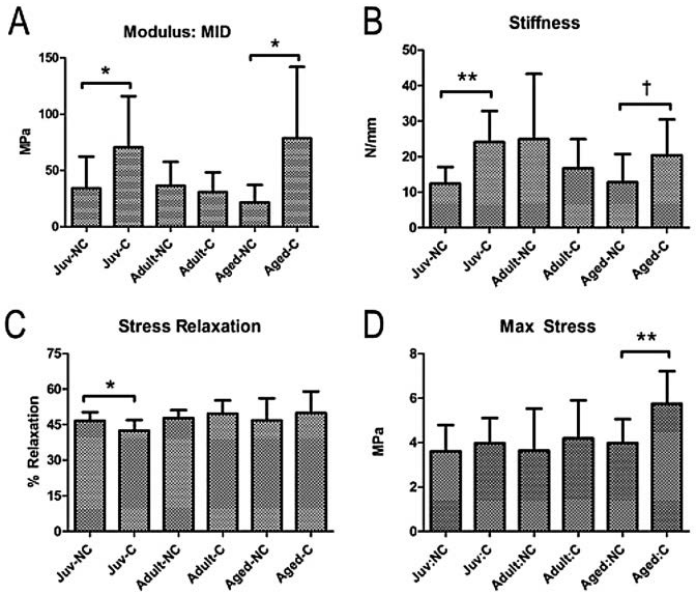

Figure 1. Augmenting repair with autologous cells caused (A) increased midsubstance modulus and (B) increased stiffness in both juvenile and aged animals, (C) decreased stress relaxation in juvenile animals, and (D) increased max stress in aged animals. (NC = Scaffold only, C = Cells).

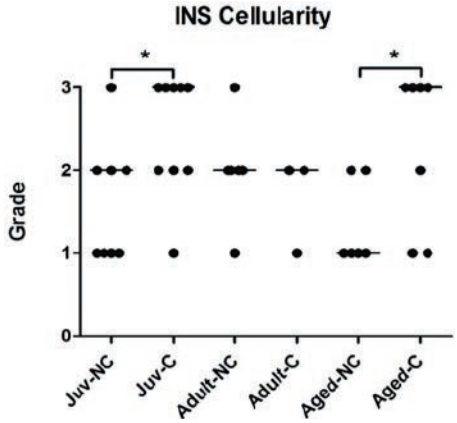

Figure 2. There was a significant increase in cellularity at the insertion of juvenile and aged tendons treated with cell-seeded scaffolds compared to scaffold-only controls.

animals, with a trend toward an increase in stiffness (Figure 1A-B). Similar improvements in insertion modulus were also noted (data not shown). Stress relaxation decreased in juvenile animals (Figure 1C), while maximum stress increased in aged animals (Figure 1D) relative to controls. Implantation of cell-seeded scaffolds also improved histological parameters. Specifically, cells were significantly more spindle-shaped in juvenile animals (insertion only, data not shown) and in aged animals (trend, midsubstance only, data not shown), while cellularity increased at the insertion in both juvenile and aged groups (Figure 2). Cell delivery decreased SafO staining in aged animals (data not shown), and increased collagen organization (decreased circular standard deviation) in both the insertion and midsubstance of aged animals, and in the insertion of juvenile animals (Figure 3). No changes were seen in any parameter in adult animals.

Discussion

Results demonstrate that delivery of autologous tenocytes to the healing supraspinatus is beneficial in juvenile and aged animals, with no effect in adult animals. As aged animals exhibit deteriorated tendon mechanical properties and healing potential,[7] a 44% increase in maximum stress with cell augmentation denotes a substantial improvement. Coupled with up to three-fold increases in tendon modulus and stiffness, the improvements in tendon mechanical strength for both aged and juvenile animals were striking. Increased numbers of cells at the insertion of treated animals demonstrates a more robust repair response, reflected in significant increases in collagen organization. These data support earlier matrix remodeling and increased collagen production after cell-augmented repair. While these findings support our first hypothesis, our second hypothesis was not substantiated. Surprisingly, both young and old animals benefitted similarly, yet no changes were seen in adult animals. Adult tendons are in relative "equilibrium" with regards to catabolic and anabolic processes, so additional tenocytes likely only sustain regular repair mechanisms. Conversely, juvenile tendons are

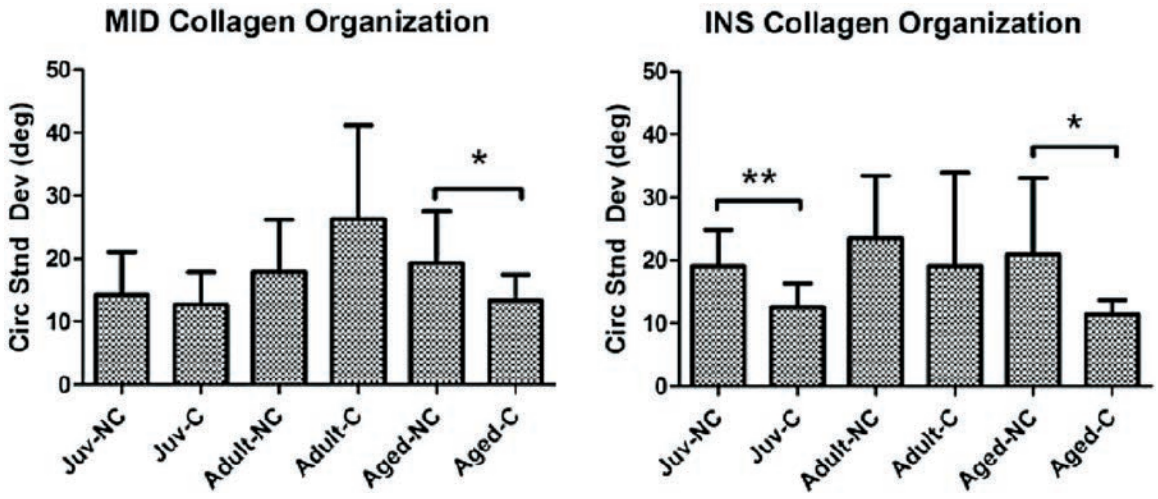

Figure 3. When comparing groups treated with cells to those without cells, collagen fiber organization was increased at the both the midsubstance and insertion in aged animals, and at the insertion in juvenile animals.

actively growing whereas aged tendons exhibit diminished cell activity and matrix turnover. In these "imbalanced" states, the addition of a supplemental cell population contributes substantially to the healing process. Further research will investigate precise mechanisms of action by which these cell populations improve tissue healing.

Significance

This research addresses regenerative medicine and musculoskeletal repair by using bioengineered scaffolds *in vivo* to improve tendon repair in a clinically relevant and well-established animal model. As this approach uses FDA approved materials and minimally manipulated autologous cell populations, it has the potential for rapid translation to clinical practice to address this important clinical problem. We have demonstrated the potential for autologous cell-seeded scaffolds to improve repairs in both the juvenile and aged population.

Acknowledgements

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Rat Supraspinatus Tendon Responds Acutely and Chronically to Exercise

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Introduction

Tendons respond to exercise by adapting their extracellular matrix (ECM). Prior studies found increased[1,2] or unchanged[3,4] tendon stiffness with chronic exercise; however, these changes have not been characterized over time, and it is unknown how acute responses following a single exercise bout lead to adaptations with repeated bouts. Previously, we showed that acute and chronic gene responses to exercise are distinct, and matrix metalloproteinase (MMP) expression is altered.[5] MMPs facilitate ECM turnover and are increased with tendinopathy. Whether MMPs are a physiologic response to load or pathologic in tendon degeneration is unknown. Therefore, the objective of this study was to identify acute responses and chronic adaptations of supraspinatus tendon to non-injurious exercise. We hypothesized that chronic exercise increases tendon mechanical properties, and MMP activity increases acutely.

Methods

One hundred fifty six male, Sprague-Dawley rats (400-450g, IACUC approved) were divided into acute or chronic exercise (EX) or cage activity (CA) groups.[5,6] Animals in acute groups were euthanized 3, 12, 24, 48, or 72 hours upon completion of a single bout of exercise (10 m/min, 1 hr) on a flat treadmill (EX3h, EX12, EX24, EX48, EX72). A control, treadmill-trained group (CA-T) did not undergo a single bout of exercise. A second control group maintained normal cage activity for the duration (CA24) and was used to measure MMP activity. Animals in chronic EX groups walked on a flat treadmill (10 m/min, 1 hr/day, 5 days/wk) for 3 days, 1 wk, 2 wks, or 8 wks (EX3d, EX1, EX2, EX8). Rats were euthanized 72 hr after their final exercise session to avoid potentially confounding acute effects of exercise. Two control groups maintained normal CA for an early (CA2) or later (CA8) time point. Tendon histology and MMP activity were measured for all groups. Tendon mechanics were determined in the EX24, CA-T, EX2, CA2, EX8, and CA8 groups. *Mechanics:* Stiffness was calculated as the slope of the linear region of the load-displacement curve during a ramp to failure at 0.3%/s. Modulus was calculated from optical strain and stress measurements. Dynamic modulus and tangent of the phase angle between the stress and strain were calculated from a frequency sweep (0.1, 1, 2, 10 Hz) of 10 sine cycles at 8% strain with 0.125% strain amplitude. Percent relaxation was calculated from a 300s stress-relaxation test at 8% strain. *Histology:* 7μm sections of paraffin-embedded bone-tendon-muscle units were stained with H&E and imaged with polarized light (chronic groups only) to determine the circular standard deviation of the collagen alignment. Cell density and shape were quantified with commercial software (Bioquant). *MMP Activity:* A SensoLyte 520 generic MMP assay kit (Anaspec) was used to determine MMP activity, following the manufacturer's protocol. Dilutions of purified human MMP-13 were used to create a standard curve. *Statistics:* To determine the effects of exercise on tendon mechanical properties, t-tests were used to compare EX and CA groups separately for each time point. Significance was set at $p \leq 0.05$ and trends at $p \leq 0.1$. To determine the effects of exercise on tendon histological properties (cell density, cell shape, organization for chronic), 1-way ANOVAs were performed for the acute groups (EX3h, EX12, EX24, EX48, EX72, CA-T) and the early chronic groups (EX3d, EX1, EX2, CA2). If the ANOVA was significant ($p \leq 0.05$) or a trend ($p \leq 0.1$), then pairwise comparisons were performed with Fisher's tests. A t-test was used to compare EX8-CA8 for tendon histology and MMP activity. A 1-way ANOVA was used to compare MMP activity in the early chronic groups. To determine the acute effects of exercise on MMP activity, after confirming no differences in MMP activity between them, CA-T and CA24 control groups were pooled to form a single control group (CA-P). Then, a 1-way ANOVA was performed for the EX3h, EX12, EX24, EX48, EX72, and CA-P groups with Fisher's tests for pairwise comparisons. All data is presented as mean + standard deviation.

Results

A single bout of exercise resulted in mild trends toward reduced tendon mechanical properties, but 2 or 8 weeks of chronic exercise led to increased tendon mechanics (Figure 1). Dynamic modulus increased after 8 weeks of exercise, and tan(δ) decreased after 2 weeks of exercise (not shown). Cell density was not affected acutely or chronically (not shown).

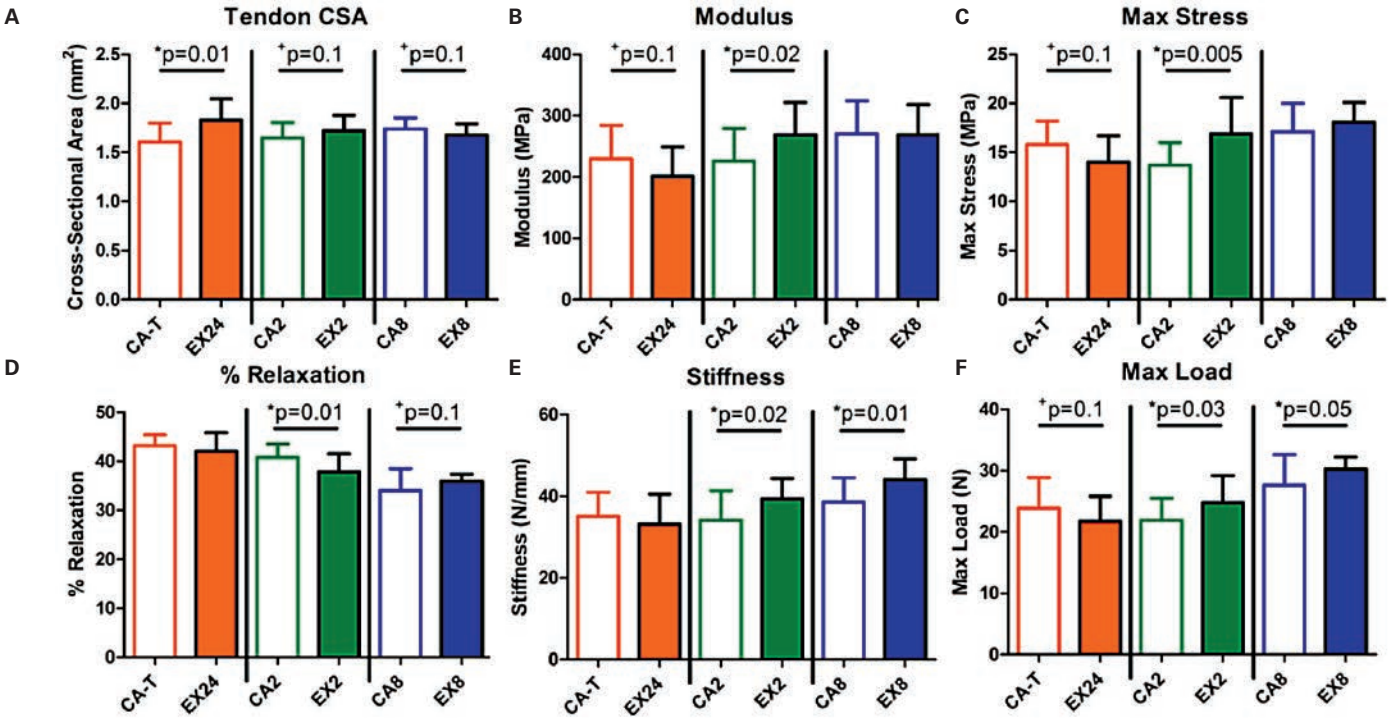

Figure 1. Tendon Mechanics. A single bout of EX followed by 24 hr of rest led to increased tendon cross-sectional area (A) and trends toward decreased modulus (B), max stress (C), and max load (F), suggesting mildly inferior properties. Chronic EX resulted in beneficial adaptations after 2 weeks and 8 weeks. n = 9-12 acute, 12-17 at 2 wks, 11-13 at 8 wks.

Cells became rounder with chronic exercise and 48 hours after a single bout of exercise (not shown). All tendons were highly organized, and chronic exercise did not affect collagen organization (not shown). MMP activity decreased 12, 24, and 48 hours after a single bout of exercise and returned to baseline by 72 hours (Figure 2A). MMP activity decreased after 8 weeks of chronic exercise (Figure 2B).

Discussion

Rat supraspinatus tendons demonstrated acute responses and chronic adaptations to exercise. The mild trends toward decreased mechanics following a single exercise bout may initiate and foster chronic adaptations. Although these differences were minor acutely, they were sufficient to result in significant beneficial adaptations chronically. Generic MMP

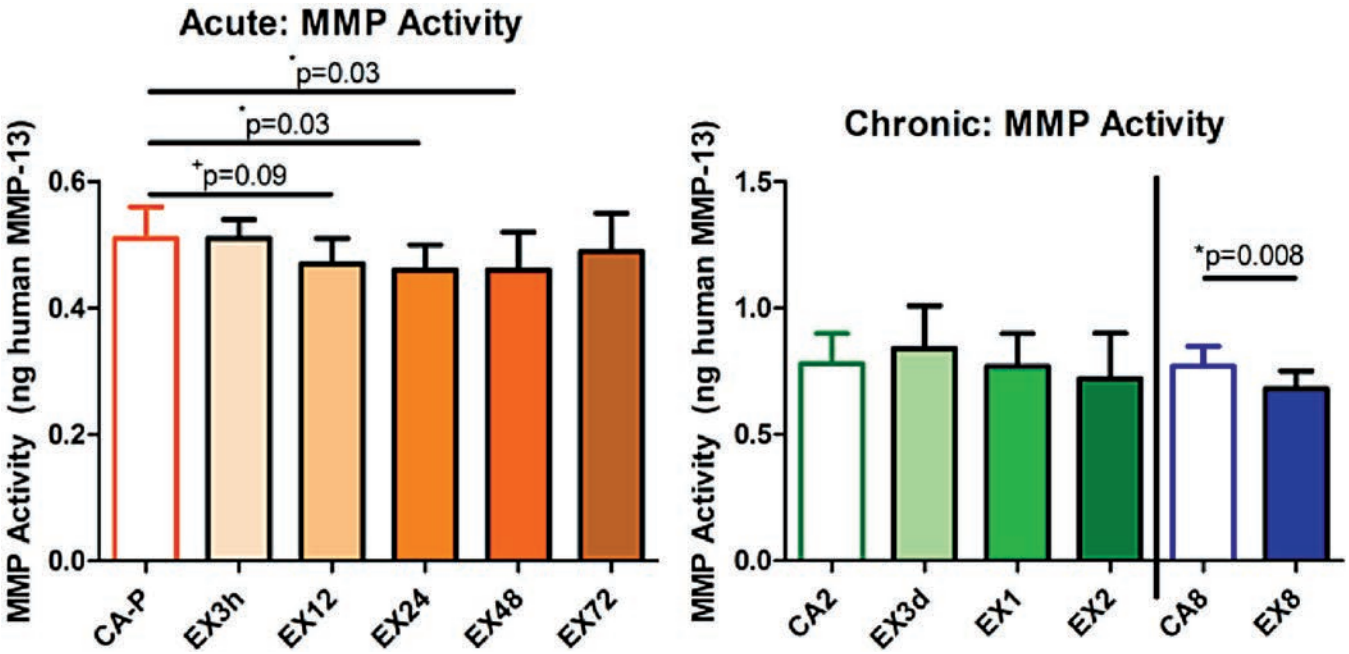

Figure 2. MMP Activity. MMP activity decreased after a single bout of EX (A) and 8 weeks of EX (B). n = 6-8 acute EX, n = 14 CA-P, n = 8-12 chronic.

activity decreased with exercise. Although some studies have found increases in MMPs following exercise, others have found decreases.[7] Decreased MMP activity may indicate an anabolic instead of catabolic response and contrasts the response seen with injury. This study investigated a single, previously validated exercise protocol, and it is unknown how results would change with increased intensity. Additionally, tendon mechanics were measured at a single acute time point, and it is unknown how exercise immediately alters these properties or when they return to baseline. Taken together, results suggest that mild, acute decreases in MMP activity and tendon mechanics following a single exercise bout lead to enhanced tendon mechanical adaptations with repeated exercise bouts. This study provides a foundation for future work to distinguish beneficial from detrimental responses to exercise to develop new strategies to prevent and treat overuse injuries.

Significance

This study helps define the acute and chronic temporal response of supraspinatus tendon to load in an *in vivo* model. Results provide a framework for future studies to develop efficient exercise protocols that minimize risk of overuse injury.

Acknowledgements

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Pulsed Electromagnetic Field Therapy Improves Tendon-to-Bone Healing in a Rat Rotator Cuff Repair Model

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Introduction

Rotator cuff tears are common musculoskeletal injuries, which often require surgical intervention. Unfortunately, post-repair prognosis is poor, with surgical repairs that fail in up to 94% of cases.[1] In an effort to improve tendon-to-bone healing, non-invasive therapies have been utilized post-operatively including ultrasound and shock wave therapy. Of note, pulsed electromagnetic fields (PEMFs) have been shown to improve bone fracture healing,[2] but the effect on tendon-to-bone healing has not yet been elucidated. Therefore, the objective of this study was to investigate the effect of PEMF on rotator cuff healing using an established rat rotator cuff detachment and repair model.[3-8] We hypothesized that PEMF exposure post-repair would improve tendon-to-bone healing and joint function.

Methods

Sixty adult male Sprague-Dawley rats (400-450g) were used in this IACUC approved study. Animals received either: 1) acute injury and repair[4] followed by cage activity and PEMF (Physio-Stim®, Orthofix, Inc., Lewisville, TX; 3hrs daily) or 2) acute injury and repair[4] followed by cage activity only. Animals were sacrificed at 4, 8, and 16 weeks (n = 10 per group per time point). Additionally, throughout the experiment prior to sacrifice, all animals in the 16 week group underwent longitudinal in vivo ambulatory[9] and passive shoulder joint mechanics assessments.[10] At sacrifice, right shoulders (n = 7 per group per time point) were dissected and processed for histological analysis.[8,11,12] All contralateral limbs (n = 10 per group per time point) were frozen at −20°C and thawed for dissection prior to tendon cross-sectional area measures and mechanical testing.[4,12,13] Following mechanical testing, proximal humeri were subjected to μCT imaging and analysis (10.5 μm resolution). Statistical comparisons were made between the PEMF and non-PEMF groups at each time point. Mechanical testing, μCT, and collagen fiber organization comparisons were made using t-tests. Histological comparisons were made using Mann-Whitney U tests. Ambulatory assessment comparisons were made using a 2-way ANOVA with repeated measures on time with post-hoc tests at each time point. Multiple imputations were calculated for a repeated measures analysis for missing data (~10%). All significance was set at $p \leq 0.05$.

Results

At 4 weeks, the PEMF group had a significantly smaller tendon cross-sectional area compared to the non-PEMF group (Figure 1A), with no differences at 8 and 16 weeks. At 4 and 8 weeks, the PEMF group had a significantly increased tendon modulus (100% increased at 4 weeks, 60% at 8 weeks) compared to the non-PEMF group, with no differences detected at 16 weeks

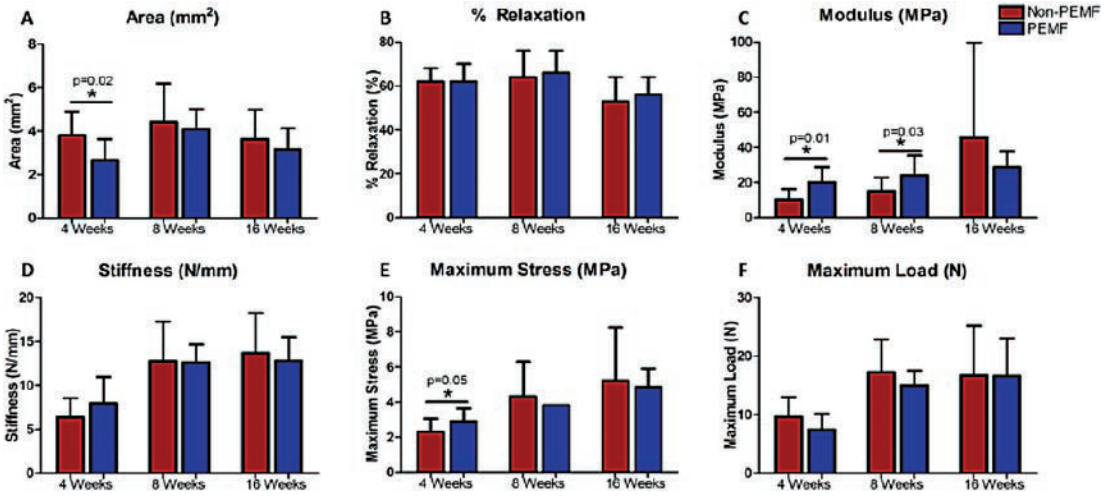

Figure 1. Tendon mechanical properties. (A) At 4 weeks, area was decreased in the PEMF group. (B) No differences were noted in % relaxation. (C) At 4 and 8 weeks, modulus was increased in the PEMF group. (D) No differences were noted in stiffness. (E) At 4 weeks, maximum stress was increased in the PEMF group. (F) No differences were noted in maximum load. Data as mean ± SD.

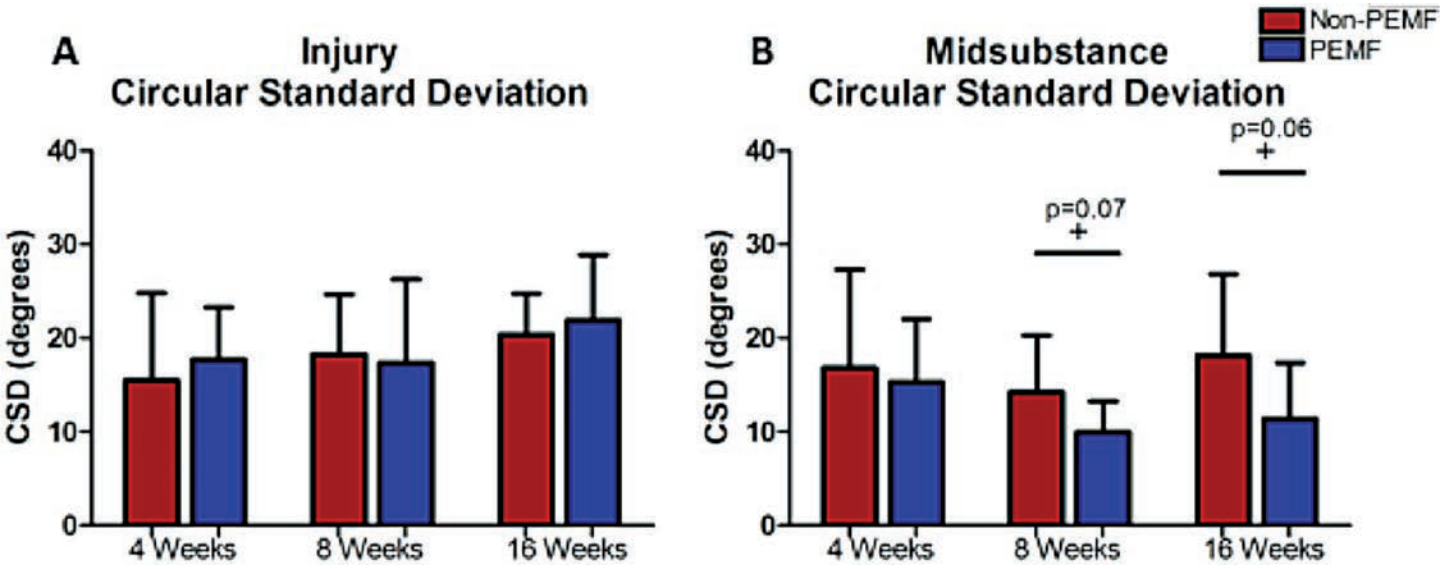

Figure 2. Collagen fiber alignment. (A) No differences were noted at the injury site. (B) At 8 and 16 weeks in the midsubstancethe PEMF group had a trend toward decreased CSD. Data as mean ± SD.

(Figure 1C). At 4 weeks, the PEMF group had significantly increased maximum stress compared to the non-PEMF group, with no differences at 8 and 16 weeks (Figure 1E). There were no differences in percent relaxation, stiffness, or maximum load at any time point (Figure 1B, D, F). For histology, at the injury site, no differences were detected in both cell shape and cellularity at any time point between groups (data not shown). Additionally, no differences were observed in collagen fiber organization at the injury site (Figure 2A). In the midsubstance at 8 weeks, the PEMF group had significantly more rounded cells (data not shown). For collagen fiber organization, the PEMF group had trends towards decreased circular standard deviation (CSD) at both 8 and 16 weeks (Fig. 2B). No differences were found in ambulatory assessment or passive joint mechanics (data not shown). For μCT analysis at 4 weeks, trabecular thickness was significantly decreased and connectivity density was significantly increased in the PEMF group (Figure 3E, H). At 8 weeks, no differences were observed in any parameter. At 16 weeks, the PEMF group had significantly increased bone volume fraction, trabecular thickness, and bone mineral density, and a trend toward increased bone mineral content (Figure 3).

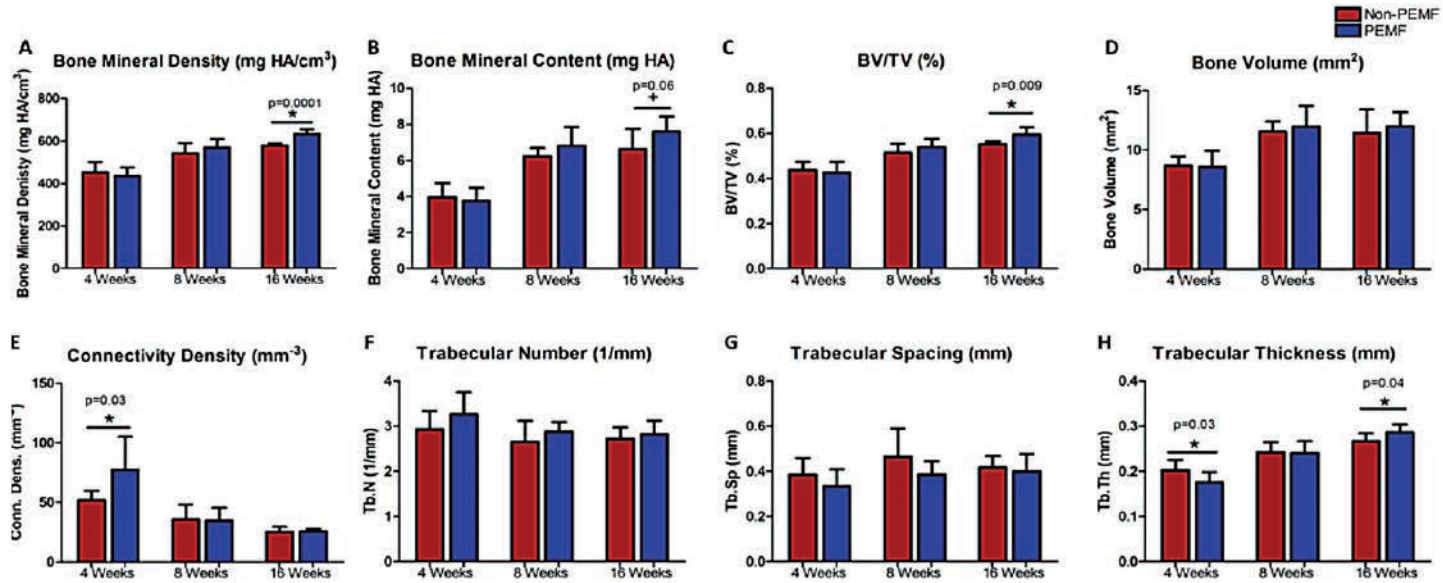

Figure 3. μCT analysis. (A) No differences were noted in bone mineral density at 4 and 8 weeks. At 16 weeks bone mineral density was increased in the PEMF group. (B) At 16 weeks bone mineral content trended toward increased in the PEMF group. (C) At 16 weeks bone volume fraction was increased in the PEMF group. (D) No differences were noted in bone volume. (E) At 4 weeks connectivity density was increased in the PEMF group. (F) No differences were noted in trabecular number. (G) No differences were noted in trabecular spacing. (H) At 4 weeks trabecular thickness was decreased in the PEMF group. At 16 weeks trabecular thickness was increased in the PEMF group. Data as mean ± SD.

Discussion

Overall, results suggest that PEMF has a positive effect on rat rotator cuff healing. Specifically, tendon mechanical properties were drastically improved in the PEMF group at both 4 and 8 weeks (100% and 60%, respectively) with a subsequent increase in bone properties at the tendon repair site. Histological analysis showed a more rounded cell shape in the PEMF group at 8 weeks in the midsubstance. This slight but significant finding might suggest inferior tissue, although this difference did not result in inferior mechanical properties. Additionally, collagen fiber organization in the midsubstance at 8 and 16 weeks showed the PEMF group trended toward decreased CSD, suggesting more organized tissue in the PEMF group, and perhaps later time points would further increase collagen organization. Overall, results demonstrate that PEMF improves tendon-to-bone healing in this animal model based on mechanical property measurements. Further studies can evaluate the mechanisms responsible for these changes.

Significance

PEMF provides a non-invasive way to improve tendon-to-bone healing in an acute rat supraspinatus detachment and repair model and shows potential for use in a clinical scenario of rotator cuff tendon to bone healing following rotator cuff repair.

Acknowledgements

Funding was provided by Orthofix, Inc. The authors thank XS Liu and WJ Tseng for assistance with μCT analysis.

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Simvastatin Recovers Supraspinatus Tendon Mechanical and Histological Properties in a Diet-Induced Hypercholesterolemia Rat Model

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Introduction

Rotator cuff tendon tears are extremely common, affecting up to 50% of Americans over 50 years of age.[1] Hypercholesterolemia, a condition which affects more than 27% of Americans over 20 years old,[2] has been shown to be a risk factor for tendon rupture, specifically in the supraspinatus and Achilles tendons.[3] Previous studies have used a high-cholesterol diet to induce hypercholesterolemia, which demonstrated an increase in stiffness and elastic modulus of the rat supraspinatus tendon.[4] In the clinic, statins are commonly prescribed to lower cholestero[1,2] but at present, little information is available examining the effect of statin treatment on the musculoskeletal system. Therefore, the objective of this study was to determine the biomechanical and histological effects of statin treatment in a diet-induced hypercholesterolemia model.[4,5] We hypothesized that hypercholesterolemic rats treated with statins would have improved tendon biomechanical and histological properties compared to untreated rats.

Methods

Thirty adult male Sprague-Dawley rats (400-450g) were used in the IACUC approved study. To induce hypercholesterolemia (HC), rats were fed a high cholesterol diet[4] (n = 20) for six months while age-matched control rats (CTL, n = 10) ate standard rat chow for six months. All rats were allowed food and water ad libitum and were weighed weekly throughout the study. After the initial six month treatment, a subset of the HC rats (n = 10) were orally dosed with simvastatin daily (20mg/kg) for three months (HC + S group). The HC and HC + S groups were fed HC chow throughout the study. All rats were sacrificed after a total of 9 months. Blood was collected from all rats at 6 months to confirm high-cholesterol in the HC groups and again at the time of sacrifice to measure total cholesterol (TC), high-density lipoprotein (HDL), triglycerides (TG) and the ratio of TC to HDL (TC/HDL). Immediately following sacrifice, right shoulders were dissected and fixed in formalin for histological analysis of collagen organization and cell morphology.[6-8] Contralateral limbs were frozen at −20°C and later thawed for mechanical testing.[4,6,9] All comparisons were made between the HC and HC + S groups only (except for serum lipid panels at 6 months to confirm HC and weight comparisons performed over time). Statistical comparisons of mechanical parameters and collagen organization were made using t-tests with significance at $p \leq 0.05$. Comparisons of cell morphology were made using non-parametric Mann-Whitney tests with significance at $p \leq 0.05$.

Results

Animals in the HC and HC + S groups were significantly lighter than the CTL rats after introduction to the HC diet throughout the duration of the study, but no differences in weight were noted between groups after induction of simvastatin treatment (data not shown). *Serum lipid analysis*: After six months, the animals in the HC diet had significantly increased TC, HDL, and TC/HDL, and significantly decreased TG (data not shown). After three months of simvastatin treatment, animals in the HC + S group had significantly decreased HDL and trended toward decreased TC. No differences were noted in TC/HDL or TG (data not shown). *Tendon mechanical properties*: At the insertion site, the HC + S group had significantly increased cross-sectional area and significantly decreased elastic modulus (Figure 1a, b respectively). In the midsubstance, no differences were detected in cross-sectional area or elastic modulus (Figure 1c, d respectively). Additionally, no differences were noted in percent relaxation and stiffness between groups (data not shown). *Histology*: No differences were observed in cell shape (Fig. 2a), cellularity (Figure 2b), or circular standard deviation (data not shown) at the insertion site between the groups. In the midsubstance, the HC + S group had significantly more spindle shaped cells (Figure 2a). No differences were observed in cellularity (Figure 2b) or circular standard deviation (data not shown) between groups.

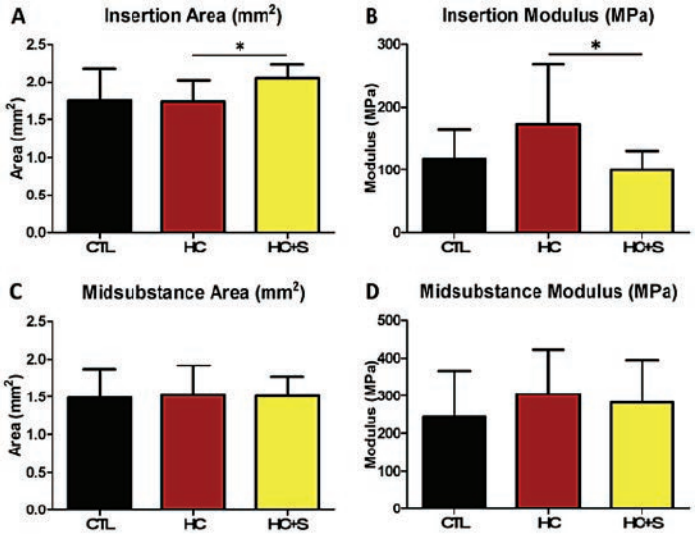

Figure 1. (A) Insertion cross-sectional area was significantly increased in the HC + S group. (B) Insertion modulus was significantly decreased in the HC + S group. (C) No differences were observed in midsubstance cross-sectional area or (D) modulus. Data presented as mean ± SD.

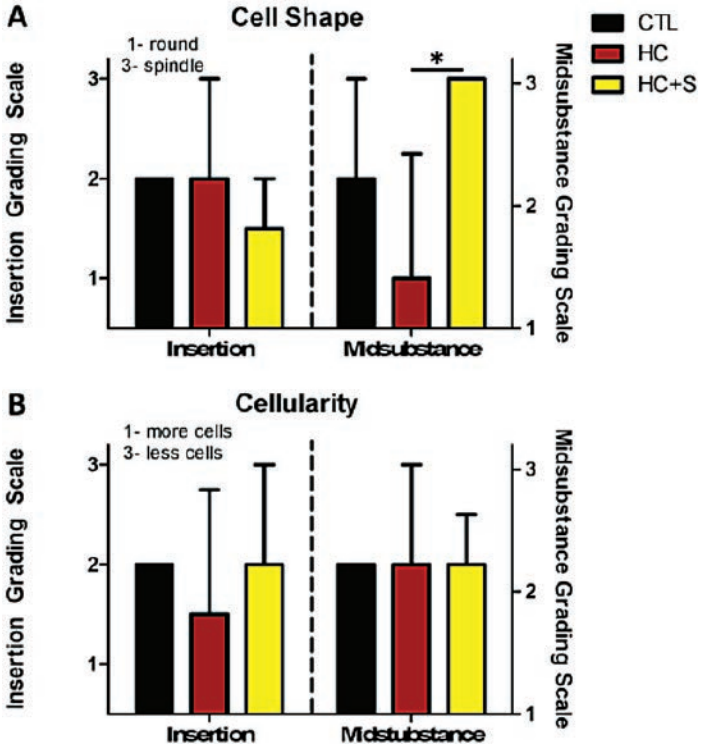

Figure 2. (A) No differences were observed in cell shape at the insertion site. In the midsubstance, the HC + S group had significantly more spindle shaped cells. (B) No differences were observed in cellularity in either region. Data as median ± IQR.

Discussion

Results suggest that treating HC rats with daily simvastatin for 3 months recovers the changes due to HC alone to baseline, control properties. Typically, a decrease in elastic modulus (Figure 1b) might be interpreted as a deleterious outcome, but when considering the modulus values in the CTL and HC + S groups (118 ± 47 MPa and 99 ± 30 MPa, respectively), it is apparent that the simvastatin treatment returned values close to baseline control values, which were elevated in the HC group (172 ± 35 MPa). Histological analysis showed the HC + S group had significantly more spindle shaped cells in the midsubstance, which indicates that the simvastatin intervention is returning the tendons back to normal cell morphology. Rounded cells in the HC group could be associated with tendinopathy and/or a more cartilaginous phenotype, which are risk factors for tendon rupture,[10] or with increased cell activity as a result of high-cholesterol in the tendon. Results suggest that simvastatin treatment tends to bring mechanical and histological parameters closer to baseline values, although the mechanisms governing these findings must still be elucidated. Further studies could also evaluate the effects of different doses or treatments.

Significance

Three months of simvastatin treatment in a diet-induced hypercholesterolemia model returns tendon mechanical and histological properties toward normal in this model system. Additionally, this data suggests that simvastatin use does not negatively affect tendon mechanical properties and might help to reduce the risk of tendon rupture.

Acknowledgements

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Revisiting Anterior Stand-Alone Fixation (ASAF) Devices for the Treatment of Single Level Lumbosacral Degenerative Disease

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Low back pain (LBP) affects 50-80% of the population at some point in their lifetime and is a frequent cause of decreased mobility and unemployment. Fusion of lumbar motion segments continues to be the foundation of treatment of intractable LBP secondary to degenerative disc disease (DDD), and can be achieved via anterior-only, posterior-only and anteroposterior approaches. Historically, the majority of fusions are performed via posterior surgical approaches. However, fusions performed via posterior surgical approaches, whether with or without instrumentation or with or without additional interbody fusion, have suffered from complications such as graft site morbidity, screw loosening, pseudoarthrosis, sagittal imbalance, high rates of adjacent level pathologies, and considerable complication and reoperation rates.[1,2] Additionally, the posterior approach has been shown to result in muscle atrophy which in itself becomes a new LBP generator.[4,5] As an attempt to mitigate these negative side effects, posterior percutaneous techniques gained some popularity as a less invasive, more muscle sparing alternative; however, minimally invasive techniques resulted in higher x-ray exposure and significantly increased rates of adjacent level facet joint violations.

In order to avoid the aforementioned complications, anterior stand-alone fusion (ASAF) devices have been introduced as an alternative method to avoid damage to the paravertebral muscles, to prevent screw displacement-related neurological and vascular complications and to reduce the rate of adjacent segment degeneration. ASAF devices provide a potential advantage by avoiding posterior muscle trauma, avoiding violation of the cranial facet joints and permitting improved sagittal balance reconstruction.

Despite reduced invasiveness and previously reported reduced infection rates, historical data exists that reports contradictory results regarding significant rates of nonunion associated with ASAF devices. This can be partially attributed to implants previously utilized to perform anterior interbody fusions, which were not stable enough to reliably achieve fusion, and thus resulted in high rates of pseudoarthroses. However, new implants have been designed which are anterior stabilized, e.g. with plates and locking screw technology, which significantly augments the overall construct strength and rigidity. The goal of the anterior stabilized stand-alone device is to negate the need for posterior fixation by promoting stability of the implant via the locking screws. The benefit of creating an anterior stand-alone fixation device is that the spine surgeon can achieve adequate stabilization to ensure fusion while avoiding the increased morbidity associated with the posterior approach. Schleicher et al published one of the early biomechanical studies using a human cadaver model comparing the Anterior stabilized stand-alone device implants to an established stand-alone interbody implant.[3] The study showed greater stiffness in lateral bending for the anterior stabilized stand-alone device compared to the established implant. Additionally, the study showed that for the anterior stabilized stand-alone device, the anterior cage takes most of the load during flexion, whereas the screws and the screw-plate junction assume most of the load during extension, and it's this augmentation of stability in extension moments that is especially important to the success of stand-alone interbody fusion.[3] In practice, the cage provides the stability lost by resection of the anterior longitudinal ligament in extension, which is the main biomechanical limitation of anterior lumbar interbody fusion (ALIF) procedures. The net effect of the implant design is that the compressive loads are evenly distributed across the implant, whereas the anterior stabilization plate and divergent locking screws serve to neutralize the tensile forces.[3]

Examining the effects of these new ASAF devices in practice, Strube et al performed a prospective cohort study comparing patients undergoing anteroposterior fusion (ALIF with transpedicular fixation: APLF) to patients undergoing anterior lumbar interbody fusion (ALIF) alone using the anterior stabilized stand-alone device. They found that the blood loss and duration of surgery were significantly lower in the ALIF group, and that while the Visual Analog Scale (VAS) and Oswestry Disability Index (ODI) scores improved in both groups, they were significantly better in the ALIF group. Additionally, rates of fusion were not statistically significant between groups at long term (41 month) follow up and only a 5% complication

rate was observed with the ALIF group[2]. Similarly, Siepe et al recently performed a prospective study including 71 patients with monosegmental DDD at the lumbosacral junction.[1] They showed an improvement in VAS and ODI at all stages of follow up, and 77.5% of patients reported satisfactory or highly satisfactory outcomes. Impressively, there was a 97.3% overall fusion rate shown on CT scan and only an 11.3% complication rate. Behrbalk et al and Burkus et al showed similar rates of fusion (90.6% and 94.5%, respectively) with the use of anterior plate in cage implant and recombinant human bone morphogenetic protein-2 and tapered interbody cages with rhBMP-2, respectively.[7,8] An additional benefit of anterior interbody fusion is its ability to restore lumbar lordosis, and to distribute it in an anatomic distribution. GuiGui et al have shown 80% of total lumbar lordosis is normally distributed between L4 and S1. This distribution of lordosis can improve sagittal balance, and may result in less pain, as the cephalad facets no longer need to hyperextend to compensate for reduced lordosis.[1,6,9]

In summary, anterior stand-alone fusion devices provide an excellent alternative to the currently more popular posterior surgical approach when treating low back pain, particularly in single level lumbosacral disease. Though historically there were concerns regarding adequate stability and an increased risk of pseudoarthroses, the advent of new technologies integrating anterior cages and plates provide the necessary stability to meet and exceed the fusion rates seen in the posterior or anteroposterior approaches for single level degenerative disease. Equivalent fusion rates and improved patient satisfaction scores are achieved with reduced complication rates, reoperation rates, surgical time and blood loss which are beneficial both to the patients and to the hospital system. ASAF of the lumbosacral junction achieved excellent rates of fusion, significant lordosis reconstruction and low reoperation rates, while avoiding weakening of the adjacent segments due to cranial facet joint violation and sagittal imbalance secondary to collateral muscle damage characteristic of the posterior approach. Thus, anterior stand-alone fixation devices for the treatment of the appropriately selected patient with lumbosacral degenerative disease provides an exciting alternative treatment modality to achieve successful fusion while minimizing complication and reoperation rates as well as reducing surgical morbidity and hospital costs.

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Fabrication, Maturation, and Implantation of a Composite Tissue-Engineered Total Disc Replacement

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Introduction

Low back pain arising from disc degeneration is one of the most common causes of limited function in adults.[1] Current treatment options are limited, favoring either physical therapy and pain management or surgical methods to fuse the motion segment. Neither approach restores native tissue structure and function, and so a number of tissue engineering strategies have emerged that focus on the creation of a composite tissue engineered total disc replacement,[2,3] with some studies showing promise in vivo.[4,5] To further this line of inquiry, we fabricated a simple composite engineered disc based on the combination of a porous polymer foam annulus fibrosus (AF) and a hyaluronic acid (HA) gel nucleus pulposus (NP). We used these constructs to determine whether the combination of native AF/NP cells or mesenchymal stem cells (MSCs) would mature to a greater extent in vitro and which cell type would best retain their phenotype after in vivo implantation in a rat tail model of disc replacement.[6]

Methods

Porous polycaprolactone (PCL) foams were fabricated by salt-leaching to form the AF regions of the engineered discs. PCL was dissolved in chloroform at a 20% (w/v) concentration and NaCl particles were sieved to yield particulate of ~106 μm that was loaded and mixed into the PCL solution with a PCL/NaCl mass ratio of 1:4 (% w/w). The resultant solidified PCL sheet with entrapped salt particles was 1.5 mm in height and individual plugs were extracted using 4 mm biopsy punch for the outer diameter and 2 mm for the inner diameter; this geometry approximates that of the rat caudal disc. To form the NP regions of the engineered discs, 1% methacrylated HA (MeHA) hydrogels were produced as in Kim, et al.[7] AF cells (AFCs) or MSCs were seeded onto the PCL foam at a density of 2×10^6 cells/construct, whereas NP cells (NPCs) or MSCs were encapsulated in HA at a density of 20×10^6 cells/ml.AF and NP regions were cultured separately in chemically defined media and combined at 2 weeks. At regular intervals over 9 weeks, compressive mechanical, biochemical, and histologic properties were evaluated. Additionally, AF/NP cell and MSC/MSC cell-seeded constructs were implanted into the rat caudal disc space after 5 weeks of pre-culture, as in Martin, et al.[6] After 5 weeks in vivo, disc height, hydration of the nucleus pulposus, and structure were assessed by μCT, fluoroscopy, and quantitative T2 MRI, and structure was evaluated via histological analyses with alcian blue/picrosirius red and collagen type II staining.

Results

By 3 weeks, the NP region of all the groups stained intensely for proteoglycans, while collagen staining in the NP increased with further culture time. In the AF region, staining gradually increased with time, though to a lesser extent than in the NP (Figure 1). There were no significant changes in the compressive modulus over 8 weeks for either group (not shown). After 5 weeks of pre-culture and 5 weeks of implantation, the disc height index (DHI) for implanted constructs was significantly greater than pre-operative levels, with only small differences between groups. Implanted discs did not result in intervertebral fusion (Figure 2A). Alcian blue/picrosirius red staining showed abundant collagen in the disc, but little

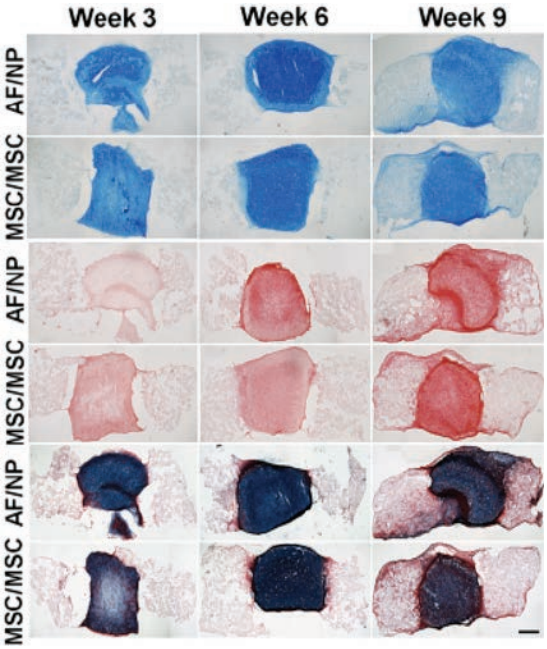

Figure 1. (A) Alcian blue, (B) picrosirius red, and (C) Alcian blue/picrosirius red staining of AF/NP and MSC/MSC engineered disc with time in in-vitro culture. (bar = 500 μm).

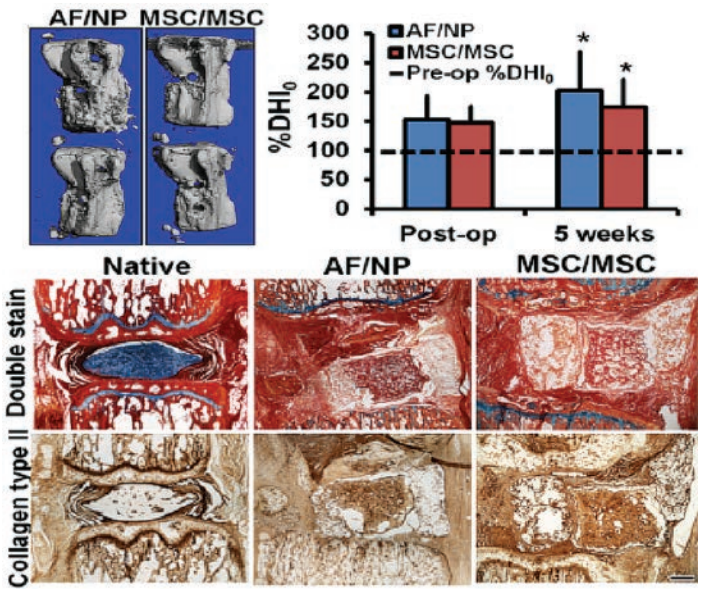

Figure 2. (A) μCT and fluoroscopic analysis of %DHI for implanted AF/NP and MSC/MSC engineered disc and (B) histology and immunostaining at 5 weeks after implantation. (*:$p < 0.05$ vs. pre-op) (bar = 500 μm).

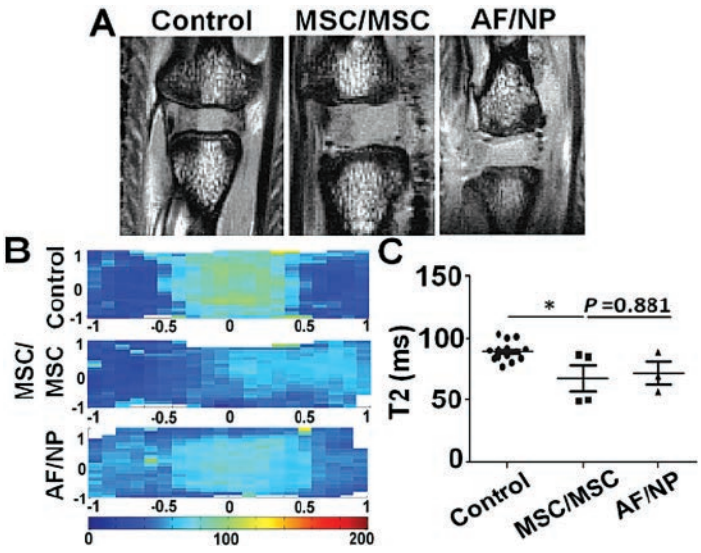

Figure 3. (A) T2 MRI images and (B) T2 maps with (C) quantification at 5 weeks after implantation (*:$p = 0.009$ vs. control).

proteoglycan in the NP region in either group. However, collagen type II staining was intense and localized to the NP at this time point (Figure 2B). MRI showed that implanted discs had a similar structure to native discs (Figure 3A, B). T2 mapping showed reduced signal in the NP for both groups compared to native discs. However, there was no significant difference between the AF/NP group and native discs (Figure 3C).

Discussion

This study demonstrated that a tissue engineered disc composed of a PCL foam AF region and a hydrogel NP region could be fabricated, matured in vitro, and implanted and maintained in the rat caudal spine. Engineered discs comprised of AF/NP cells and MSCs performed similarly, maintaining their structure after 5 weeks in vivo, though loss of proteoglycan was evident in the NP region for both groups. This suggests that, following 5 weeks of implantation, water and proteoglycan content are less than in the native disc, perhaps reflecting the inflammatory nature of the operative site and unwanted remodeling post-implantation.

Significance

This work demonstrates the successful fabrication, maturation, and in vivo function of a composite engineered disc composed of a PCL foam AF and a hydrogel NP using both native disc cells or MSCs.

Acknowledgements

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Recapitulating the Spectrum of Intervertebral Disc Degeneration in a Large Animal Model

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Introduction

Intervertebral disc degeneration is a progressive cascade that leads to structural and mechanical failure, and is frequently associated with low back pain. Patients present in a variety of degenerative states, classically defined by the Pfirrmann grading system, based on qualitative interpretation of magnetic resonance images (MRI).[1] Current treatments focused on alleviating the symptoms of discogenic pain are limited, as they do not restore disc mechanics or structure. As a result, there is considerable interest in developing biologic regenerative therapies to treat disc degeneration. For these therapies to be successful they must be tailored to the degenerative state of each disc. For example, they may include injection of stem cells, hydrogels or growth factors for mild to moderately degenerated discs, or engineered total disc replacements for severely degenerated discs.[2,3] A critical aspect of evaluating and translating these regenerative approaches into clinical use is a size-relevant large animal model that recapitulates the spectrum of degeneration seen in humans. The objective of this study was to establish a goat model of intervertebral disc degeneration in which a gradient of degenerative changes, from mild to severe, could be reproducibly achieved through mechanical (nucleotomy) or chemical (chemonucleolysis) perturbation.

Materials & Methods

With IACUC approval, 9 goats underwent a surgical procedure to induce the degeneration of the lumbar intervertebral discs. Using an open, lateral, retroperitoneal transpsoatic approach, L1-2, L2-3 and L3-4 lumbar discs were randomized to receive either subtotal nucleotomy (n = 10) or injection of 200μL of either 0.1U (n = 5), 1U (n = 10) or 5U (n = 5) chondroitinase-ABC (ChABC) via a 22G spinal needle. The L4-L5 (n = 5) disc received a sham saline injection, and the T13-L1 and L5-L6 discs served as intact controls (n = 10). Lateral plain radiographs of the lumbar spine were obtained pre-operatively, immediately post-operatively, and at 1, 2, 4, 6, 8, 10 and 12 weeks post-operatively for quantification of disc height index (DHI). After 12 weeks, the animals were euthanized, and the lumbar spines harvested and imaged using a 3T MRI scanner. Images for quantitative T1δ and T2 mapping[4] were obtained, as well as T2 weighted images for Pfirrmann grading. Correlations between quantitative MRI parameters and Pfirrmann grade were established by linear regression.

Individual motion segments were then imaged using high resolution microcomputed tomography (μCT) to visualize and quantify morphologic changes to the vertebral bony endplate. Alcian blue (glycosaminoglycans) and picrosirius red (collagen) stained, mid-sagittal, histological sections were used to visualize degenerative changes. Sections were graded on a visual analog scale by three blinded observers in five categories: organization of the annulus fibrosus, nucleus pulposus matrix, annulus fibrosus/nucleus pulposus border, nucleus pulposus cellularity, and cartilage endplate structure. Five samples from each of the intact control, nucleotomy and 1U ChABC groups were utilized for biomechanical testing. Following overnight equilibration in PBS, samples were subjected to a testing protocol consisting of 20 cycles tension compression (−230N to +115N), followed by 1 hour of creep at −230N (~0.48 MPa).[5] Tension/compression data was fit to a sigmoid function and creep data was fit to a five parameter viscoelastic constitutive model for analysis.[6] For quantitative outcome measures, statistically significant differences ($p < 0.05$) were established via one or two-way ANOVAs with Tukey's post-hoc tests.

Results

Histological evaluation (Figure 1) revealed advanced degenerative changes to the disc in the 5U ChABC group, with moderate changes in the nucleotomy and 1U groups, and mild changes to the 0.1U ChABC group. Histologic scores were highest in the 5U and 1U ChABC group, followed by the nucleotomy and 0.1U ChABC groups across all categories. There were no statistically significant differences in histologic score between control and sham discs. Discs injected with 1U or 5U of ChABC exhibited a progressive decrease in DHI, with 28% and 34% reductions in DHI in the 1U and 5U groups, respectively, after 12 weeks ($p < 0.05$). Compared to the sham group, DHI was also significantly reduced in the 5U group at 10 weeks, and in the 1U group at 2, 4, 6, 8, and 10 weeks ($p < 0.05$). Discs subjected

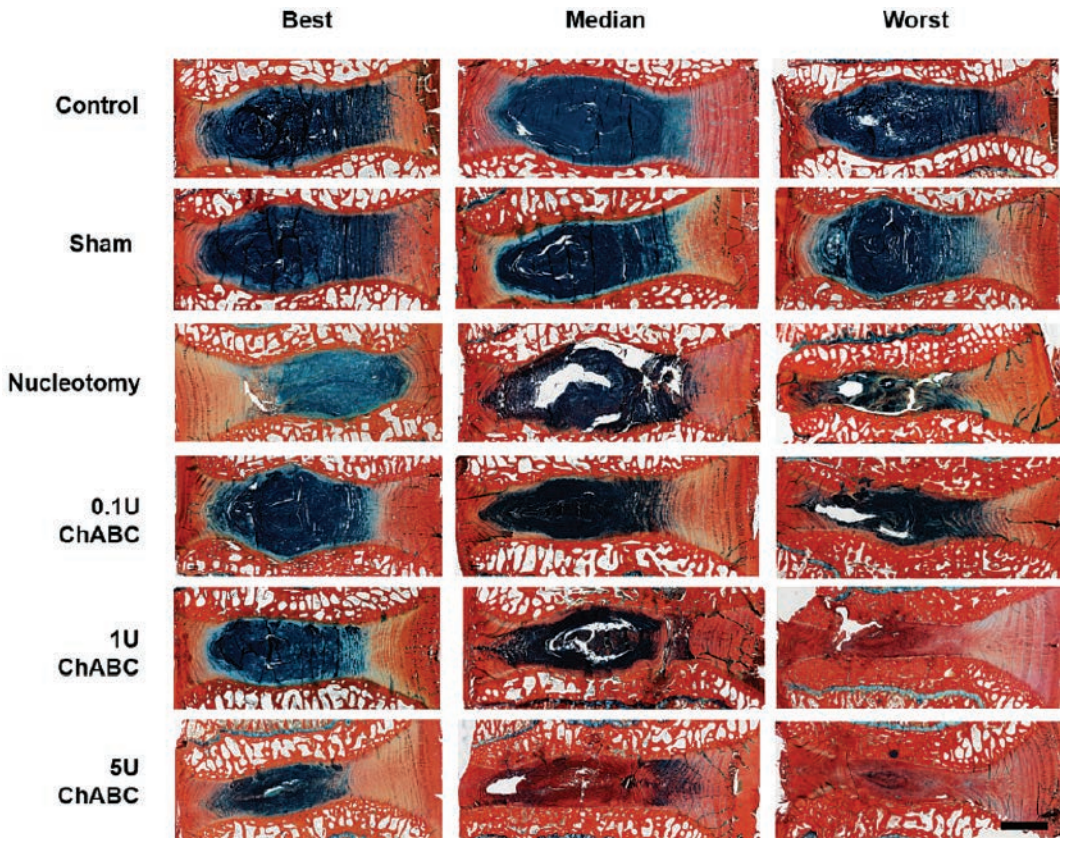

Figure 1. Histology. Degeneration was characterized by fibrosis of the nucleus pulposus, disorganization of the annulus fibrosus, loss of disc height and reduction in alcian blue staining. No degenerative changes were observed in sham discs compared to control. Alcian blue and picrosirius red stain, scale bar = 3 mm.

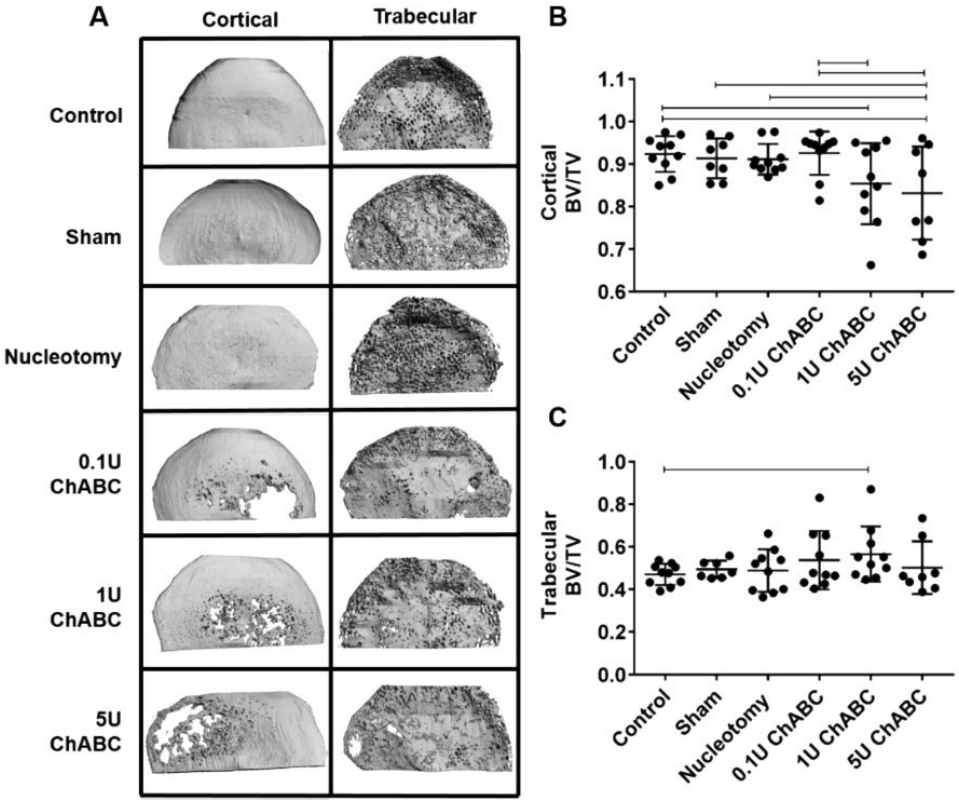

Figure 2. Microcomputed tomography (μCT). 3D reconstructions (A) of the subchondral cortical endplate and the adjacent trabecular bone illustrate alterations to the cortical (B) and trabecular (C) bone volume fraction. The cortical endplate with the lowest BV/TV is shown in (A). Bars denote significance, $p < 0.05$, ANOVA, Tukey's post-hoc test.

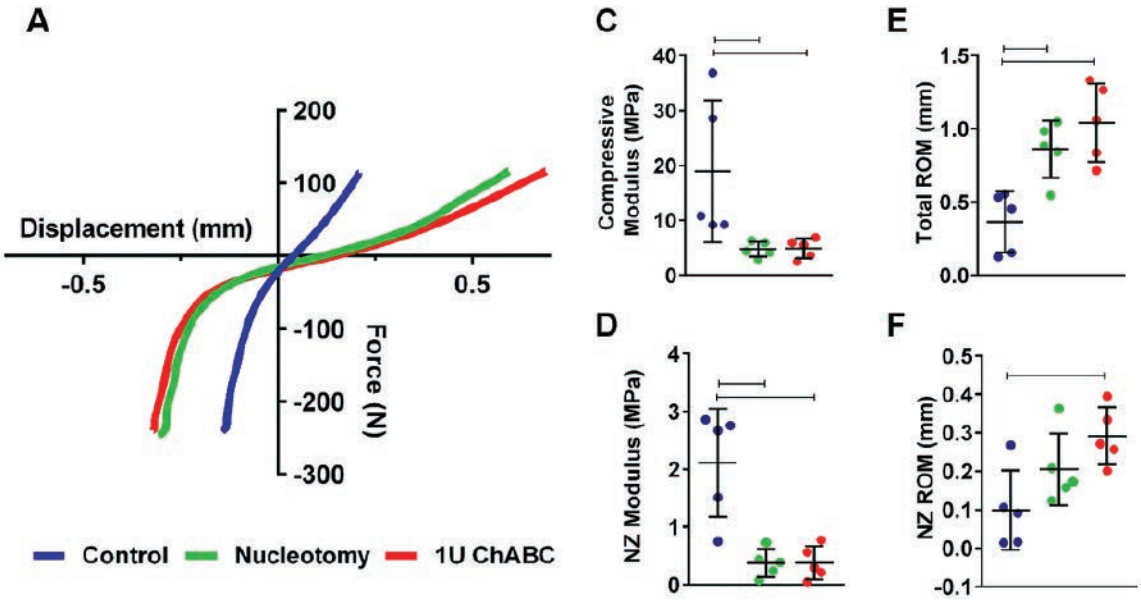

Figure 3. Biomechanical properties. Average force displacement curves (A) generated by LOWESS smoothing demonstrate aberrations in disc mechanical properties with degeneration. Significant differences in compressive modulus (C), NZ modulus (D), Total ROM (E) NZ ROM (F) were observed in degenerative versus control discs. Bars denote significance, $p < 0.05$, ANOVA, Tukey's post-hoc test.

to nucleotomy or injection of 0.1U ChABC also exhibited an initial decrease in DHI (significant at 1, 4, 6, 8 and 10 weeks); however, partial recovery of disc height was observed at 12 weeks in both groups. DHI for control and sham injected discs was unchanged over 12 weeks.

Three-dimensional μCT analysis (Figure 2) demonstrated significant cortical bone loss in the vertebral endplate of the 1U and 5U groups. The bone volume fraction of the adjacent trabecular bone was significantly increased for these same groups. T1δandT2 values in the nucleus pulposus (NP) were significantly lower in the 1U and 5U groups compared to sham and control discs. Nucleus pulposus T2 values were significantly lower in the nucleotomy group compared to sham.T1δandT2 values correlated significantly ($r^2 = 0.63$ and $r^2 = 0.53$, respectively) with Pfirrmann grades. Average force displacement curves (Figure 3) illustrated alterations to the mechanical response of the disc to loading in the 1U ChABC and nucleotomy groups compared to control. Compressive and neutral zone modulus were significantly lower, and range of motion greater, in nucleotomy and 1U ChABC discs compared to controls.

Discussion

A large animal goat model of disc degeneration was established that exhibits a gradient of degenerative changes from mild to severe. This work advances previous caprine animal models of degeneration, which have mainly achieved only mild degenerative changes.[7] Observed changes to both the disc and the bone in the adjacent vertebral endplate are consistent with the changes reported in human disc degeneration.[8,9] Over the 12 week study, nucleotomy and injection of low dose (0.1U) ChABC induced mild to moderate degenerative changes to the disc, as seen via disc height changes, quantitative MRI, and histology. Injection of higher doses (1U and 5U) ChABC induced moderate to severe disc degeneration. Structural derangement of the disc with degeneration in the 1U ChABC and nucleotomy groups was associated with significantly altered disc mechanical function.

Conclusions

In this study, we established a large animal model that replicates the spectrum of disc degeneration seen in humans and provides the basis for future studies of the biological mechanisms underlying disease progression. Moreover, this model can be used to evaluate the therapeutic potential and safety of a wide range of novel biological therapies designed to treat disc degeneration at a variety of stages.

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Biomechanical Estimation of Elbow Valgus Loading in Throwing Athletes as a Means to Reduce Injury Risk

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Introduction

Throwing athletes competing in sports such as baseball, javelin, cricket and handball are exposed to greater ulnar collateral ligament (UCL) injury risks due to accumulated overhand repetitions and elevated arm accelerations.[1-3] Repetitive loads fatigue and damage the elbow's dynamic stabilizers placing greater stress demand on the UCL as a tensile restraint to valgus opening.[4]

Medial elbow instability and chronic pain typically ended careers of competitive athletes prior to 1974; when Dr. Frank Jobe and his surgical team performed the first UCL reconstruction on a professional pitcher, Tommy John, coining the name "Tommy John surgery." Surgical repair has become quite successful with 83% of patients demonstrating excellent results with highest efficacies associated with the muscle-splitting approach.[5] Both primary and UCL revision surgeries have been increasing over the past several decades.[6] Despite the public's perception, primary reconstruction does not enhance performance and tends to decrease quality of play.[7] Furthermore, revision surgeries report longer recovery periods, lower rates of return to play, decreased competitive durability and shortened career length.[6] This abstract outlines how biomechanical assessments of overhand throwing can approximate joint loads for the medial elbow—a surrogate for UCL loading—and how modifying other aspects of the pitching motion may mitigate medial elbow loading. Further understanding of biomechanical influences on the elbow joint in throwers has the potential to reduce primary and revision UCL surgery rates.

Background

Elbow pain is one of the most common injuries associated with overhand throwing—nearly 50% of all baseball players report elbow pain[2, 8] and similar trends are expected in other throwing sports.[8] While managing throwing frequency and intensity is vital for protecting athletes from overuse injuries,[2,3,8] biomechanical analyses of joint and ligament loading during the delivery may identify risk factors for elbow pain and injury.

Question

How are biomechanical screenings established for coaches, parents and athletes to estimate medial elbow loads and how can mechanics be modified to lessen UCL stress?

Discussion

Elbow loading mechanics are estimated using throwing arm kinematics acquired using high-speed motion capture systems (Figure 1A). Throwing arm kinematics are tracked via retroreflective markers adhered to the skin to determine joint centers of rotation, body segment orientations, velocities, and accelerations.[9, 10] These kinematic data are then used to solve Newton's equations[10] to approximate the reaction loads at each joint of the throwing arm. While these joint reaction loads do not account for internal factors, like muscle force or ligament engagement, it does provide insight into the external demands placed on the joint. Computational modeling can then be employed to approximate how muscles and ligaments stabilize the joint.[11]

Ulnar collateral ligament tension stabilizes the elbow when the joint is exposed to valgus loads.[5, 10,12] Valgus torque has been reported as high as 100 Nm during maximal external shoulder rotation,[5, 10, 13] which would overload and cause UCL failure without contributions from active stabilizers.[4, 10, 14] Elbow loading can be exacerbated with increases in throwing velocity, competitive level, and physical size.[15] Elbow valgus moments are continued through acceleration as the humerus internally rotates toward home plate following the late cocking stage, or maximal external shoulder rotation. Forearm inertia resists forward acceleration where it continues to lay back causing flexor-pronator mass stabilizers and the UCL to counteract with varus moments (Figure 1B).[10] Internally generated elbow varus moments compress the medial elbow compartment thereby accelerating the forearm and hand forward in the direction of the throw. Repetitive loading and inadequate rest can impair valgus resistance offered by the flexor-pronator mass muscles causing the UCL to assume a greater role in stabilization.[4]

Modifying other aspects of pitching biomechanics can mitigate elbow loading while simultaneously improving performance.[12, 13, 16] Youth throwing athletes throw with greater variability and gross mechanical flaws—characterized by less elbow flexion at peak

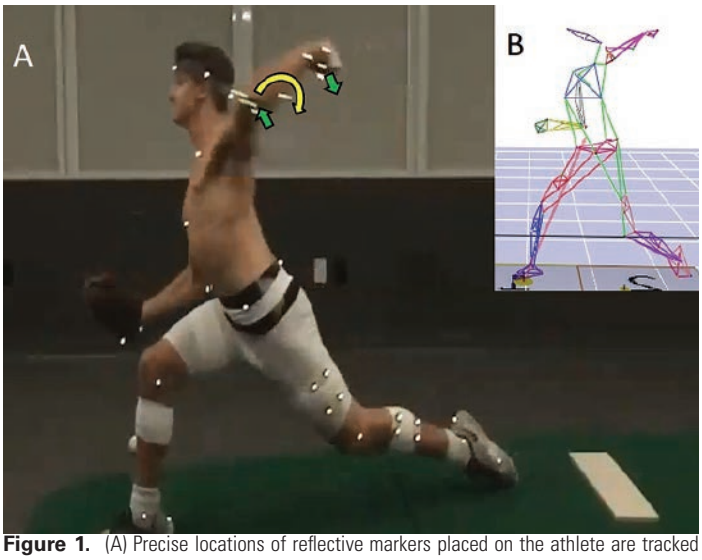

Figure 1. (A) Precise locations of reflective markers placed on the athlete are tracked to quantify throwing kinematics. These data are used to approximate throwing-arm elbow loading (B, yellow arrow), along with modifiable biomechanical variables like stride-length and trunk-tilt.

shoulder external rotation and increased lateral trunk tilt at releasemaking them susceptible to medial epicondylitis and UCL strains.[17] Adult pitchers are more biomechanically consistent, deliver with higher velocities, and are at greater risk of overuse injuries that lead to ruptures of the UCL. Professional pitchers monitored over the course of three seasons showed that elbow injuries were directly related to higher elbow valgus loads.[15] Mechanical consequences owing to greater valgus loading among the injured pitcher group were attributed to higher shoulder external rotation torques and ball velocities.[15] Throwing at high velocities with deeply engrained injurious mechanics from youth development may be the strongest contributors to adult elbow injuries.[15, 17]

Lower body and trunk mechanics impact medial elbow loading.[12,13,18] Stride length, the basis of generating, bracing and transferring energy in the throwing delivery[16] is perhaps the most important modifiable behavior that affects elbow loading. Short strides relative to body height may elicit aggressive external rotation moments for the shoulder in preparation of arm acceleration.[16, 18] Stride length must regulate the degree of rotational opening of the pelvis and trunk relative to foot contact.[12, 13, 16] Early opening of the pelvis decreases elbow valgus loading,[13] while the early initiation of trunk rotation in the transverse plane has been linked to increasing elbow valgus rates of loading.[12] Excessive trunk tilt to the non-throwing arm side tends to increase elbow valgus loading.[19, 20] Quantified changes of every 10 degrees of contralateral lean increases elbow valgus by 3.7 Nm in baseball pitcher.[20]

Closing Remarks

Biomechanical analysis has the potential to decrease injury susceptibility while increasing throwing performance, as pathomechanic risks can be communicated to coaches, parents, and athletes. Motion capture techniques offer measurable means to reduce elbow loading by monitoring linked segment motion; for example, optimizing stride length, stride orientation (stride foot placement), decreasing axial and lateral trunk orientation relative to stride foot contact can decrease elbow valgus loading[12, 16]. Ultimately, identified increases in elbow valgus loads during throwing warrant mechanical changes to minimize demands placed on the dynamic stabilizers and UCL while maximizing ball velocity. The Human Motion Lab at Penn offers a biomechanical and evidence-based approach to identify and correct maladaptive pitching mechanics and should be considered an important tool in the prevention of throwing injuries.

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U·P·O·J

Low-Fidelity Simulation: An Emerging Trend in Orthopaedic Surgical Education

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Introduction

Current and future surgical residents face a vastly different learning environment than those of previous generations. While the number and complexity of required surgical skills continues to increase, institutional changes such as work-hour restrictions, heightened emphasis on patient safety, and efforts to improve efficiency to reduce health-care costs pose challenges to learning in the operating room (OR).[1,2] In response, many surgical residency programs have developed innovative methods to teach surgical skills in non-traditional venues. Simulation training offers residents the opportunity to acquire and practice skills in a learner-centric environment. Its favorability among orthopaedic teachers and trainees is demonstrated by a 2013 national survey reporting that 86% of orthopaedic surgery residents and 80% of program directors agreed that surgical skills simulations should become a required part of training.[3] Yet, 87% of program directors identified a lack of available funding as the most substantial barrier to developing such programs at their institutions. Nonetheless, both the American Board of Orthopaedic Surgery (ABOS) and the Accreditation Council for Graduate Medical Education (ACGME) have mandated use of surgical simulation in orthopaedic residency curricula.[4]

Benefits of Simulation Training

Simulation training has improved operative performance of trainees in a variety of surgical disciplines.[5-9] Moreover, whereas evaluation of resident performance can be difficult in the operating room setting, simulation training allows instructors to prioritize resident learning and focus on evaluating and teaching specific components of complex procedures. Deliberate practice in a simulation setting may be a more efficient teaching modality than experience alone, especially for learning basic psychomotor skills.

Development of technical skills in an ex-vivo setting can lead to a synergistic improvement in trainees' intraoperative educational experience. In a randomized trial, Palter et al[10] demonstrated that residents trained in fascial closure on a low-fidelity simulator not only exhibited better technical skills in the OR, but performed better on an examination of clinical material discussed during the case. These findings are consistent with the Fitts-Posner theory of motor skills acquisition, which proposes that novice learners must devote more active attention to performing tasks. In contrast, trainees proficient in basic surgical skills have sufficient cognitive attention to devote to simultaneously learn surgical decision-making, a critical component of surgical training that is less amenable to learning outside of the OR.[11-13]

Low-Fidelity Simulation: Goals and Challenges

In light of such benefits, surgical simulation technology, and associated costs, have grown rapidly in recent years. For example, Blyth et al[14] developed computerized simulators in which learners perform hip fracture fixation in a three-dimensional virtual environment. In contrast to the realism depicted in such a high-fidelity virtual reality program, low-fidelity simulation aims to develop interventions that incorporate essential elements of a surgical skill into a cost-effective model that can be replicated and implemented in a wide variety of training environments.[9] Low-fidelity interventions are rooted in educational theory, which suggests that fidelity is less relevant for novice learners.[15] Therefore, low-fidelity interventions are best targeted for junior trainees to develop basic psychomotor skills or to learn the sequence of multi-step procedures.

In developing and validating a low-fidelity simulation tool, it is critical to establish a direct relationship between a learner's proficiency using the low-fidelity tool and improvement in the analogous intraoperative skill. For example, while Butler et al[16] successfully trained medical students to perform a diagnostic knee arthroscopy on an anatomic dry model, this skillset did not translate significantly into improved proficiency for knee arthroscopy in cadaver specimens. The authors concluded that the model could supplement, but not replace the cadaveric specimens. Ultimately, researchers in surgical education are challenged to correlate simulation training with improved patient outcomes.

Recent Advancements

Although orthopaedic surgery has lagged behind other surgical disciplines in developing

simulation tools, recent guidelines have spurred interest in the field, as evidenced by three studies published in 2015.

Lopez et al[17] created the Fundamentals of Orthopaedic Surgery (FORS) board (Figure 1), which trains junior-level residents in six skills, including fracture reduction, three-dimensional drilling accuracy, simulated fluoroscopy-guided drill accuracy, depth-of-plunge minimization, drill-by-feel accuracy, and suturing. The FORS board is composed of supplies purchased at a local hardware store for a cost of less than $350. After longitudinal training using the FORS board, a group of 25 medical students outperformed a control group of junior residents in four of the six skills.

Similarly, Coughlin et al[18] developed and validated a simple box model for training and evaluating learners on specific fundamental psychomotor skills of arthroscopy (Figure 2).

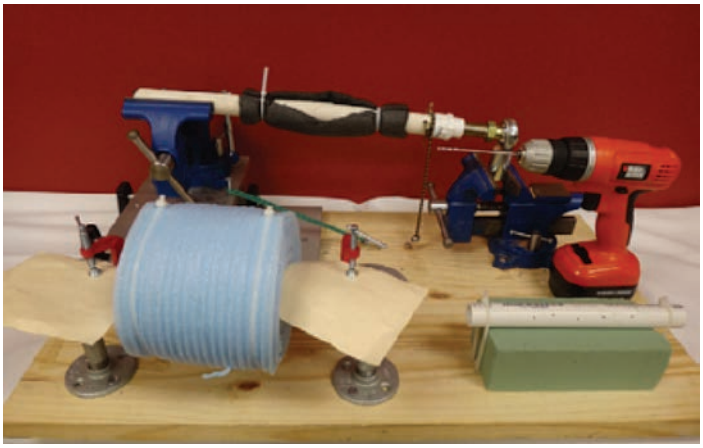

Figure 1. The Fundamentals of Orthopaedic Surgery (FORS) Board.

[Re-printed with permission from Lopez G, et al. A cost-effective junior resident training and assessment simulator for orthopaedic surgical skills via fundamentals of orthopaedic surgery: AAOS exhibit selection. *J Bone Joint Surg Am.* 2015;97(8):659-666.]

The study demonstrated that increased level of experience correlated with improved performance in probing, grasping, tissue resection, shaving, suture-passing, and knot-tying. High intra-rater and inter-rater reliability, evidenced by an intraclass correlation coefficient of 0.99, supports the model's use as a learner assessment tool.

Dedicated time for simulation offers a training ground not only for learners, but also for teachers, as coaching strategies can be designed and validated in this setting. In a randomized trial, Levy et al[19] compared two modalities for teaching two different basic surgical skills: tying a locking, sliding knot and making a low-angle drill hole. In comparison to the learners taught by demonstration alone, those receiving real-time acoustic feedback under operant learning principles demonstrated significantly greater precision in both surgical tasks. Taken together, these studies suggest an emerging interest in leveraging simulation resources for researching educational initiatives.

Future Directions

The University of Pennsylvania is well-suited to be at the forefront of this emerging field. The Human Tissue Lab (HTL) has served as an exemplary model for integrating surgical skills instruction into an orthopaedic residency curriculum. With routine, frequent time devoted to resident education already established in this state-of-the-art facility, Penn can serve as a model program for developing and testing simulation interventions. As one example, a randomized trial that compares two modalities for teaching ACL graft preparation is planned for a resident teaching session in HTL in 2016. In addition, a low-fidelity model to teach the skill of pin placement when drilling a convex surface, simulating an osteochondral lesion, is also in development. Such work promotes the development of orthopaedic surgery trainees amidst a new learning environment.

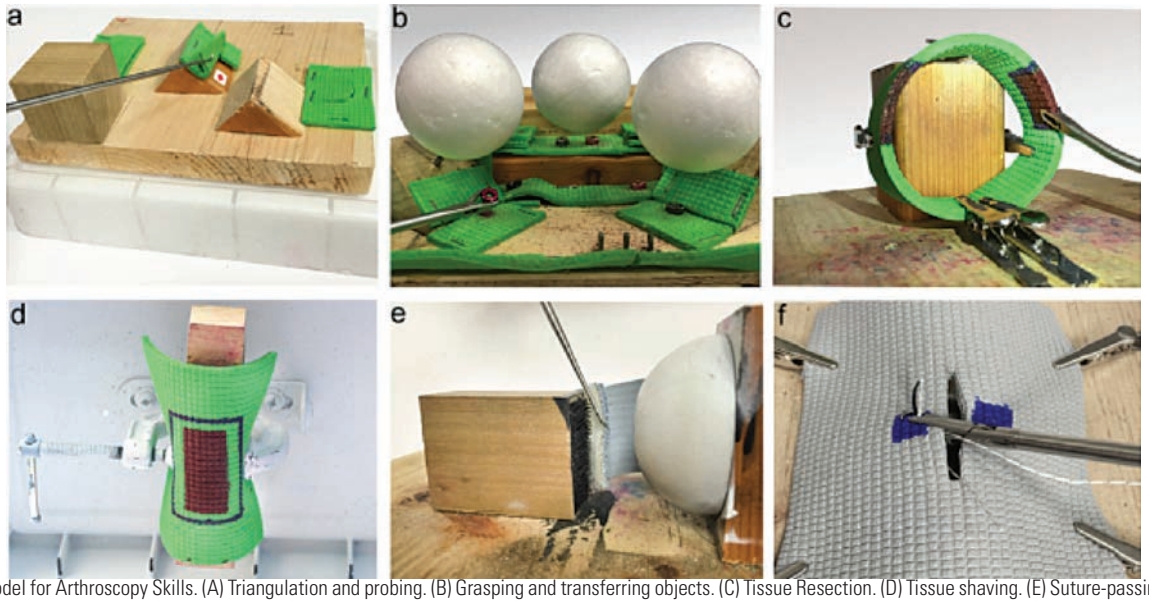

Figure 2. Box Model for Arthroscopy Skills. (A) Triangulation and probing. (B) Grasping and transferring objects. (C) Tissue Resection. (D) Tissue shaving. (E) Suture-passing. (F) Arthroscopic knot-tying.

[Re-printed with permission from Coughlin RP, et al. A validated orthopaedic surgical simulation model for training and evaluation of basic arthroscopic skills. *J Bone Joint Surg Am.* 2015;97(17):1465-1471.]

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Perfectionism—The Foe of Happiness

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The demands of education and occupation appear to be increasing. Performance standards in any given arena are becoming more competitive. In response to increasing stressors, many turn to perfectionism, believing such an approach will ensure success and shield oneself from failure.

Nothing could be further from the truth.

Ambitious individuals tend to label themselves "perfectionists," and many wear this identity as a badge of honor. Rendering perfectionism this positive connotation can conceal larger, more deep-seated issues. A distinction must be made between the "pursuit of excellence" and perfectionism.[7] Working hard or pushing oneself relentlessly to achieve a goal is the sign of a dedicated individual, not necessarily perfectionism. In most cases, perfectionism has its origins in anxiety or self-esteem issues, which themselves have been linked to less personal satisfaction and an increased risk of suicide.[7,9] Perfectionists have a higher risk of eating disorders, anxiety disorders, and depression.[3] The life of one who strives to be 'perfect' is riddled with fear and extreme caution. Creativity, joy, inspiration, and even productivity are throttled when perfection is the only option. Ironically, according to Flett and colleagues,[5] successful people actually are less likely to be perfectionists, as the symptoms of perfectionism are more likely to thwart higher levels of success one might achieve.[2,7]

Many of us try to attain perfection. We try to cultivate (or at least project) perfect marriages, and yes, we strive to perform perfect surgical procedures, even though we may know that perfection is an illusion. Yet, we all have tales of surgeons spending more than 6 hours in an operating room attempting to achieve the "perfect" fracture reduction in a case that typically requires a fraction of the time.

In a vocation as demanding as orthopaedic surgery, perfectionism can sap a surgeon's energy—leaving little room for self-care and relationships. Perfectionists tend to overcommit themselves, and are generally exceedingly sensitive to criticism. They procrastinate, waiting for the 'perfect' time to attend to tasks.[5] For the surgeon, challenging cases may be deferred. Cases that an average surgeon could readily handle on a given day are often referred elsewhere. Instead of doing five excellent procedures, the perfectionist surgeon may spend hours trying to produce the "perfect reduction" in a case that usually requires about one hour of manipulation.

In essence, perfectionists simply fear imperfection, and equate any error with personal defectiveness. They lead their lives convinced that perfection is the only means to self-acceptance.[6]

Origins of Perfectionism

Beneath perfectionism usually lies a self-esteem issue. During formation, the perfectionist likely received messages of conditional acceptance from a significant caretaker, usually a parent.[6] The message was interpreted loud and clear: "I will love you if …" The demands for academic, behavioral, or athletic perfection from a parent can forge a wounded self-image in a child. The presence of affectionless and over controlling parental figures, coupled with a tendency for neuroticism have been found as common denominators in the childhood of perfectionists.[10] Our childhood experiences, in addition to some genetic influences, largely determines the burden of intrusive thinking we each experience. The pressure to perform generates dysfunctional thoughts in the young mind and will linger for the remainder of their lives, unless recognition of distorted thought patterns are recognized and addressed.

Cognitive Distortions

Clearly, perfectionism is a byproduct of dysfunctional and distorted thinking. Cognitive behavioral psychologists have characterized faulty, inaccurate thinking into several cognitive distortions or patterns of erroneous thoughts.[1] Each "cognitive distortion" is merely a lie our brain sends to our conscious mind. Common distortions include ignoring the positive whereupon one's mind is prepossessed with thoughts of all that is wrong with a particular situation, rather than positive aspects of a given occurrence. For example, a preoccupation of the one errant screw in an otherwise superb fracture reduction is a classic example of ignoring the positive. A distortion commonly found in perfectionists is all-or-nothing thinking. That is, one negative event may trigger a cascade of intrusive thoughts which generalize misfortune

into all aspects of one's life. For instance, a difficult surgery to the perfectionist may generate a stream of negative thoughts along the lines of "I am no good," "I am a lousy surgeon," or even "I don't deserve to be called orthopedic surgeon." Even when a perfectionist achieves success, they do not experience the delight of the accomplishment. Instead, there is only relief that this time they did not fail.

Perfectionists also are prone to several other patterns of distorted thinking including personalization and blame—the tendency to blame oneself for something he or she was not entirely responsible for. Another is labeling, whereby one tends to base his or her entire identity on their shortcomings. Instead of acknowledging a mistake, 'labelers' are quick to identify themselves as "losers" or abject failures. Perfectionists may experience as many as 10 common thought distortions, which all lead to diminished personal happiness and joy (Table 1).

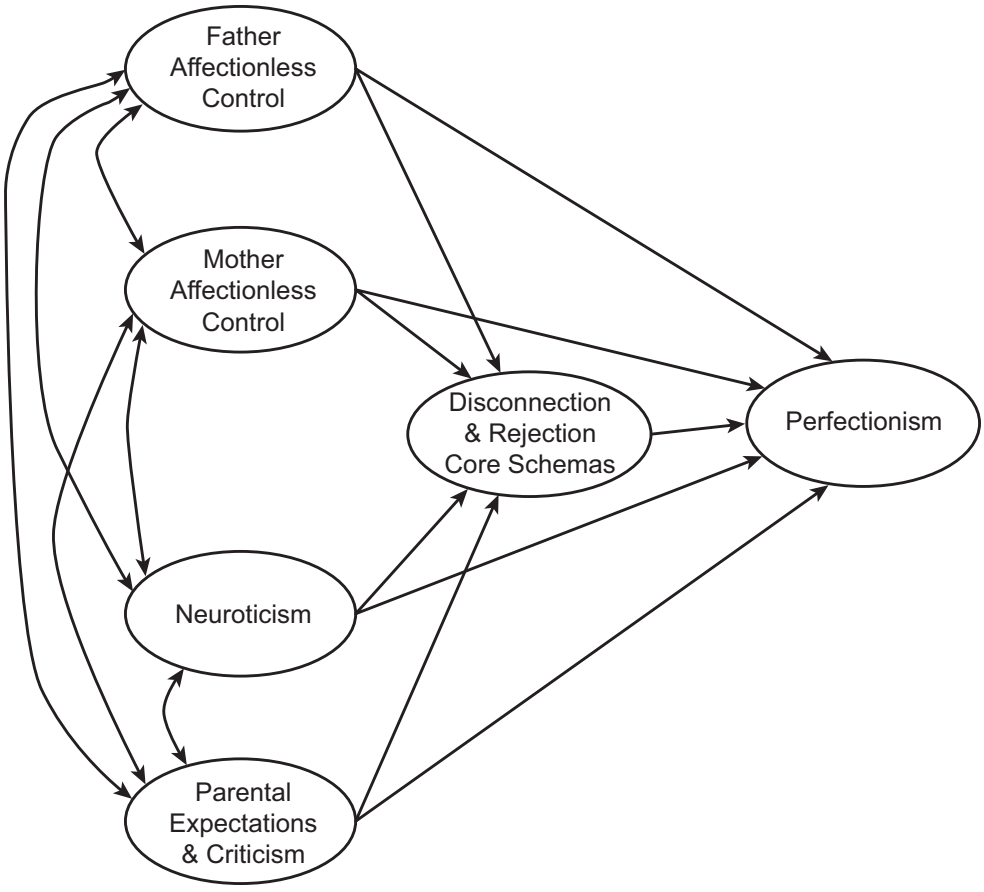
The recognition and awareness of these distorted thoughts is the beginning of the road to recovery. Create space with perfectionistic thoughts by observing them and not *becoming* them. When they arise, simply breathe and let these intrusive thoughts pass. Recognize that perfectionistic thoughts and perfection-driven emotional movements are lies that your mind is presenting to you. The compulsions and neurotic movements that distorted perfectionistic thoughts are to be observed as simply tricks your mind is playing. Much has been written on mindfulness, or living in the moment.[8] When we are entirely present, intrusive and compulsive thinking wanes. Reading books and attending courses on mindfulness, coupled with daily practice, will yield great benefits in managing our minds.

Recovery: Courage to Accept Imperfection

Recovery from perfection requires an overhaul of improper thinking which may take some considerable time. In his 2008 study, psychologist and marriage and family therapist Thomas S. Greenspon PhD proposes "building an environment of acceptance" through self-empathy, encouragement, self-reflection, and dialogue.[7] These are not steps, Greenspon argues, but rather elements of an approach that will help an individual move beyond thoughts of perfectionism.

"Perfectionism, in this approach, is seen as a self-esteem issue arising from emotional convictions about what one must do to be acceptable as a person," Greenspon writes in the study. "It reflects a perfectionistic person's basic sense of reality, not simply a set of irrational beliefs that can be changed by deciding to think differently. There is a great deal at stake emotionally, for which perfectionism is a defense. Overcoming perfectionism is a recovery process, more like nurturing a flower's bloom than like fixing a broken object."[7]

Obviously recovery can be hastened with the help of a therapist, and cognitive behavioral therapy has been shown to be especially effective.[4] A trained therapist can help examine thoughts that evoke anxiety and fear and reframe them into more realistic cognitions. In addition, seeking a mentor who has the right balance of self-compassion and acceptance



may serve as highly effective patterning for one's life. An appreciation that others will accept us more fully when we are authentic and real, rather than a "perfect" pseudoself that our minds have constructed out of fear, may help us become more tolerant of ourselves.

Suggestions to Overcome Perfectionism

1. In the words of David Burns MD:[1] "Dare to be average" for the next 30 days. Accept that you are imperfect and resist the temptation to give into fear. Just *be*, and reconnect with your creative self. Let inspiration and passion rule rather than "shoulds."
2. Make a list of pros and cons on a piece of paper about your perfectionism. Burns uses this exercise to convince his patients that they are less productive when perfectionism takes hold.[1]
3. Another tactic Dr. Burns recommends is to become more "process oriented" rather than results oriented.[1] For example, focus on a good consistent effort in the operating room and release the compulsion to attain the perfect surgery. Implicit with a process orientation is the setting of realistic time limits to each task. Be sure to adhere to them. You will be surprised at the satisfaction and productivity boost you will realize.
4. Look at mistakes as opportunities for growth, rather than as signs of failure. We learn from errors, not successes. Each apparent step backward merely brings us closer to our goals.

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Trauma Tips and Tricks: Measurement and Management of Compartment Syndrome

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Acute Compartment Syndrome (ACS) results when an enclosed fascial compartment surrounding skeletal muscle experiences pressure sufficient to decrease tissue perfusion. While most commonly seen following fractures, it can be seen in many clinical conditions, including but not limited to blunt trauma, crush injuries, iatrogenically from tight wrappings such as casts and splints, arterial injuries, reperfusion following ischemia, and fluid extravasation. Furthermore, it is most commonly encountered in the clinical setting in the lower leg compartments, but can occur in any osseo-fascial compartment.

Compartment syndrome is considered a surgical emergency, as the inability to properly decompress fascial compartments and permit tissue perfusion can result in permanent and irreversible damage to muscle and nerves. Typically, this is a clinical diagnosis, and is characterized by the famed "6 P's." These include Pain, Passive stretch, Paresthesias, Poikilothermia, Pallor, and Pulselessness. Recent studies have shown that manual palpation of compartments is not an effective method of determining compartment tenseness. While this physical exam finding may play a part in the diagnostic process, it cannot be reliably used to confirm or rule out acute compartment syndrome.

It is important to note that many of these "6 P" findings are unreliable, and may only be present after compartment syndrome has been present for a significant amount of time. However, pain with passive stretch, disproportionate pain, and loss of two point discrimination are early signs of acute compartment syndrome that may be used to aid in clinical diagnosis. In pediatric populations, an increased pain medication requirement may be the only indication of impending compartment syndrome.

Physical exam, while a mainstay in the diagnosis of compartment syndrome, is often not possible. This is especially true in the case of comatose or obtunded patients, the mentally ill or demented population, and pediatric patients. In such cases, the use of an inter-compartmental pressure device may be helpful in the diagnosis of impending compartment syndrome and the need for intervention. While significant controversy still exists, recent evidence supports the usage of an absolute Intra-Compartmental Pressure (ICP) $>$ 30 mm Hg or within 30 mm Hg of the resting diastolic blood pressure (ΔP = Diastolic BP - ICP). Diastolic blood pressure measurements should be prior to anesthesia induction, as anesthetics can artificially lower the diastolic blood pressure.

One of the popular devices used at our institution for ICP measurements is the Stryker Intra-Compartmental Pressure Monitor (Figure 1). This device involves the usage of a pressure monitor attached to a small needle and syringe. By introducing a small of amount of fluid into individual compartments, the device is able to measure the pressure of the compartment. This gives the user the ability to gain objective data supporting, or refuting, the diagnosis of possible compartment syndrome.

The use of the Stryker Monitor does have several important considerations, however. First, the disposable needle, chamber and pre-filled syringe should be assembled and placed correctly into the device. Assembly instructions should be referenced. Proper technique is crucial to obtain accurate measurements. Once trajectory is decided, the device should be held at a constant angle with the horizon at all times when measuring. Some prefer keeping the device parallel with the ground for consistent measurements. Prior to measuring pressures, the device needs to be calibrated. While in the trajectory for measurement, a small drop of water should be expressed from the needle tip to create a continuous column of water within the bevel. Then the zero button is pressed, and a "0" on the screen is confirmed prior to measurement.

Depending on the location of investigation, every compartment should be separately

Figure 1. Stryker Monitor.

investigated, as ACS can occur in single or multiple compartments. Consequently, a firm understanding of the anatomy of the various compartments of the body is paramount in the investigation of compartment syndrome. For example, in the lower leg, the anterior, lateral and superficial posterior compartment are measured based on external anatomy and their superficial location. The deep posterior compartment is measured by a medial approach, having the needle hug the posterior tibia to avoid the neurovascular bundle. Measurements should be performed within 5 cm of the fracture, if one is present, as it is the location of the highest compartment pressures. Fracture hematoma can give false pressure reading and should be avoided. Finally, the amount of fluid injected is user dependent, and should be kept consistent. Ideally, less than 0.5 cc of fluid is injected for each measurement, but is dependent on the model used and the compartment being investigated. Injecting excess fluid can result in falsely elevated readings. Multiple measurements in each compartment should be performed to confirm accuracy.

In the treatment of acute compartment syndrome, a thoughtful history and attentive physical exam should remain the mainstay of diagnosis. However, certain patient populations may not be amenable to such investigation; in such cases, the treating physician may require the use of intra-compartmental measuring devices. It also remains a useful adjunct to unclear physical exam findings. Such devices remain a powerful tool in the armamentarium of clinicians that may encounter acute compartment syndrome.

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Locking Cap Designs Improve Fatigue Properties of Polyaxial Screws in Upper Extremity Applications

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Introduction

The advent of polyaxial screw and locking plate systems has provided a new framework for fragment-specific fixation of upper extremity fractures. Previous research has suggested that the ultimate failure strengths of polyaxial screws can be compromised due to excessive screw angulation or inferior locking design. Although this information is valuable, clinical failure of these implants is more likely due to fatigue from cyclic loading rather than an acute event. There is a paucity of data regarding fatigue properties of these implants. This study sought to examine fatigue characteristics of two implant types: 1) locking cap (LC) designs, and 2) cross-threaded (CT) designs. Our goal was to compare LC and CT implants at 0, 10, and 15 degrees of angulation to determine the effect of locking mechanism on screw-plate interface failure. We hypothesized that LC implants would have superior fatigue properties in comparison to CT designs and that increased angulation of the screw would have a negative impact on the fatigue life of CT implants, but would not have any effect on LC implants.

Materials and Methods

A total of 72 screws were tested in four upper extremity implants. Two implants were LC designs (Miami Device Solutions (LC1), Zimmer Biomet NCB (LC2)) and two implants were CT designs (Smith and Nephew PERI-LOCVLP (CT1), Stryker VariAx (CT2)). Using a Bose 3550 with a 49Nm torque cell, screws were locked into place with a rotational speed of 45 degrees/sec and a linear speed (mm/sec) that was determined by the pitch of the threads on the shaft of the screw. All screws were aligned with a custom-built jig to 0, 10 or 15 degrees relative to the plate and torqued to exact manufacturer specifications (n = 6 for each group). Screw-plate assemblies were potted in a low temperature metal alloy (Cerralow 117) such that there was 10mm of vertical clearance between the surface of the alloy to the center of the screw head. The potted implant was securely held within the test frame such that a hardened steel actuator with a 3mm diameter cylindrical tip had a line of action 4mm away from the nearest face of the implant. Implants were fatigue tested on a Bose 3330 universal test frame with a 4450N load cell. For fatigue testing, a staircase method consisting of 11 steps was employed. The first step imparted a 100N cyclic force on the screw for 5000 cycles at a rate of 2Hz. Each subsequent step increased the linear force by 50N, increasing the moment applied to the screw-plate interface by 0.2 Nm up to 2.4Nm. Failure of the implant was defined as screw displacement exceeding 5 degrees from the original axis. If the implant survived

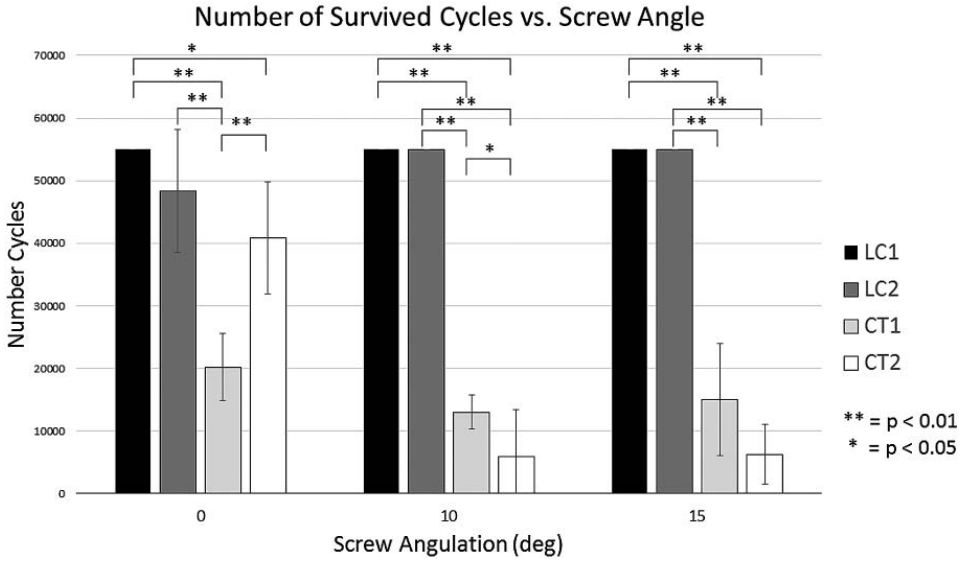

Figure 1. Comparisons of survived cycles before failure. In all but one case (LC2 vs CT2 at 0 degrees), the LC designs sustained a significantly higher number of cycles in comparison to CT designs. With the exception of one case (CT2 at 0 and 10 degrees), there was no significant decrease in survived cycles due to changes in angulation for a particular design. Error bars are +/− 1 standard deviation.

the fatigue test, a ramp to failure was performed at a rate of 0.1mm/s. Cycles to failure were statistically compared using one-way analysis of variance and Tukey honestly significant difference post hoc comparisons with a critical significance level of $\alpha = 0.05$.

Results

Fatigue testing demonstrated that both LC designs were consistently able to sustain a significantly higher number of cyclic loads than either of the CT designs (Figure 1). There was only one comparison (LC2 and CT2 at 0 degrees) in which the LC design did not sustain a significantly higher number of cycles. Further, there were no significant differences in the number of cycles sustained by LC designs due to changes in screw angulation.

Discussion and Conclusions

Likely because of the spherical screw head geometry, LC fatigue characteristics are not influenced by the orientation of the screw relative to the plate. Application of a locking cap in the operating room requires extra time, but provides significantly more robust fixation of the screw to the plate and provides a more predictable and consistent result. It should be noted that this study was limited to upper extremity implants. Lower extremity implants may perform differently than those in this study and warrant further investigation.

Use of a "Kickback" screw in olecranon fractures stabilization increases stability

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Background

Plate fixation continues to be an effective tool to address unstable olecranon fractures, but the presence of osteoporotic bone often leads to complications that result in undesirable fixation failure when subjected to external loads. Previous studies have sought to elucidate the differences between various implants to determine if certain design elements provide superior stability during loading, but no significant differences were found. The use of an additional screw, placed out of plane, in the proximal fracture segment may increase stability. The screw is targeted from distal to proximal through the plate and is aimed towards the tip of the olecranon ("kickback" screw—Figure 1). No biomechanical testing has been performed to determine whether the addition of this screw provides additional stability to the ulna during dynamic loading. The goal of this study was to determine the efficacy of the kickback technique by applying a series of increasing loads to the distal ulna during prescribed elbow flexion/extensions. We hypothesized that utilization of a kickback screw would improve the stability of the construct in comparison to implants that did not receive the screw.

Materials and Methods

Eight paired, fresh–frozen, cadaveric forearm specimens were used for this study (2M, 2F, average age: 88.25). All soft tissues were removed except the elbow capsule, triceps, and radioulnar interosseous ligament. Four Synthes 3.5mm LCP olecranon plates were implanted using the standard surgical technique, while the other four plates were implanted with an extra "kickback" screw. To simulate a comminuted fracture, a transverse osteotomy was created at the center of the sigmoid notch of each specimen and a second osteotomy was made 3 mm distal to the first osteotomy. The bone between the osteotomies was removed so that there was no bony contact between the proximal and distal portions of the ulna. The triceps tendon was sutured to a looped nylon strap to enable a flexion/extension motion of the elbow during displacement-controlled motion of the actuator of the Bose 3550 test frame. To examine the performance of the plates, biomechanical testing followed a previously published protocol. Briefly, the sectioned ends of the humerus and radius/ulna were potted in poly(methyl methacrylate). With the humerus secured to the test frame, the radius/ulna pots were fitted to a custom-built aluminum fixture (mass = 1.2kg) that allowed for the incremental attachment of hanging masses at a distance of 22.5cm from the olecranon fossa. 20mm of displacement of the triceps tendon corresponded to a range of motion between 90° and 60° of flexion. Motion between bone fragments was tracked with a 3-D motion tracking system (Optitrack) to within 0.1mm of accuracy. The arm was initially cycled 30 times at 0.2 Hz with an empty fixture, weighing 1.2kg. This process was repeated by increasing the hanging mass in 0.5kg increments until failure occurred. Failure was defined as 1) permanent relative displacement of the proximal and distal fragments of more than 3 mm, or 2) catastrophic failure of the bone or implant.

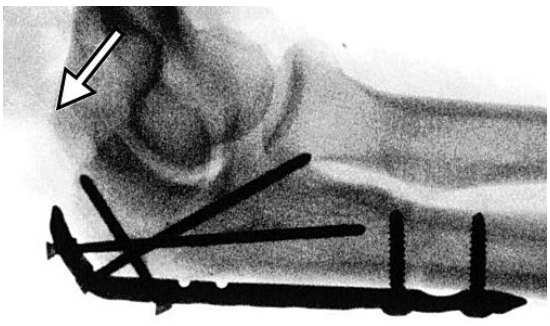

Figure 1. Location and direction of the additional "kickback" screw is shown by the white arrow on the radiograph. The standard group received the same screw pattern, but without the additional screw.

Results

The addition of the "kickback" screw increased the number of survived cycles and maximum load sustained in three out of four cases (Table 1). Briefly, the standard group survived an average of 128 cycles before failing at an average of 3.33kg, while the kickback group survived an average of 174 cycles before failing at an average of 3.83kg. There was no statistical difference between the groups in terms of cycles survived or maximum loads sustained. Three out of four samples from the standard treatment experienced gradual failure due to fracture displacement, while three out of four samples from the kickback group experienced catastrophic failure prior to 3mm of fracture displacement.

Table 1

Group	Specimen	Final Load (kg)	Final Cycle #	Failure mode
Standard	1	5.2	211	Catastrophic Failure
	2	2.2	90	>3mm Fracture Displacement
	3	4.7	211	>3mm Fracture Displacement
	4	1.2	1	>3mm Fracture Displacement
	Average	3.33	128	
Kickback	1	6.2	316	Catastrophic Failure
	2	3.7	174	Catastrophic Failure
	3	2.2	61	Catastrophic Failure
	4	3.2	144	>3mm Fracture Displacement
	Average	3.83	174	

Discussion and Conclusion

Although the results from this experiment do not reach statistical significance, we are encouraged by the improvements in survived cycles and sustained load in three out of four cases. Further, it is interesting that the "kickback" screw seems to mitigate the magnitude of relative bone fragment migration. These results reinforce our clinical decision to allow for early range of motion in patients with a "kickback" screw to allow for earlier return to activity. The study requires more samples in order to increase statistical power and therefore further tests will be conducted in the future to fully determine the utility of a "kickback" screw in olecranon repairs.

Arthroscopic Lysis of Adhesions Improves Knee Range of Motion after Fixation of Intra-articular Knee Fractures

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Introduction

Intra-articular fractures about the knee often necessitate surgical reduction and stabilization. Arthrofibrosis requiring surgical intervention after intra-articular trauma involving the knee has been reported to be as high as 14.5%, and patients with prolonged application of external fixation are at the highest risk for developing arthrofibrosis.[1-10]

The average knee range of motion (ROM) motion has been reported to be 107 degrees after intra-articular distal femoral fractures and 105 degrees after proximal tibial fractures, while the arc of motion of a normal knee is 0 to 135 degrees.[8,12,13] In general, flexion from 0 to 125 degrees is sufficient for daily activities, including sitting and stair climbing.[13] Small impairments of ROM of the knee can cause gait disturbances and significantly increase the energy expenditure required for daily activity. [14]

Traditional treatments for arthrofibrosis after intra-articular fractures about the knee include manipulation under anesthesia (MUA), open quadricepsplasty, and surgical arthroscopic lysis of adhesions (SALKA). Although SALKA has been described to treat posttraumatic knee arthrofibrosis, to date there are no studies describing this procedure's efficacy.

The purpose of this study was to examine the immediate and sustainable range of motion (ROM) changes after surgical arthroscopic lysis of knee adhesions for post-traumatic knee stiffness after open reduction internal fixation.

Materials & Methods

This study is an IRB approved retrospective review of a consecutive series of patients at a single institution with arthrofibrosis after internal fixation about the knee (tibial plateau, patella, distal femur) who underwent SALKA from 2009-2014. 13 qualifying patients were identified (mean of 35 years, range 22-67 years) that underwent SALKA for posttraumatic knee stiffness. The primary outcome variable was change in knee ROM following SALKA both immediately after surgery and at latest follow up. Factors including gender, age, body mass index, tobacco use, laterality, associated injuries, immunocompromised status, and times from surgical fixation to SALKA and from SALKA to last follow up were evaluated. Statistical analysis was performed using one tailed paired Student's *t* test for pre- and post-operative group comparison. An *a priori* power analysis was performed.

ROM was assessed under general anesthesia prior to SALKA in all cases. Standard anterolateral and medial portals were made with occasional use of posterior, trans-septal, and suprapatellar portals as deemed necessary. Adhesions were lysed using a radiofrequency ablative device in the medial, lateral, posterior, and suprapatellar compartments. Special care was taken to release the quadriceps off the femur with an elevator through additional superior parapatellar portals. After lysis, manipulation was performed using the proximal tibia as the primary lever. Force was applied in a controlled graduated fashion at the knee. Manipulation was used to achieve a minimum of 110 degrees of flexion. ROM was assessed after SALKA while the patient was still sedated under anesthesia.

Results

The patients included 9 (69%) males and 4 (31%) females. Mean time between ORIF and SALKA was 245 days (range, 84-509 days). Average follow up after SALKA was 137 days (range 8-782 days). Injuries leading to SALKA included 9 (69%) tibial plateau fractures, 3 (23%) patella fractures and 1 (8%) distal femur fracture. Patient demographics are summarized in Table 1. In order to detect a large effect size, Cohen's D 1.15, assuming a type-I error rate of 0.05 and a power of 0.80, we would need to enroll a minimum of 11 patients in this study to compare preoperative to postoperative ROM.

Prior to SALKA, the mean ROM arc was 75 degrees (range 5-118 degrees; SD = 30 degrees) in the office. The mean ROM arc was 72 degrees (range 40-110 degrees; SD = 23 degrees) under sedation immediately prior to surgery. The mean intraoperative arc of motion achieved at the time of SALKA was 128 degrees (range 40-110 degrees; SD = 23 degrees). The mean intraoperative improvement was 56 degrees (range 25-95 degrees; SD = 25) ($p < 0.0001$). At the most recent follow up, the mean ROM arc was 101 degrees (range 30-140 degrees; SD = 29 degrees). The mean improvement of ROM arc from office visit prior to SALKA to latest follow up visit was 26 degrees (range 0-69 degrees; SD

Table 1: Descriptive Statistics of All Patients in the Study Population

Average Age (years)	35(22–26)
Gender	
Male	9 (69%)
Female14 (31%)	
Time between ORIF and Lysis (days)	245 (84–509)
Follow up time since Lysis (days)	137 (8–72)
Fracture Type	
Tibial Plateau	9 (69%)
Patella	3 (23%)
Intra-articular distal femure	1 (8%)
Laterality	
Right	6 (46%)
Left	7 (54%)
Comorbidities	
Smoker	3 (23%)
Diabetes Mellitus	0 (0%)
Cardiac Disease	0 (0%)
Polytrauma	
Obesity	0 (0%)
Immunocompromised	0 (0%)

= 25 degrees)($p = 0.001$). An average of 27 degrees (range -5-95 degrees; SD = 27, $p = 0.002$) was lost between immediate post-operative ROM and that at the most recent follow up (Figure 1). One patient gained 5 degrees of motion from immediately after SALKA to the latest follow up.

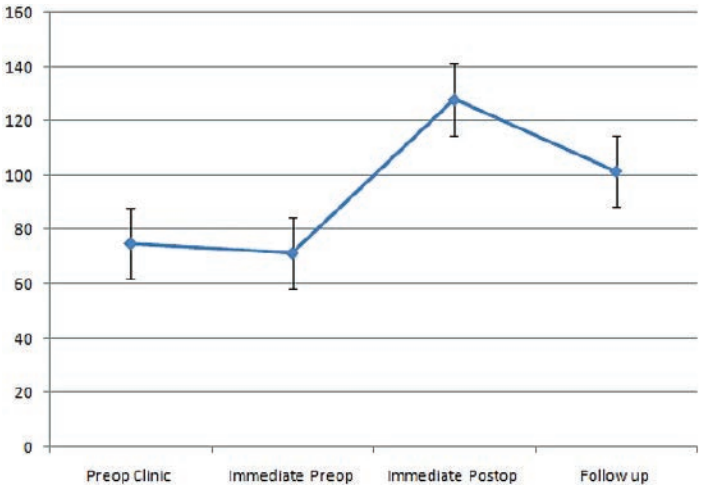

Figure 1. Range of Motion Before and After SALK.

Discussion

Traditional treatments for arthrofibrosis after intra-articular fractures about the knee include MUA, open quadricepsplasty, and SALKA. While there are previous series on the efficacy of MUA and open quadricepsplasty for treatment of posttraumatic knee arthrofibrosis, to our knowledge, there are no reports regarding the efficacy of SALKA.[15-16]

This study is limited as a retrospective chart review with a small sample size (n = 13). Despite an *a priori* power analysis showing that this study was adequately powered, our study was powered only to detect large effect sizes. As a surgical procedure, SALKA can be quite variable with patients having a variety of demographics, co-morbidities, and types of previous internal fixation procedures. These factors limited our ability to make specific conclusions regarding the true impact of patient risk factors on ROM before and after SALKA. Nevertheless, given that each patient were measured against themselves, the efficacy of SALKA can be assessed.

SALKA improved ROM intra-operatively from an average of 72 degrees immediately before surgery to 128 degrees directly after surgery or a 56 degree (78%) improvement in total ROM. All patients in this cohort showed improvement of ROM immediately after surgery. At latest follow up visit, mean ROM was 101 degrees or 26 degrees (35%) of sustained improvement in total ROM from the preoperative visit. At latest follow up, patients lost an average of 27 degrees of ROM from immediately after SALKA. This decrease in ROM from immediately after SALKA to latest follow up visit is biased by the fact that the immediate post-operative measurement was obtained intra-operatively while the patient was still anesthetized.

Our results are comparable to MUA and open quadricepsplasty for posttraumatic knee arthrofibrosis where an average improvement of 64 degrees and 76.3 was seen immediately after these respective procedures. MUA and open quadricepsplasty lost 13 degrees and 25 degrees respectively from immediately postoperatively to latest follow up.[15-16] Although there are no reports for SALKA for posttraumatic arthrofibrosis after ORIF, our results are comparable to SALKA for arthrofibrosis after total knee arthroplasty (TKA). A systematic review reports that that SALKA increases average ROM ranging from 16.5 - 60 degrees for treatment of the stiff TKA.[17]

Conclusions

SALKA for arthrofibrosis of the knee after articular fracture fixation increases range of motion. This improvement in ROM is similar to results found in MUA and open quadricepsplasty. While an improvement from pre-operative ROM is obtained, the ROM gains in all 3 procedures diminish over time. SALKA offers an advantage over MUA in that arthroscopy allows the surgeon to examine and treat soft tissue impingement, loose bodies, or adhesions under direct visualization. SALKA is also less morbid than open quadricepsplasty. Although SALKA shows promising results, this technique may be technically challenging as it may require a surgeon to use posterior,

trans-septal, and suprapatellar portals. Indications for MUA, quadricepsplasty versus SALKA for treatment of posttraumatic arthrofibrosis are unclear. Future research may directly compare these three procedures and develop a protocol for their specific indications in treatment for posttraumatic knee arthrofibrosis after ORIF.

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Peroneal Nerve Palsy I: Evaluation and Diagnosis

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Background

Palsy of the peroneal, or fibular nerve, can be a devastating injury and is the most common mononeuropathy of the lower extremity. The clinical manifestations of peroneal neuropathy range from mild cutaneous symptoms to widespread sensory loss, pain and foot drop leading to ambulatory dysfunction. Injury to the nerve can be acute or chronic, and may result from intrinsic dysfunction or extrinsic manifestations. While most commonly injured at the knee, dysfunction may occur due to compression or injury of nerve fibers anywhere from the spine to the hip or ankle. This article reviews the anatomy of the peroneal nerve, the clinical findings, etiology and evaluation of suspected peroneal nerve injury, and treatment options.

Anatomy

The peroneal nerve contains fibers from L4-S1 spinal roots from the lumbosacral nerve plexus and is a direct branch of the sciatic nerve. In the distal posterior thigh, the sciatic nerve branches into the tibial nerve and the common peroneal nerve; the latter courses proximally between the biceps femoris muscle and the lateral head of the gastrocnemius muscle. Exiting the popliteal fossa posterior to the knee joint, the nerve then wraps around the head of the proximal fibula deep to the peroneus longus muscle, which is a frequent site of compression or injury.

The proximal nerve supplies three articular sensory branches to the knee, as well as the lateral sural cutaneous nerve (LSCN). The LSCN supplies sensation to a portion of the posterolateral leg as well as anastomosing with the medial sural cutaneous nerve (a branch of the tibial nerve) to form the sural nerve.

Below the level of the head of the fibula, the common peroneal nerve then branches into superficial and deep branches; the superficial peroneal nerve (SPN) innervates the two muscles of the lateral leg compartment, the peroneus longus and brevis, which aid in eversion and plantar flexion of the foot. The SPN supplies cutaneous sensory innervation to the majority of the dorsal foot and the lateral ankle.

The deep peroneal nerve (DPN) travels deep to the peroneus longus muscle and innervates the anterior leg compartment, responsible for toe extension and foot dorsiflexion, namely the tibialis anterior, extensor hallicus longus, extensor digitorum longus, and peroneus tertius muscles. At the ankle the nerve supplies an articular branch to the joint itself, and then splits into a lateral and medial branch. The lateral branch innervates intrinsic foot extensors, namely the EDB and EHB, while the medial branch supplies cutaneous sensory innervation to the first web space of the dorsum of the foot.

Etiology

There are numerous ways in which the peroneal nerve can be injured, either directly or indirectly. Compression of the common peroneal nerve at the level of the fibular head is the most common modalities of injury to the nerve. This occurs due to the position over the bony prominence, combined with its relatively superficial position and frequent tethering of the nerve by the origin of the Peroneus Longus tendon. Tight clothing, thin body habitus, compressive casts/splints/pneumatic devices have all been reported to contribute to direct compression of the peroneal nerve at the level of the fibular head with resulting nerve palsy. Positioning during surgical intervention, as well as in cases of prolonged bedrest, has been shown to cause peroneal neuropathy secondary to prolonged direct compression. In addition, direct compressive injury due to rare mass lesions, varus malalignment of the knee (causing repetitive stretch) and lateral osteophytes (causing direct traction injury) has been reported in the literature.

Direct injury to the peroneal nerve, as well as its antecedent and descending branches, can occur due to penetrating wounds, iatrogenically during surgery, compartment syndrome of the leg, or musculoskeletal injuries with displaced osseous fragments. A concomitant injury rate to the peroneal nerve in tibial plateau fractures has been reported in as high as 1% of cases, with nerve injury occurring with both initial trauma and iatrogenically during surgical fixation. Injuries to antecedent nerve fibers in the sciatic nerve can occur in the setting of acetabular and hip fractures, as well as posterior hip dislocation.

Furthermore, peroneal nerve injury has been reported in the setting of acute ligamentous injury to the knee, most commonly when seen

with rupture of the anterior cruciate ligament. A recent study has shown that posterolateral corner injuries of the knee with concomitant bicep avulsion or fibular head fractures have a 90% incidence of peroneal nerve displacement, which could potentially predispose to injury during surgical exploration.

The peroneal nerve can also experience dysfunction in the setting of acute stretching or traction injuries. This can occur with dislocations of the knee as the nerve is stretched over the posterior condyle of the femur, and can frequently coincide with vascular compromise. Additionally, total knee arthroplasty does have a rare complication of peroneal nerve palsy due to increased traction of the nerve, either during surgery or with post-operative positioning. While rare, release of flexion contractures or correction of a valgus deformity in total knee arthroplasty places new demands on the excursion distance of the peroneal nerve fibers and may cause clinical symptoms. A similar concept is seen in the sciatic nerve with total hip arthroplasty procedures that produce increased leg length and offset.

Ankle sprains have also been shown to cause direct injury to the peroneal nerve, proportional to the grade of the sprain itself. The typical injury pattern of plantar flexion and inversion of the ankle places stretch not only on the everting muscles of the lateral compartment but also on the superficial peroneal nerve, due to its proximal tethering at the level of the fibula. While the use of potentially compressing splints and casts is a further cause of peroneal neuropathy, a study of 66 patients with ankle sprain showed that over eighty percent of patients with a grade III ankle sprain had electrodiagnostic evidence of peroneal nerve injury 2 weeks later.

Clinical findings

Depending on the level of injury, different clinical manifestations can be seen in peroneal nerve injury. Injuries to the L5 nerve root itself can frequently be seen with disc herniation, and may be accompanied by low back pain as well as peripheral symptoms to the nerve. In addition, patients may complain of weakness of ankle inversion, controlled primarily by the tibialis posterior and the tibial nerve.

Injuries to the proximal nerve fibers, either as they exit the spinal column or in the sciatic nerve, will cause a global injury that affects the entire peroneal nerve and its descending branches, causing widespread cutaneous and muscular deficits, and may vary depending on the location of the injury and the specific fibers affected.

A proximal injury to the common peroneal nerve itself will cause foot drop and an inability to extend the toes, due to the lack of innervation of the anterior compartment by the DPN. The SPN will also be affected, causing a deficit in foot eversion due to palsy of the SPN, which innervates the lateral compartment of the leg. Patients with foot drop will frequently complain of a "slapping gait," and that their toes drag the ground while walking. In addition, cutaneous sensation will be lost over the dorsum of the foot and the lateral ankle.

As many common peroneal nerve injuries occur at or below the level of the fibular head, it is important to differentiate if the the lateral sural cutaneous nerve (LSCN) is still intact. Absense of LSCN will cause a loss of cutaneous sensation to the posterolateral proximal calf, as well as affect fibers in the Sural Nerve.

Injuries to SPN and DPN will cause discrete clinical manifestations that correspond to their muscular compartments and sensory maps. Superficial peroneal neuropathy will cause cutaneous symptoms of the lateral ankle and dorsal foot, and will result in an inability to evert the foot. Conversely, DPN palsy will cause sensory deficits of the first dorsal webspace, as well as a lack of dorsiflexion and toe extension resulting in the characteristic "foot drop." An isolated lesion to the distal DPN may cause a deficit in toe extension only.

Testing modalities

When a peroneal nerve injury is suspected, a detailed history and physical exam is paramount to proper diagnosis. Cutaneous manifestations such as burning, tingling, and numbness may be seen upon provocative testing such as Tinel's sign, while gait and strength analysis may show muscular deficits when compared to the contralateral side. Reflexes of the lower legs should be tested and compared, as the presence of a pathological reflex (i.e. a positive Babinski reflex) may indicate a central nerve lesion. However, several modalities exist to elucidate the precise cause and level of possible injury.

Imaging studies such as X-rays, CT's and MRI's are particularly useful in the diagnosis of nerve injuries. Spinal conditions such as herniated disks as well as mass lesions may require an MRI, while arthritis, fractures, or dislocations of the knee and surroundings structures can cause direct injury to the Common Peroneal Nerve and can be elucidated on Xray or CT-scan.

In the absence of extrinsic injuries to the nerve, it may be necessary to consider intrinsic factors involved in nerve palsy. Systemic abnormalities such as diabetes, polyarteritis nodosa, Hereditary Neuropathy with Liability to Pressure Palsy (HNPP), and Charcot-Marie-Tooth can cause demyelination and dysfunction of the nerve at any point along its length as well as predispose to increased susceptibility to pressure palsy. Electrical studies such as EMG (electromyography) or NCS (nerve conduction study) can test the ability of electrical impulses to travel along individual muscles and nerves, respectively. Nerve conduction studies are particularly useful in determining if neuropathy is due to demyelination (which shows slowing of action potential propagation) or axon loss (illustrated by decreased amplitude of action potentials).

Biopsy of a muscle or nerve itself can be considered definitive proof of instrinsic dysfunction; however, the above testing modalities have all but obviated the need for invasive biopsy.

Conclusion

Peroneal nerve palsy is a common lower extremity injury after trauma. It requires a thoughtful approach by the treating physician, and in **Part II** we will discuss treatment options.

For a full list of references, please contact the primary author.

Considering Cartilage Lesions in the Evaluation of the Adult with Joint Pain

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Introduction

Patients commonly present to orthopaedic surgeons for evaluation of "joint" pain. The etiology of the pain is generally articular or peri-articular; however, occasionally the pain may be referred from a regional or distant location. A thorough history and physical examination in conjunction with appropriate radiographic imaging usually leads to an accurate diagnosis. Common causes of large joint (shoulder, hip and knee) pain with which orthopaedic surgeons are familiar and can consistently diagnose accurately include osteoarthritis, infection, avascular necrosis, rotator cuff injury of the shoulder, and meniscus tears of the knee. Several additional entities more specific to the individual joints exist for which the familiarity and level of diagnostic expertise varies based on pathology prevalence and level of surgeon experience and sub-specialization. Occasionally, a bone lesion will be identified in the workup of joint pain. Determining whether the bone lesion is the source of the patient's symptoms is crucial to ensuring the patient receives appropriate management. Misdiagnosed and/or inappropriate management of malignant bone lesions leads to poor outcomes.[1-4] Alternatively, attributing a patient's symptoms to a benign cartilage lesion should occur only after ruling out other, more common, causes of joint pain in the adult patient. This article will focus on common cartilage lesions that may be encountered in the evaluation of the adult with joint pain.

Benign Cartilage Lesions

Osteochondroma is the most common benign bone lesion and it is not uncommon for a person with an osteochondroma to have multiple lesions.[5] Osteochondromas have a stalk that is confluent with the normal medullary canal attached to a cartilage cap. Typically, they arise from the metaphysis of long bones, may be associated with metaphyseal broadening and are directed away from joints (Figure 1a). Osteochondromas usually present and grow in childhood or adolescence; growth or the development of pain in adulthood warrant further evaluation, as malignant degeneration is known to occur at a rate of $< 1\%$ for solitary lesions and in 1%-10% of patients with multiple osteochondromas (Figure 1b).[6,7] Pain does not necessarily mean a lesion is malignant; osteochondromas can cause pain due to tendon or soft tissue irritation (bursitis), irritation of an adjacent nerve or if the stalk fractures. Advanced imaging (CT +/− MRI) can be helpful to evaluate a painful osteochondroma. A cartilage cap $>$ 2cm in adulthood has been shown to be suggestive of secondary chondrosarcoma, as the cartilage cap should decrease with the termination of skeletal growth as growth factors disappear.[7,8]

Enchondromas are benign tumors of hyaline cartilage that typically occur in the medullary canal in the metaphysis of long bones (particularly proximal humerus and distal femur) or in the distal appendages. Almost always,

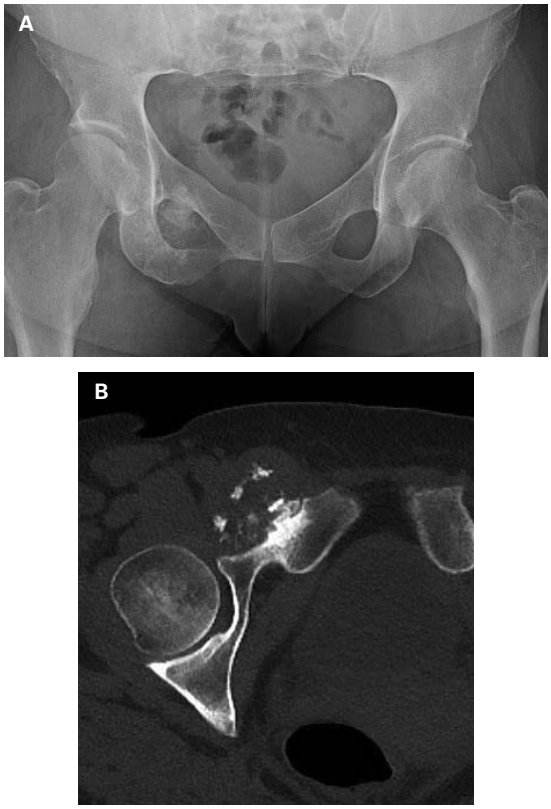

Figure 1. (A) An AP pelvis radiograph demonstrating a sessile osteochondroma of the proximal right femur as well as a secondary chondrosarcoma arising from an osteochondroma of the right superior pubic ramus in a 61-year-old female with Multiple Hereditary Exostoses (MHE). The patient presented with a slowly progressive painful mass in her right anterior pelvis. (B) Axial CT image of the right hip reveals the superior pubic ramus secondary chondrosarcoma. Note the stippled calcifications and loss of "regular" osteochondroma architecture. Surgical pathology confirmed the presence of a Grade 1 chondrosarcoma.

enchondromas are asymptomatic and found incidentally.[5] Radiographically (Figure 2a), they are well-marginated and have a central area with a variable amount of mineralization (*i.e.* "popcorn calcification"). Imaging findings concerning for a more aggressive lesion include an eccentric location, irregular calcification, endosteal scalloping, cortical thickening, soft tissue extension, bone expansion, and/or intralesional lucencies (Figure 2b and c). If present, these findings may warrant further advanced imaging (CT and/or MRI). Treatment of enchondromas is observation. If a pathologic fracture occurs through an enchondroma, nonoperative management is often possible due to the high healing rate.

Chondroblastoma is a rare benign cartilage tumor that almost exclusively occurs in the pediatric population and is usually epiphyseal in location. They tend to be painful and can lead to joint effusion and stiffness, particularly when there is cortical breakthrough. On plain radiographs, chondroblastomas are radiolucent with a sharply demarcated border (Figure 3a and b). Chondroblastomas are benign but can extremely rarely metastasize to the lungs. Management is surgical curettage and bone grafting.

Synovial Chondromatosis is a metaplastic process whereby synovial cells produce intra-articular loose bodies comprised of hyaline cartilage.[8] The nodules are typically small and numerous; however, they can become confluent. They may also become lodged in the synovial lining and no longer appear as "loose" bodies. There is a clear predilection for large joints, with the knee being most commonly affected. Synovial chondromatosis can be painful and present similar to osteoarthritis. Pain is mediated through mechanical damage and inflammatory molecules. Plain radiographs usually demonstrate small intra-articular calcified nodules but may be negative if the nodules have not calcified. Treatment of synovial chondromatosis is removal of loose bodies and synovectomy. Rarely, malignant progression can occur.[9]

Malignant Cartilage Lesions

Chondrosarcoma is the malignant form of a cartilage lesion and may arise primarily or be secondary to a benign cartilage lesion. The pelvis and proximal appendicular skeleton are the most common sites and it is very rare in the hands and feet. Chondrosarcoma in the long bones infrequently is associated with a soft tissue mass and differs from most sarcomas in that they are usually slower in progression and low to intermediate-grade. Thus, the typical presentation for chondrosarcoma is long-standing history of pain with mild swelling and progressive limitation in daily activities and sports—strikingly similar to the presentation of a number of common arthropathies, such as osteoarthritis.[10] Dedifferentiation of chondrosarcoma is possible, which may present with a rapid conversion from indolent to fulminant disease (and symptomology). Chondrosarcomas are designated as low- (Grade 1), intermediate- (Grade 2), or high- (Grade 3) grade. Sixty-percent are low-grade and can be difficult to distinguish from enchondromas radiographically and histologically; interobserver agreement by pathologists between enchondroma and low-grade chondrosarcoma is only moderate.[11] Because of this, history, physical examination and critical review of imaging is critical to making the diagnosis of chondrosarcoma. Subtle scalloping may be the only radiographic finding suggestive of a malignant lesion. A change in appearance of a previously stable lesion is suggestive of possible malignant transformation. Anatomical location also matters. Two pathology specimens from the hand and pelvis may be identical; however the specimen from the hand will likely be an enchondroma whereas the pelvic specimen is more likely a low-grade chondrosarcoma. Grade 2 chondrosarcomas usually have a more aggressive radiographic appearance with cortical breakthrough and bony destruction evident. Grade 3 chondrosarcomas appear similar to other high-grade lesions with significant bony destruction, cortical breakthrough, associated soft-tissue mass, and periosteal changes. High-grade

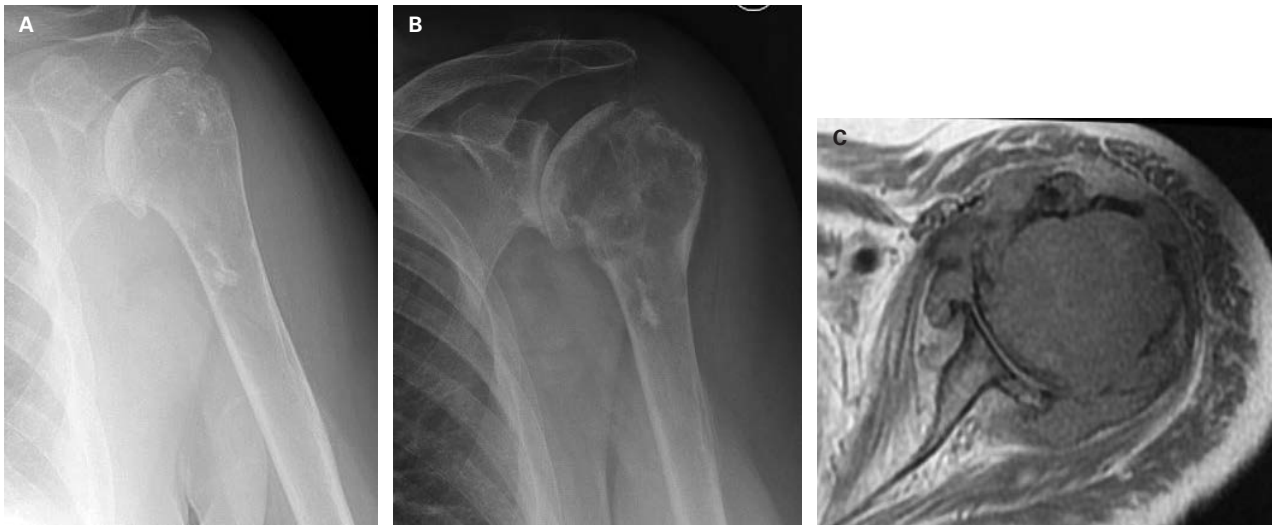

Figure 2. (A) AP radiograph demonstrating left proximal humerus enchondroma in a 79-year-old female being evaluated for left shoulder pain deemed to be due to cuff tear arthropathy. Note the medullary-based lesion with "popcorn" calcification pattern. (B) AP radiograph demonstrating malignant degeneration of the proximal humerus enchondroma into a chondrosarcoma. Note the expansile and destructive nature of the lesion as well as multiple focal lucencies within the lesion. (C) Axial T1-weighted MRI showing associated soft tissue mass. The patient, now 85-year-old, presented with increased shoulder pain and dysfunction. The patient ultimately underwent surgical resection and endoprosthetic reconstruction. Pathology revealed a Grade 2 chondrosarcoma.

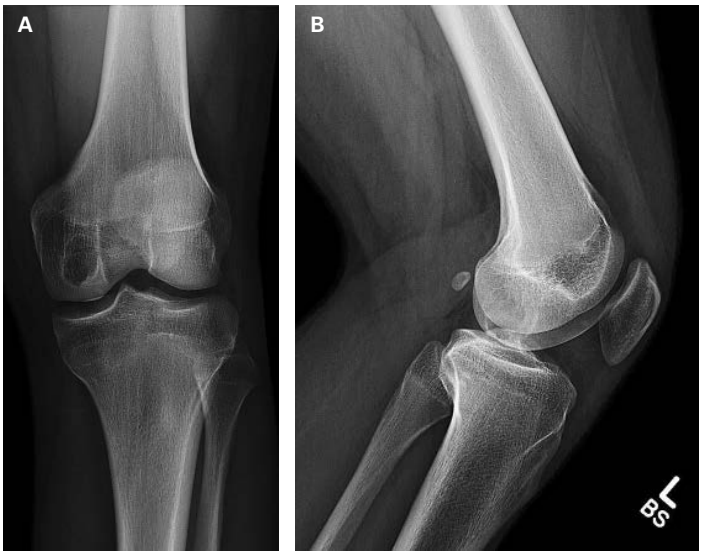

Figure 3. (A) AP and (B) Lateral radiographs of the left knee in a 17-year-old male demonstrate a chondroblastoma of the distal posterior medial femoral condyle. Note the characteristic epiphyseal location and the classic findings of radiolucency with a sharply demarcated border. The patient presented with knee pain, swelling and painful range of motion.

primary chondrosarcomas have minimal calcification unless they are dedifferentiated from low-grade lesions. Treatment of chondrosarcomas is focused on wide surgical resection, as these tumors are notoriously resistant to chemotherapy and radiation therapy.

Discussion

Bone lesions frequently generate stressful situations for both patients and physicians; arriving at an accurate diagnosis for the bone lesion as well as the patient's presenting symptoms is critical to assuaging anxiety and helping ensure proper management is implemented. A diligent history, physical examination, and critical review of imaging studies can frequently help determine whether a patient has an incidental benign bone lesion associated with real joint pathology that fits the patient's symptomology or whether there is concern that the bone lesion might be the source of the patient's symptoms and might warrant further workup and/or referral to an orthopaedic oncologist.

One common scenario encountered by orthopaedic surgeons is a middle-aged patient presenting with shoulder pain. The vast majority of such patients will have rotator cuff +/− biceps tendon pathology or pain referable to degenerative changes of the glenohumeral or acromioclavicular joints. Onset, location, character, intensity, duration and aggravating/alleviating factors of pain are important. Reproducible pain with specific provocative maneuvers is unlikely to be from a cartilage lesion. Plain film radiographs may show characteristics of enchondroma; however, if the exam and other advanced imaging (MRI is often the imaging modality of choice to evaluate various shoulder pathology) are consistent with a benign enchondroma and a separate shoulder pathology, the patient should be treated independently of the lesion. If there is concern, a diagnostic intra-articular or subacromial injection may be performed; pain from an intraosseous cartilage lesion will not be alleviated, whereas pain from rotator cuff pathology or osteoarthritis should be significantly decreased after therapeutic injection. If there is a change in symptoms, the patient should be reassessed and new imaging should be obtained. It is possible that the patient has developed worsening or new shoulder pathology, but it is possible that the previously identified benign cartilage lesion is now symptomatic due to malignant transformation (Figure 2b and c).

Another common scenario involves patients being evaluated and treated for degenerative joint disease of the hip or knee. The overwhelming majority of older adults presenting with hip or knee pain will have osteoarthritis and it is tempting to focus solely on the classic radiographic findings of degenerative joint disease (joint space narrowing, subchondral sclerosis, osteophyte formation and subchondral cyst formation). Careful attention must be paid, however, to radiographs, particularly around the hip, as chondrosarcomas involving the acetabulum may present quite similarly to hip osteoarthritis and can be subtle due to overlapping tissues in the pelvis. Chondrosarcoma diagnosed at the time of reconstructive hip surgery may compromise an opportunity for a limb salvage procedure and ultimately lead to a poorer prognosis.[3] If the degree of radiographic degenerative joint disease does not match the level of symptoms, one must be confident that there are no other potential etiologies. Articular based lesions of the hip in adults that do not fit classic descriptions of common pathology (*eg.* avascular necrosis) often warrant further workup or referral to an orthopaedic oncologist as there is a greater chance for primary or metastatic malignancy in this location.

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U·P·O·J

Study of the Different Methods of Management of Defects after Curettage of Benign Cystic Bone Lesions

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Introduction

Currently, treatment of benign bone cysts includes: observation, injection of bone marrow or demineralized bone matrix,[1-4] curettage combined with bone or synthetic grafting,[5-7] decompression with intramedullary nailing or cannulated screw,[8-10] or a combinations of these approaches.[11]

Bone grafting can be done with either autologous bone or with allograft bone. Autologous bone grafts have osteogenic, osteoinductive, and osteoconductive properties. Autologous bone grafts have an excellent success rate, low risk of disease transmission, and histocompatibility; however, there is a limited quantity of autogenous bone graft available, especially in children.[12,13] Allografts do not contain the same growth potential as autograft and are associated with an increased risk of infection, causing some to limit its use in filling bone cysts, especially in children.[1,2]

Calcium phosphate ceramics can be used to fill bone defects. These materials act as osteoconductive bone void filler that completely resorbs as newly formed bone remodels and restores anatomic features and structural properties.[12,13]

Chemical adjuvants such as phenol, ethanol, hydrogen peroxide and alcohol have been used to extend the margin of resection in benign aggressive bone lesions. The advantages of chemical adjuvant include lowering recurrence rates and necrosis.[14-20] The goal in the use of chemical adjuvants such as hydrogen peroxide is to create a balance between exclusive mechanical methods while limiting the higher toxicity of more aggressive adjuvants. Hydrogen peroxide has a direct cytotoxic effect on active cells through denaturation of protein, controlling microscopic disease in the reactive zone after curettage has removed the gross pathologic tissues.[16-18]

Filling of the resulting cavity after removal of the pathological tissues is not always necessary and healing of the cavity can occur in a reasonable time.[21] The goal of this work was to evaluate the different methods of management of defects following curettage of benign cystic bone lesions.

Methods

Forty-two patients were diagnosed as having benign bone lesions. There were 21 (50%) males and 21 females. Age ranged from 5 to 62 years (mean: 12.65). The proximal metaphysis of the femur was affected in 15 patients (36%), the proximal humerus in 10 patients (24%), the distal tibia in 6 (14%), the proximal tibia in 3 (7%), the pelvis in 3 (7%), the distal femur in 2 (5%), the scapula in 2 (5%) and the distal humerus in one (2%).

All procedures were performed under general anesthesia. The surgical approach was chosen according to the site of the lesion to allow for adequate exposure. The lesions were thoroughly curettaged. The tissues obtained were sent for histopathological study. Five patients had received incisional biopsy prior to definitive treatment and were diagnosed as aneurysmal bone cyst (ABC).

The diagnosis was ABC in 30 patients (71%), non-ossifying fibroma in 7 (17%), fibrous dysplasia in 4 (10%) and eosinophilic granuloma in one patient (2%). There were 33 primary and 9 recurrent lesions.

The patients were classified into three groups (Table 1) according to the method of management of the defect after the curettage and saline lavage.

Table 1. Grouped methods of treatment.

Group	No	%	Method of treatment
Group 1	24	57%	Curettage + Hydrogen Peroxide (nonvascularised fibular strut grafts were used in 9/24 cases)
Group 2	13	34%	Curettage + Autogenous Iliac Crest Bone Graft + G-Bone Hydroxyapatite Granules
Group3	5	12%	Curettage + Autogenous Iliac Crest Bone Graft
Total	42		

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Group 1 consisted of 24 patients (57%) in whom hydrogen peroxide (20% solution) was used as an adjuvant, continuously lavaged for three to five minutes. Care was taken to protect the surrounding soft tissues from thermal and chemical injury during lavage. In this group, the resultant cavity was not filled (Figure 1a and b). In nine cases, autogenous non-vascularized fibula was used as a strut graft to stabilize the weak cystic wall after curettage. The fibular graft was rigidly impacted inside the cavity. If it was difficult to impact the strut graft, minimal fixation methods were used to stabilize it (Figure 1c and d). Cast immobilization used in 11 cases, internal fixation was used in two cases

Group 2 consisted of 13 patients (34%) in whom the resultant bone defect was filled with a combination of autogenous iliac crest bone graft (ICBG) and G-Bone (hydroxyapatite) granules (Figure 2a and b). In one patient a non-vascularized fibular graft was additionally used. Internal fixation was used in two patients; one for pathologic femoral neck fracture through fibrous dysplasia, and the other for recurrent fibrous dysplasia with coxa vara deformity (Figure 2c and d).

Group 3 consisted of five patients (12%) in whom the resultant defect was filled with autogenous ICBG. Postoperatively, physical activities were restricted until there was radiographic evidence of bone healing.

Radiological changes were evaluated according to the modified Neer classification system.[22] Neer I were determined to have a healed cyst filled with new bone with or without small radiolucent area < 1 cm. Neer II had a healing cyst with a defect $< 50\%$ of the bone diameter. Neer III had a persistent cyst with a radiolucent area $> 50\%$ of the bone diameter. Neer IV had a recurrent cyst.

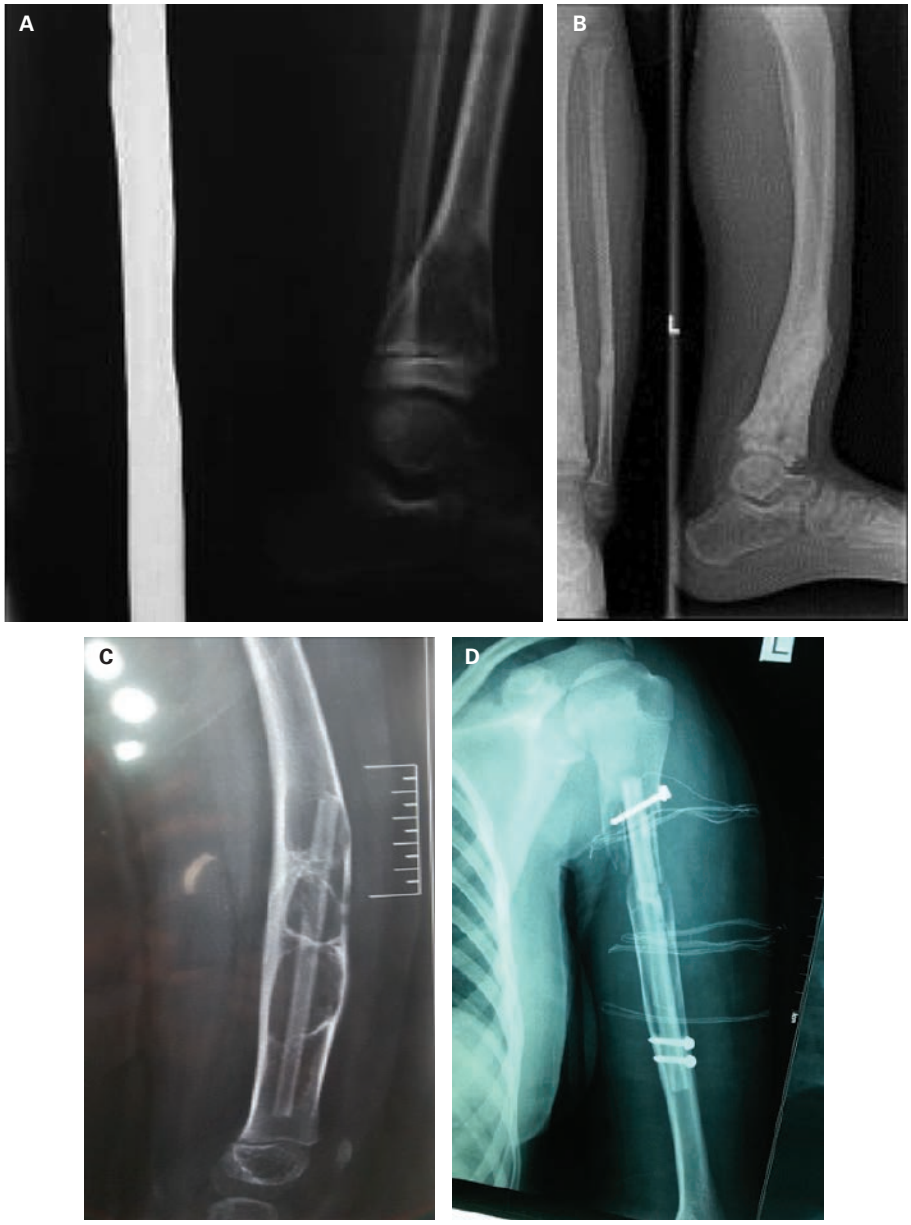

Figure 1. (A) Radiological presentation of a case of recurrent solid ABC of the distal tibia in a 9-year-old boy. (B) Follow-up at 18 months after curettage and hydrogen peroxide lavage; near complete healing and consolidation are observed. (C) 6-year-old boy with large ABC of the distal distal femur treated by curettage, hydrogen peroxide lavage and fibular strut autograft. (D) 15-year-old girl with pathologic fracture through a humeral shaft ABC treated by curettage, hydrogen peroxide and strut fibular autograft with internal fixation for added stability.

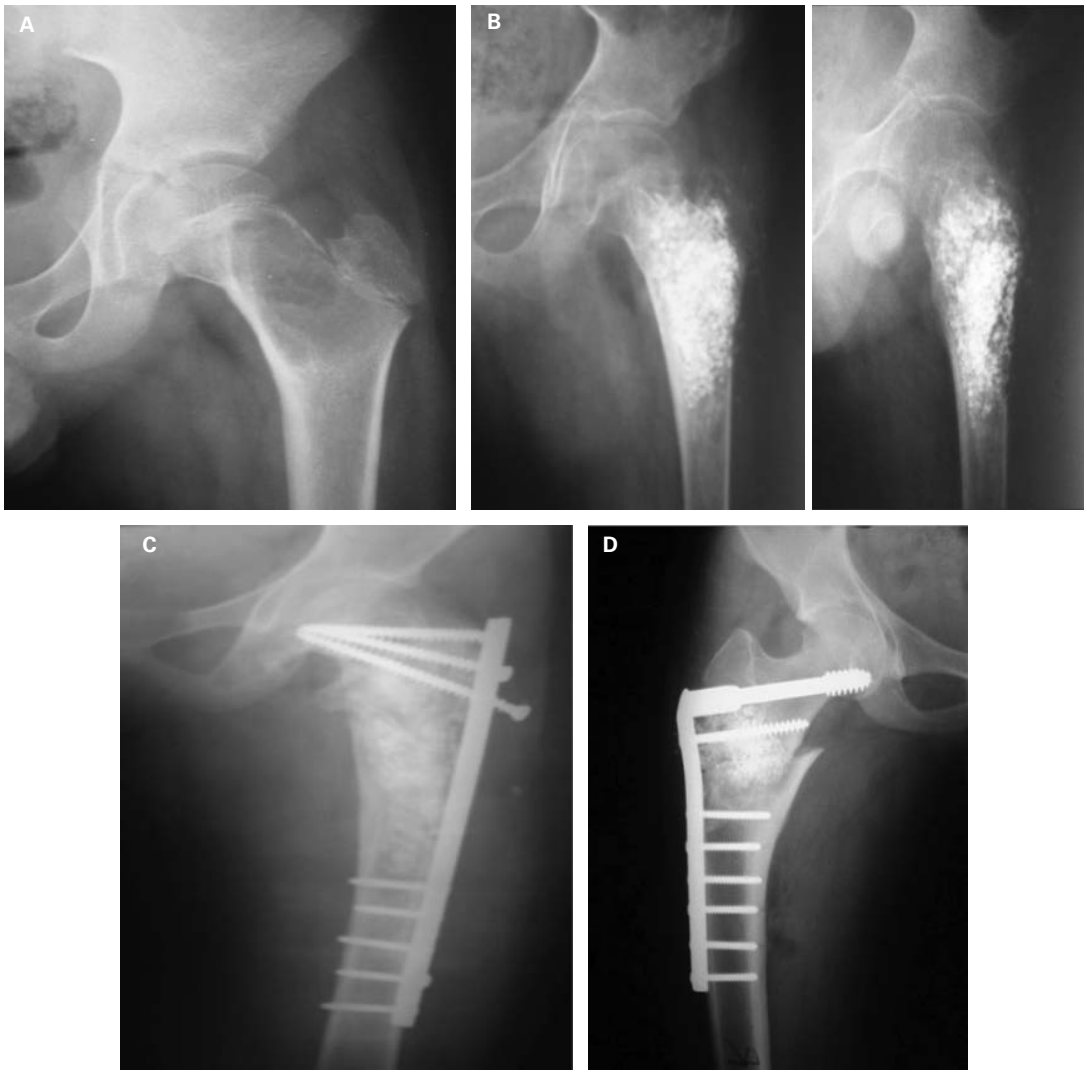

Figure 2. (A) ABC in the proximal femur. (B) Combined autogenous ICBG and hydroxyapatite granules. (C) Femoral neck fracture through fibrous dysplasia of the proximal femur treated by combined grafting and with internal fixation. (D) Corrective osteotomy, combined grafting and DHS fixation for recurrent fibrous dysplasia with severe coxa vara deformity.

The procedure was considered successful if the lesion was completely healed or healed with a small radiological defect. When evidence of consolidation of the lesion or cortical thickening was absent six months after the initial procedure or when recurrence occurred, the procedure was considered unsuccessful.[22]

An approval was given by the institutional review board (IRB) and informed consent was obtained from each patient or designated power of attorney.

Results

Mean clinical follow up was 25.1 months for Group 1, 18.7 months for Group 2, and 14.6 months for Group 3.

In Group 1, all lesions demonstrated complete radiographic healing (Neer Class 1). Full functional recovery occurred in all cases except one; a patient with a recurrent proximal femoral lesion had a coxa vara deformity leading to a Trendelenberg gait. In lower extremity cases, full weight bearing was achieved by three months postoperatively in 9/16 cases. Autogenous fibular grafts were used in nine cases (Figure 3); in all cases the fibula was completely incorporated within six months postoperatively. No local recurrence or pathological fractures occurred in Group 1.

The overall success rates were 92% for Group 2 and 80% for Group 3 (Figure 4). Recurrence occurred in 1/13 (8%) patients in Group 2 and in 1/5 (20%) patients in Group 3. At final follow-up, 10/13 (77%) of patients in Group 2 were classified as Neer Class I. In Group 3, 4/5 (80%) of patients were considered Neer Class I (Table 2).

The patient included in Group 3 who sustained recurrence following ICBG for ABC of the proximal femur subsequently underwent a combined technique and was included in the analysis for Group 2, with no evidence of recurrence at 26-months post-op.

Recurrence occurred in one patient in Group 2; this patient presented with a fracture through fibrous dysplasia in the femoral neck and was treated by the combined technique together with internal fixation using plate and screws. The fracture and the cyst healed; however, the lesion recurred.

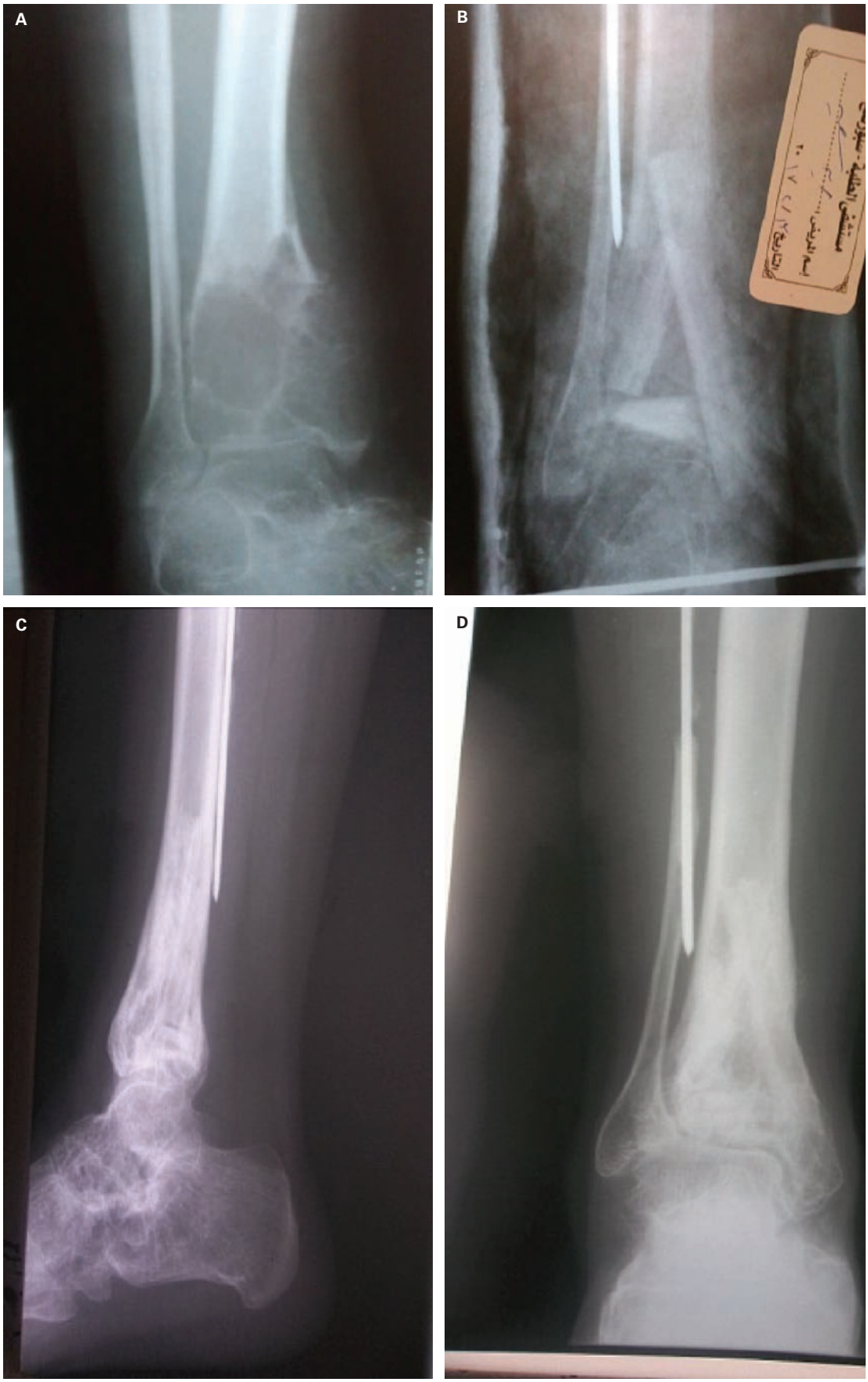

Figure 3. (A) 14-year-old girl with an aggressive distal tibia ABC. (B) Aggressive curettage + hydrogen peroxide lavage + fibular strut graft impacted in place without filling of the cavity. (C and D) X-rays at 18 months follow-up showing almost complete healing and consolidation of the lesion.

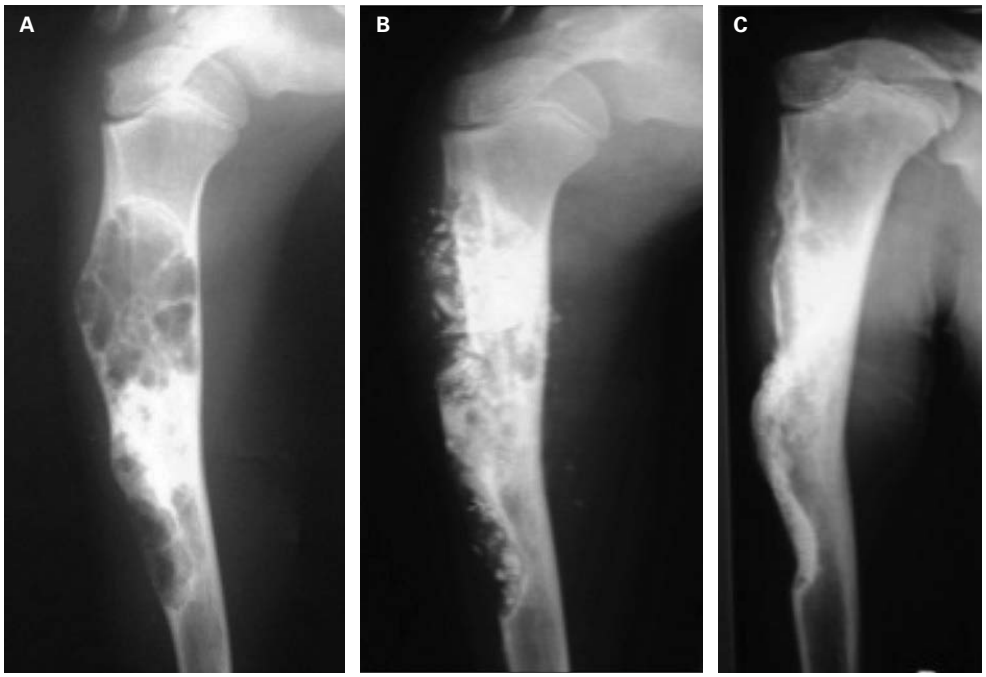

Figure 4. (A) Preoperative X-ray showing recurrent ABC, following two prior attempts at bone grafting. (B) Combined autogenous ICBg, fibular allograft, and hydroxyapatite granules. (C) 30 month follow-up showing healing without recurrence.

Table 2. Results of different treatment groups.

	Neer I	Neer II	Neer III	Neer IV	Total
Group 1	24	0	0	0	24
Group 2	10	2	0	1	13
Group 3	4	0	0	1	5
	38	2	0	2	42

Another surgery was performed 14-months postoperatively removal of hardware and curettage and G-bone grafting. Unfortunately the lesion again recurred.

Discussion

Autogenous ICBG is considered the gold standard for filling bone defects after curettage of benign cystic bone lesions; this graft type is osteoconductive, osteoinductive and osteogenic.[23,24] Autogenous ICBG has known complications such as limited graft volume (especially in children), donor site morbidity, increased pain, hematoma and wound infection.[23] To address these concerns, allografts and biologics have been processed and used. These too have limitations: cost, histo-incompatibility, infection risk, lack of growth potential compared to ICBG.[23]

Several authors used various forms of adjunctive therapy in conjunction with curettage, including phenol, hydrogen peroxide, liquid nitrogen and alcohol with variable reults.[14,18].

In our study, ICBG was used to fill smaller sized defects, while a combination of ICBG and G-bone (hydroxyapatite) was used to fill larger defects. The graft only group (Group 3) had recurrence in 1/5 cases, while the combined graft group (Group 2) had recurrence in 1/13 cases. The no graft group (Group 1) had recurrence in 1/24 cases.

We found a similar rate of recurrence among patients with ABC as found by Mankin, *et al*, who fount that 20% of their 150 cases of ABC had recurrence after treatment with curettage and allograft bone chips or Polymethylmethacrylate. Autogenous ICBG was used with large lesions or for recurrent lesions.[25]

One out of four (25%) patients with fibrous dysplasia had recurrence. The recurrence occurred in the only case of polystotic fibrous dysplasia included in this study. We were obliged to do bone grafting because she presented with a transcervical femoral neck fracture through a large lesion. Attempts to completely remove polyostotic disease with curettage and bone grafting are rarely successful.[26]

Uchida[27] used hydroxyapatite blocks and granules to fill defects in 60 cases of benign bone tumors after resection. The implants were well incorporated into the host bone in

all cases. Evaniew, *et al*[23] treated 24 benign bone tumors with intralesional curettage followed by reconstruction with a calcium sulphate/calcium phosphate composite. Recurrence occurred in two cases and deep infection in four cases.

In this study, hydrogen peroxide was routinely used as an adjuvant after curettage in Group 1 patients. It is believed that hydrogen peroxide has both local thermal and chemical effects. The use of hydrogen peroxide as an adjuvant treatment of non-malignant active and aggressive bone tumors has been rarely discussed in the literature.[18] To our knowledge, no reports exist in the literature on its use alone as an adjuvant after curettage of benign bone lesions. Hydrogen peroxide has the advantages of being cheap and is usually readily available. It has the benefit of not being harmful to skin or the local healthy tissues as has been reported with cryotherapy; additionally, our results show superior results than similar studies using other adjuvants.[28]

Conclusion

Thorough curettage with hydrogen peroxide (20% solution) lavage could be an effective and inexpenisve method to control local recurrence after treatment of benign bone lesions. Strut fibular autografts can provide good mechanical stability especially in huge lesions. The combined use of hydroxyapatite synthetic granules with autogenous bone graft is a safe and effective option for the situations when a large amount of graft is needed, especially in children with large and/or recurrent benign bone lesions.

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Progression of Vertebral Bone Disease in Mucopolysaccharidosis VII Dogs from Birth to Skeletal Maturity

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Disclosures: MEH (BioMarin Pharmaceutical Inc.)

Introduction:

The mucopolysaccharidoses (MPS) are inherited lysosomal storage disorders characterized by mutations in hydrolases that degrade glycosaminoglycans (GAGs). GAGs accumulate in cells leading to multi-systemic clinical manifestations.[1] Many subtypes, including MPS VII (Sly Syndrome, beta-glucuronidase deficiency), exhibit severe skeletal abnormalities that are prevalent in the spine.[2-4] Previously, we showed the presence of cartilaginous lesions in MPS VII vertebrae that represent failed secondary ossification during postnatal development[4,5] and contribute to progressive spinal deformity.[6] To effectively target and optimize timing for therapeutic intervention for vertebral bone disease in MPS VII, it is critical to first elucidate temporal patterns of disease manifestation during postnatal development. Therefore, the objective of this study was to establish the nature, timing, and progression of vertebral bone disease in MPS VII from birth to skeletal maturity, using the naturally-occurring canine disease model.

Methods:

For this study, we used the naturally-occurring MPS VII canine model that mimics both the progression and pathological phenotype of the skeletal abnormalities found in human patients.[7] With IACUC approval, control and MPS VII dogs (n = 1-5) were euthanized at 9, 14, 30, 42, 90, 180, and 365 days, and lumbar and thoracic vertebrae were excised. Progression of vertebral bone formation of primary and secondary ossification centers were analyzed using μCT. Bone formation in vertebral secondary ossification centers was visualized through reconstructed images of the regions cranial and caudal to the growth plates. To quantify trabecular bone content in the primary ossification centers, standard 3D morphometric analyses were performed and bone volume fraction (BV/TV) and bone mineral density (BMD) were determined.[8] For non-invasive assessment of bone formation, serum was collected at 90, 180, and 365 days-of-age and bone-specific alkaline phosphatase (BAP) activity was measured using an ELISA kit. Significance for 9 and 14-day BV/TV and BMD measurements (n = 5 for each group) was established using 2-way analyses of variance and post-hoc Tukey's test ($p < 0.05$). Significance for 30 and 42-day BV/TV and BMD measurements (n = 2 for each group) were determined with unpaired t-test ($p < 0.05$).

Results:

Initiation of vertebral secondary ossification was markedly delayed in MPS VII animals compared to controls (Figure 1). While secondary ossification commenced by 14 days-of-age in controls, in MPS VII vertebrae, this did not occur until 30 days-of-age. Further, when it did commence, secondary ossification in MPS VII animals was highly irregular compared to the smooth, symmetrical phenotype in controls as seen from the axial images in Figure 1. Examining midsagittal cross-sectional images (Figure 2), bone formation in secondary ossification centers of the MPS VII animals was observed to cease progressing between 42 and 90 days-of-age. At 180 and 365 days-of-age, bone content in secondary ossification centers was greatly diminished compared to controls. Additionally, at 365 days, in control animals, growth plates were closed but remained open in MPS VII animals. Examining primary ossification centers, trabecular bone content was normal at early ages, but

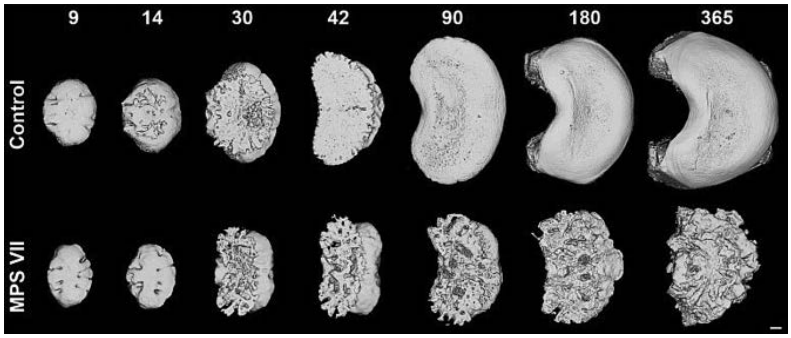

Figure 1. Representative axial μCT images showing delayed, incomplete, and non-uniform progression of secondary ossification in MPS VII vertebrae compared to controls. Numbers indicate postnatal days-of-age. Scale = 1mm.

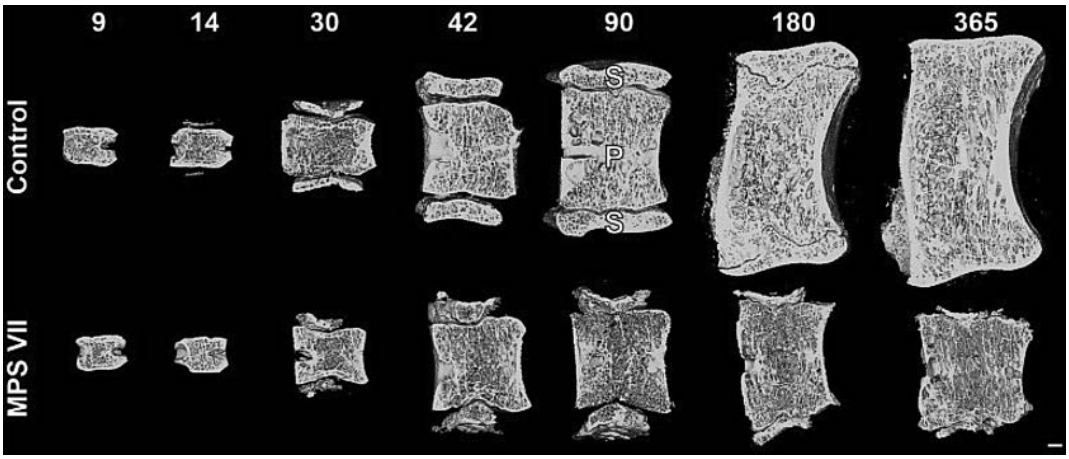

Figure 2. Representative midsagittal μCT images showing lower trabecular bone content in MPS VII vertebral primary ossification centers at older ages compared to controls. Numbers indicate postnatal days-of-age. Scale = 1mm. S: Secondary ossification center; P: Primary ossification center.

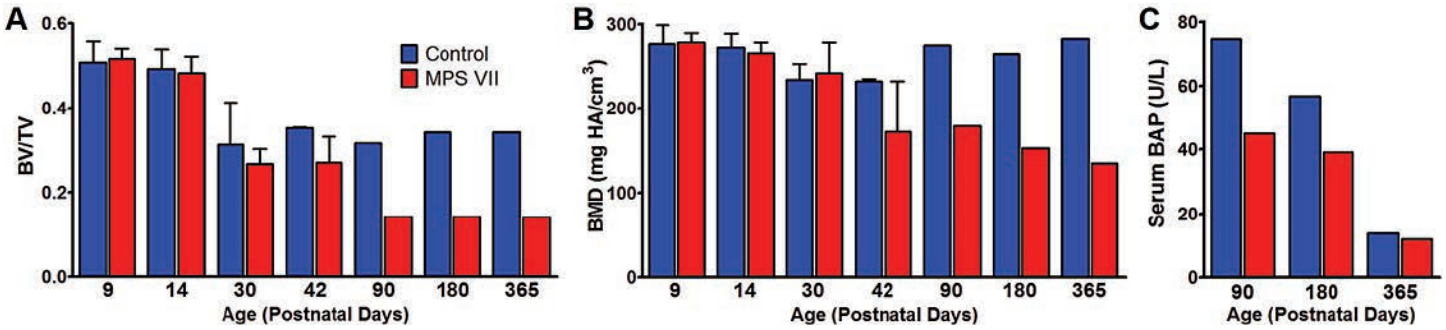

Figure 3. (A) Bone volume fraction (BV/TV) and (B) Bone mineral density (BMD) for control and MPS VII vertebral primary ossification center trabecular bone from birth to skeletal maturity. (C) Serum bone alkaline phosphatase (BAP) activity for control and MPS VII animals at older ages. 9 days (n = 5), 14 days (n = 5), 30 days (n = 2), 42 days (n = 2), 90 days (n = 1), 180 days (n = 1), and 365 days (n = 1).

beyond 30 days, MPS VII vertebrae exhibited lower BV/TV and BMD (Figure 2, Figures 3A and B). Finally, serum BAP levels were lower in MPS VII animals at 90 and 180 days (during skeletal growth), but not at 365 days (skeletal maturity) (Fig 3C).

Discussion:

This work establishes that vertebral bone disease in MPS VII manifests differently in primary and secondary ossification centers in a temporally-dependent manner, informing optimal targeting and timing for potential therapeutic interventions. Vertebral secondary ossification in MPS VII was found to be not only markedly delayed, but also highly non-uniform, suggesting that early developmental signals for bone formation are both impaired and spatially dysregulated. While primary ossification centers were not significantly affected at early ages, pathological changes (lower trabecular BV/TV and BMD) in MPS VII animals were evident at older ages. Lower serum BAP activity levels in MPS VII animals at 90 and 180 days may indicate reduced osteoblast activity during skeletal growth. Results also suggest that serum BAP may be a robust, non-invasive diagnostic tool for assessing bone disease progression in MPS patients.

Significance:

MPS VII is associated with severe skeletal disease for which there are currently no treatments. This study contributes to identification of optimal therapeutic windows for targeting bone disease in MPS VII and suggests a new diagnostic tool for assessing bone disease in MPS patients.

Acknowledgments

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U·P·O·J

The Critical Role of Mesenchymal Progenitors in Initiating the Secondary Ossification Center at the Epiphyseal Cartilage

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Introduction

Endochondral ossification in long bones proceeds via the development of a diaphyseal primary ossification center (POC) in embryo and an epiphyseal secondary ossification center (SOC) after birth. While POC formation has been extensively studied, the initiation and expansion of SOC are largely uncharacterized. In particular, whether mesenchymal progenitors, which have been shown to move into developing POC along with invading blood vessels,[1] play a similar role in the SOC formation is not known. Tomato (Td)+ cells of *Col2-Cre Rosa-Td* mice were recently identified as mesenchymal progenitors that constantly replenish osteoblasts and osteocytes in POC.[2] In this study, we used this lineage tracing method to investigate the potential actions of mesenchymal progenitors in SOC formation and development.

Methods

All animal work was approved by the Institutional Animal Care and Use Committee at the University of Pennsylvania. *Whole mount staining of bone tissues- Col2-Cre Rosa-Td* mice were generated by breeding *Col2-Cre* mice with Rosa-Td homozygous mice. Femurs and tibiae were harvested at various time points after birth, fixed in 2% PFA, decalcified in 15% EDTA, immersed into 20% sucrose and 2% polyvinylpyrrolidone, and processed for thick frozen sections, which underwent immunofluorescent staining with antibodies against Endomucin, VEGF, PDGFRβ, osterix (Osx), and DIPEN. Fluorescent signals were acquired from Zeiss LSM-710 laser scanning confocal microscope with a depth of 100 μm and 3D images were processed by Volocity. *Cell migration-* Bodyen chamber was used to study the migration of mouse primary endothelial cells towards the conditioned media from mouse bone marrow mesenchymal progenitors. *Fibroblast colony-forming cells (CFU-F) and differentiation assays-* Td^{high} cells from the epiphysis of *Col2-Cre Rosa-Td* pups were sorted and seeded at a density of 6,000 cells/T25 for CFU-F assay. Those cells after expansion were cultured in osteogenic and adipogenic media for differentiation assays. *Statistics-* Data are expressed as means ± SEM and analyzed by unpaired, two-tailed Student's t-test.

Results

We observed that in P4 *Col2-Cre Rosa-Td* pups, while most epiphyseal chondrocytes were Td+, those located at cartilage surface (Td^{high} cells) had much higher Td signal than those inside cartilage and therefore were the only visible cells after elevating the threshold of Td signal (Figure 1A). Since later all osteoblasts, osteocytes, and Osx+ osteoprogenitors within SOC were Td^{high} (Figure 1B), those Td^{high} cells at cartilage surface likely contain mesenchymal progenitors responsible for subchondral bone formation. Indeed, sorted Td^{high} cells had very high CFU-F frequency (6×10^{-3}) and were able to differentiate into

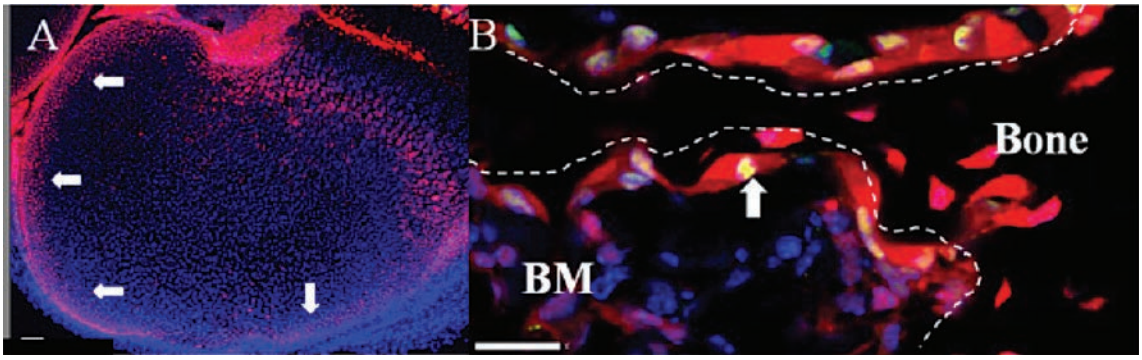

Figure 1. Td^{high} cells in the epiphyseal perichondrum contain mesenchymal progenitors that reconstitute subchondral bone after SOC formation. A. At P4, chondrocytes at perichondrium (arrow) express a high level of Td. B. At P18, osteoblasts on the bone surface (dash line), osteocytes within bone, and Osx+ cells (arrow) were all Td^{high} cells (Td: red; Osx: green; DAPI: blue).

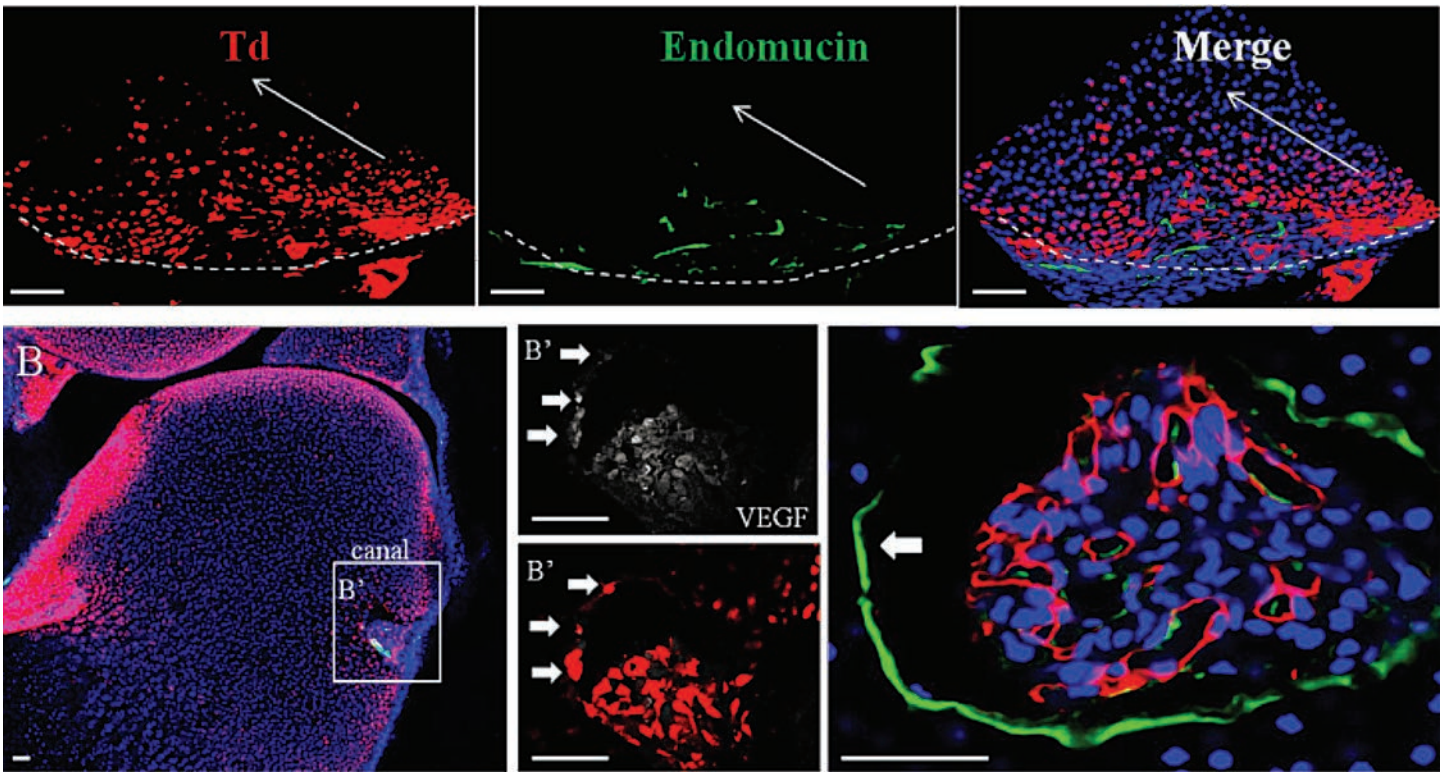

Figure 2. Td^{high} cells promote vasculogenesis during the initial stage of cartilage canal formation. A. Td^{high} cells penetrate into cartilage, followed by individual Endomucin+ endothelial cells. Arrow points to the direction of canal advancement, and perichondrium is labeled with dash lines. B. Td^{high} cells at the leading edge were VEGF+. B': zoom in images. Arrows point to Td+VEGF+ cells at the front edge of a cartilage canal. C. DIPEN staining at the front edge (arrow) and wall of a cartilage canal (DIPEN: green; Endomucin: red; DAPI: blue; scale bar = 50 μm).

osteoblasts and adipocytes in culture. At P5, using a whole mount staining with 3D image reconstruction, we observed that Td^{high} cells started to penetrate into cartilage at discrete surface sites, followed by individual Endomucin+ endothelial cells (Figure 2A). TUNEL staining identified that chondrocytes within these sites undergoes apoptosis. At P6, cartilage canals were initiated at these sites. The canal wall, which consists of Td^{high} cells, endothelial cells, and chondrocytes, was again preceded by VEGF+ Td^{high} cells leading the front edge (Figure 2B). At this stage, surrounding chondrocytes were VEGF−. Inside the canal, there was a cone-shaped and dense cell cluster, including Td^{high} cells, single endothelial cells, vessels, and CD45+ cells, with the base at the cartilage surface. Those Td^{high} cells became proliferative (BrdU+) and migrated either as perivascular cells or as individual cells. Interestingly, they only expressed mesenchymal progenitor marker PDGFRβ, but not other markers such as Osx, CD44, and CD105, implying that they are probably at the early stage of stem cells. There was a space about 30 um long between the canal wall and the cell cluster where only erythrocytes were detected. The canal wall was distinctly labeled by antibodies against the aggrecan-degraded product DIPEN (Figure 2D) and apoptotic chondrocytes. The unique initiation and structure of cartilage canal strongly suggest that mesenchymal progenitors play a leading role in promoting vasculogenesis, a de novo vessel formation process, and together with chondrocytes create a path for canal protrusion. In vitro, mesenchymal progenitors greatly stimulate the migration of endothelial cells (Figure 3). After P8, all hypertrophic chondrocytes were VEGF+ so the expansion of SOC was led by blood vessels growth into chondrocytes followed by Td^{high} progenitors (Figure 4), the same mechanism by which POC is developed.

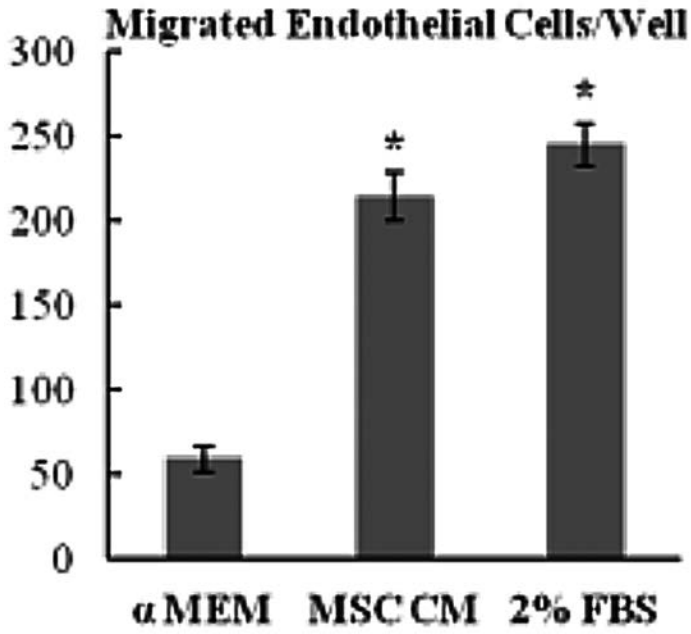

Figure 3. Endothelial cells migrate towards the conditioned media (CM) from mesenchymal progenitors (Mean ± SEM, n = 4, *: $p < 0.01$ vs αMEM).

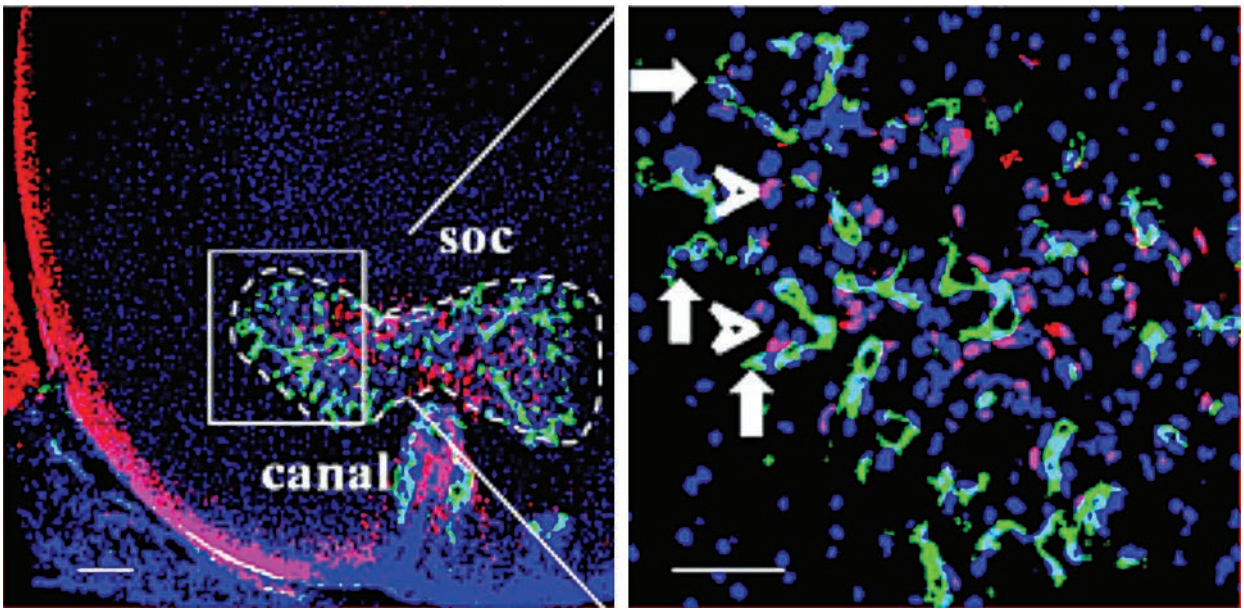

Figure 4. Vessel protrusion (arrows) precedes Td^{high} cells (arrow head) during SOC expansion at P9 (Endomucin: green; Td: red; DAPI: blue).

Discussion:

Our studies demonstrate a critical function of mesenchymal progenitors within the perichondrial Td^{high} cells in initiating SOC formation. Those cells are the first to move towards the center of epiphyseal cartilage and are part of the front edge of cartilage canal. They also chemoattract endothelial cells to promote vasculogenesis, possibly through a VEGF− dependent pathway, followed by cartilage canal formation. This mechanism is distinct from how POC is formed, by which angiogenesis precedes the moving of mesenchymal progenitors into cartilage.[1] This difference might be due to the different expression pattern of VEGF. In cartilage, only hypertrophic chondrocytes are VEGF+ cells. In contrast to POC where mesenchymal progenitors and vessels move into VEGF+ hypertrophic chondrocytes, the superfacial layer of articular cartilage before SOC formation does not contain VEGF+ chondrocytes. Instead, VEGF+ Td^{high} cells play a pivotal role in promoting vessel invasion. In addition, our studies revealed those mesenchymal progenitors undergo phenotypical changes during their migration from perichondrum to cartilage canal, and eventually become osteoblasts and osteocytes within SOC.

Significance

Using lineage tracing and 3D imaging approaches, we discovered a novel mechanism for mesenchymal progenitors to initiate SOC formation.

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Suppression of Sclerostin Alleviates Radiation Damage to Bone by Protecting Bone forming Cells

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Introduction:

Radiation damage to the skeleton within the radiation field results in a spectrum of bone changes from mild osteopenia to osteoradionecrosis.[1] We have demonstrated that radiation markedly suppressed bone turnover and that bone tissue atrophy is the most common outcome after radiation. We hypothesize that functional recovery of bone architecture in this adverse environment will be possible by the use of anabolic bone forming agents. As a proof of principle, we showed that recombinant human parathyroid hormone (rhPTH1-34, teriparatide), the only FDA-approved anabolic treatment for osteoporosis, completely abrogated the damaging effects of radiation in rat bones.[2] Due to a tumorigenic concern with the use of PTH during radiotherapy, an alternative was sought after. Mechanistic studies revealed that Wnt pathway played an essential role in teriparatide function in protecting bone against radiation. Sclerostin is a Wnt antagonist which binds with the Wnt co-receptor LRP5/6 and its expression in adults is largely restricted in osteocytes. Loss of Sclerostin function in human patients, as in the case of sclerosteosis and van Buchem disease, or in SOST knockout mice, result in high bone mass phenotype with no increased tumor formation. Therefore, we hypothesize that suppression of Sclerostin renders structural and functional protection to the radiated bones, while minimizing the potential risks.

Methods:

Small animal radiation research platform (SARRP) radiation and Scl-Ab treatment- All procedures were approved by our institution's Animal Care and Use Committee. Sclersotin antibody (Scl-Ab) and *SOST* knockout (KO) mice were provided by Novartis pharmaceuticals. Two-month-old male *WT* (C57BL6) mice and *SOST-KO* mice received two 8 Gy doses delivered on days 1 and 3 to the distal metaphyseal region of the right femurs from SARRP (Xstrahl), a clinically relevant focal irradiator for rodents. This was designed to mimic the typical femur dose constraints for whole pelvis intensity modulated radiotherapy for patients with prostate, rectal, or endometrial cancers. Following radiation, *WT* mice were subcutaneously injected with either vehicle or Scl-Ab (100 mg/kg/week) for 4 weeks. On day 28, bilateral femurs (radiated and non-radiated) were harvested for μCT, histology, and histomorphometry, and serum were collected for bone markers. For dynamic labeling, calcein (15 mg/kg) and xylenol orange (90 mg/kg) were injected at 9 and 2 days, respectively, before euthanization. *μCT and finite element analysis (FEA)-* The metaphysis of distal femur was scanned by μCT 35 (Scanco Medical AG) at 6 μm resolution followed by calculation of trabecular bone structural parameters and stiffness (n = 7/group). *Histomorphometry-* After μCT scanning, femurs were processed for plastic embedding for static and dynamic histomorphometric analysis. Osteocyte (Ocy.) and adipocyte (Ad.) number were also quantified. *Histology-* At 2 weeks post radiation, femurs (n = 5/group) were harvested for paraffin embedding followed by TUNEL staining (Apoptag® TM, Millipore). At 1 week post radiation, femurs were processed for frozen embedding followed by Alexa-488-labeled phalloidin staining to visualize F-actin fibers. *Colony Forming Assay-* Two month old *WT* mice (n = 3/group) and *SOST-KO* mice (n = 3) were radiated and injected with Scl-Ab as described above. At 2 weeks, femurs were harvested, cleaned and the metaphyseal bones were digested with collagenase. Cells were seeded at 1×10^6/T25 flask in the growth medium for CFU-F assay. *Statistics-* Data are expressed as means ± SEM and analyzed by paired, two-tailed Student's t-test for comparison of radiated and non-radiated legs and by unpaired, two-tailed Student's t-test for comparison of vehicle and Scl-Ab-treated samples and for comparison of *WT* and *SOST-KO* samples.

Results:

Focally irradiated adult mice at distal femoral metaphysis that received 16 Gy radiation generated from SARRP induced a significant trabecular bone loss and structural deterioration in irradiated femurs compared to contralateral ones in vehicle-treated mice (BMD: −20%; BV/TV: −21%; Tb.N: −10%; SMI: +30%; Stiffness: −75%) (Figure 1). Remarkably, Scl-Ab injections increased trabecular BMD, BV/TV, Tb.N, decreased SMI and increased stiffness to a similar level regardless of radiation, implying that Scl-Ab treatment is able to reverse radiation-induced bone damage in a clinical setting. Interestingly, *SOST-KO* mice were markedly resistant against any structural damage

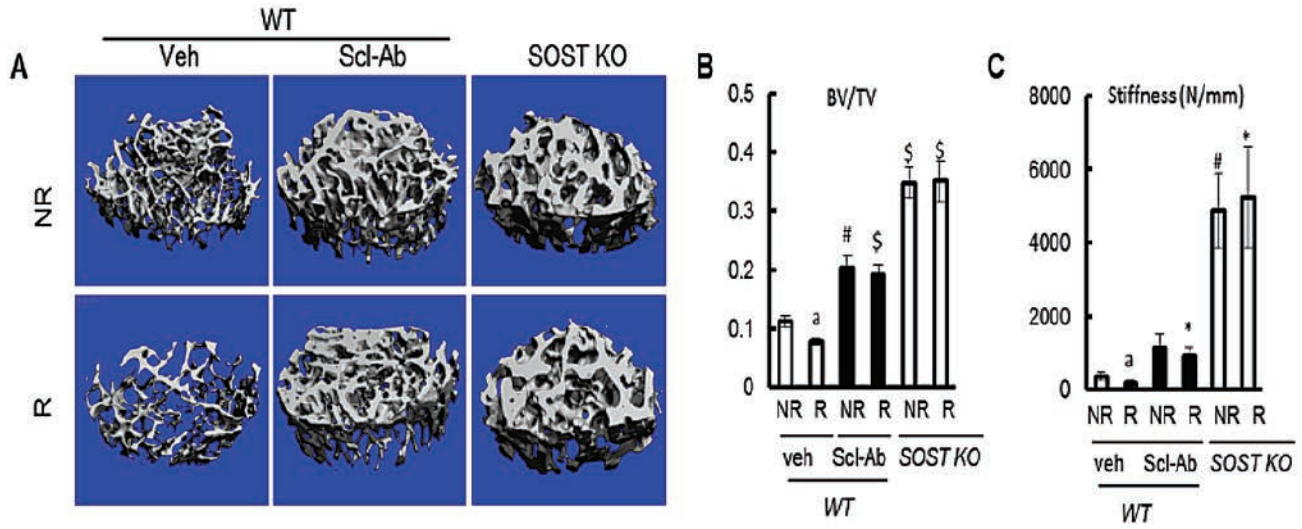

Figure 1. Suppression of Sclerostin greatly improves bone turnover of radiated bones. A. 3-D μCT images at 6μm resolution, revealing the trabecular architecture. B. Bone volume fraction as a ratio of total volume. a; $p < 0.05$ NR vs R. #; $p < 0.01$ vs veh-NR, $; $p < 0.01$ vs veh-R. C. Finite element analysis, a; $p < 0.05$ NR vs R. #; $p < 0.01$ vs veh-NR, *; $p < 0.01$ vs veh-R.

from ionizing radiation. Static histomorphometry indicated that radiation negatively regulated the bone forming cells (Ob.N: −30%, Ocy.N.: −33%, empty Ocy. lacunae: +6-fold, Ad.N.: +10-fold). Dynamic histomorphometry revealed a 10-fold suppression in active bone formation post-irradiation. TUNEL staining revealed a 4-fold increase in apoptotic osteoblasts in the radiated bones. Amazingly, suppression of *SOST* not only protected the osteoblasts, but also partially but significantly suppressed the adipocyte formation after radiation. As compared to the 10-fold reduction in the control, Scl-Ab had a significantly higher bone formation rate, which was correlated by an overall increase in serum osteocalcin levels in the Scl-Ab treated animals. To assess the early stage effect of radiation on osteocytes, F-actin staining revealed a shortening of canaliculae, leading to impaired osteocytic connections with the bone surface and neighboring osteocytes (Figure 2). In contrast, Scl-Ab treatment, not only improved the length of canaliculae, but also protected the inter-osteocytic connections, which is considered important for the overall bone turnover and health. In bone, osteoblasts and osteocytes are derived from bone marrow mesenchymal progenitors (MP's). CFU-F assay clearly revealed that, while radiation greatly diminished the progenitor numbers (−90%), Scl-Ab treatment significantly increased these progenitor numbers by 2-fold.

Discussion:

This study provides proof-of-principle that Scl-Ab blocks bone loss and microarchitecture deterioration after radiation. Furthermore Scl-Ab not only protects the bone forming cells, including MP's, osteoblasts and osteocytes from cell death, but also inhibits the differentiation of MP's towards adipocytes on exposure to radiotherapy.

Conclusions

Scl-Ab treatment minimizes or fully rescues bone loss and damage associated with radiotherapy, and therefore provides a potential therapeutic treatment for radiation induced osteoporosis.

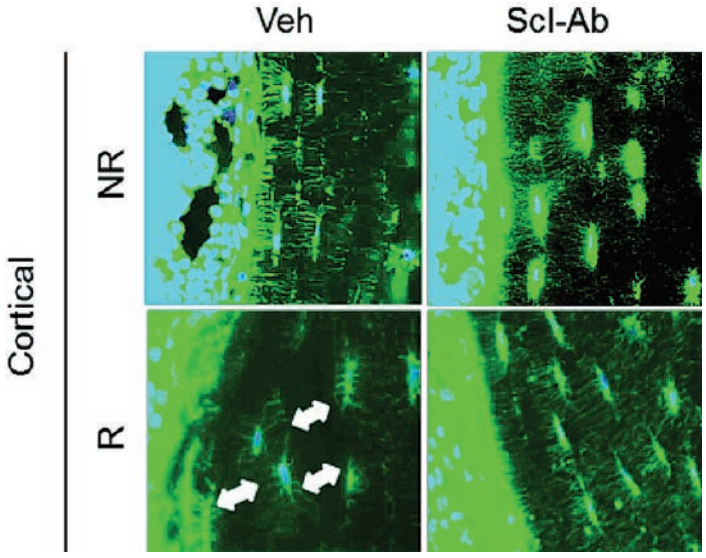

Figure 2. Scl-Ab protects the osteocyte canalicular-network from ionizing radiation. Double headed arrows indicate the lack of canalicular connections.

Acknowledgements

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Temporal Dynamics of Nascent ECM Production by Chondrocytes and MSCS via Multi-Color Protein Labeling

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Introduction

The extracellular matrix (ECM) of articular cartilage provides mechanical resilience over a lifetime of load-bearing use. Matrix synthesis and turnover occur continuously, and are requisite for cartilage homeostasis. In the context of engineered cartilage, the rates of ECM production, retention, and degradation define how rapidly an engineered construct can mature. However, most traditional methods to quantify the temporal dynamics of ECM formation rely on bulk biochemical measures that mask differences within the cell population.[1] Methods that resolve matrix at the single cell level, such as traditional staining or autoradiography coupled with histology, are unable to measure matrix synthesis rates in a single sample at multiple time points.[2] To overcome these limitations, we used a novel metabolic labeling approach in which artificial methionine analogs are included in the culture media. These analogs incorporate into proteins during synthesis and, following fixation, are fluorescently labeled via a 'click' reaction.[3] Sequential culture in the presence of different analogs allows for multi-color visualization of ECM formed at different times (analogous to how bone apposition rate is monitored by sequential exposure calcein and alizarin red.[4] This method allows one to quantify the temporal characteristics of ECM formation and visualize the structure and distribution of nascent ECM elements. In this study, we used this novel technique to quantify differences in the timing, heterogeneity, and localization of ECM produced by chondrocytes and mesenchymal stem cells (MSCs) in 3D culture.

Methods

Cell culture- Juvenile bovine MSCs and chondrocytes were isolated as in.[5] Passage 1 cells were encapsulated in 2% agarose micro-gels (μ-gels, ~400 μm in thickness) at 2×10^6 cells/mL. These μ-gels were cultured in a chemically defined, methionine-free media containing 10 ng/mL TGFβ-3 and 50 mM of the methionine analog homopropargylglycine (HPG) for days 1 to 7. On days 8 to 9, the HPG was replaced with 50 mM of a different methionine analog (azidohomoalanine (AHA)). Following fixation, Alexa488-azide and Alexa594-alkyne were reacted with HPG and AHA, respectively, to label proteins that had incorporated the methionine analogs during the two culture periods.[3] Nuclei were stained with Hoechst. *Imaging & analysis-* Individual cells were located via nuclear staining, and confocal sections of the cell midplane were captured at 100× (n = 20-30 cells/group). For each cell, 20 radial intensity profiles emanating from the cell center were mapped, truncated to include only the extracellular domain, and averaged over each cell. Total matrix intensity was calculated by integrating intensity over distance from the cell membrane. *Statistics-* Matrix radius and total intensity were compared via t-test.

Results

Methionine analogs metabolically labeled both intracellular proteins as well as proteins incorporated into the forming ECM (Figure 1A). At day 1, both cell types demonstrated intracellular labeling, but little extracellular labeling. During the first 7 days, ECM progressively extended from the cell border and into the extracellular space (Figure 1B-C). By day 7, both chondrocytes and MSCs accumulated a metabolically labeled extracellular matrix comprised of discrete fibers (Figure 1D). The chondrocyte matrix was both more extensive (radius = 19.8 vs 3.1 μm, $p < 0.01$) and more intensely labeled (total intensity = 165 vs 49 a.u., $p < 0.01$) than the MSC matrix. To compare the relative variability of the matrix, we computed the coefficient of variation (CV) of total matrix intensity. On day 7, MSCs demonstrated greater matrix variability than chondrocytes (MSC CV = 1.84, chondrocyte CV = 1.24), likely reflecting the heterogeneity that is characteristic of MSCs, as well as the slower kinetics of their differentiation and matrix formation process.

When the media was switched at day 8 from HPG- to AHA-containing media, two temporally distinct protein populations were labeled green and red respectively (Figure 2A). In chondrocytes, the nascent matrix accumulated over days 8-9 and labeled with AHA (red) was strikingly different from the matrix accumulated earlier in culture ('pre-existing' matrix incorporating HPG, green). In contrast to the discrete fibers formed at earlier time points, the nascent matrix formed by chondrocytes during this period lacked clear organization and

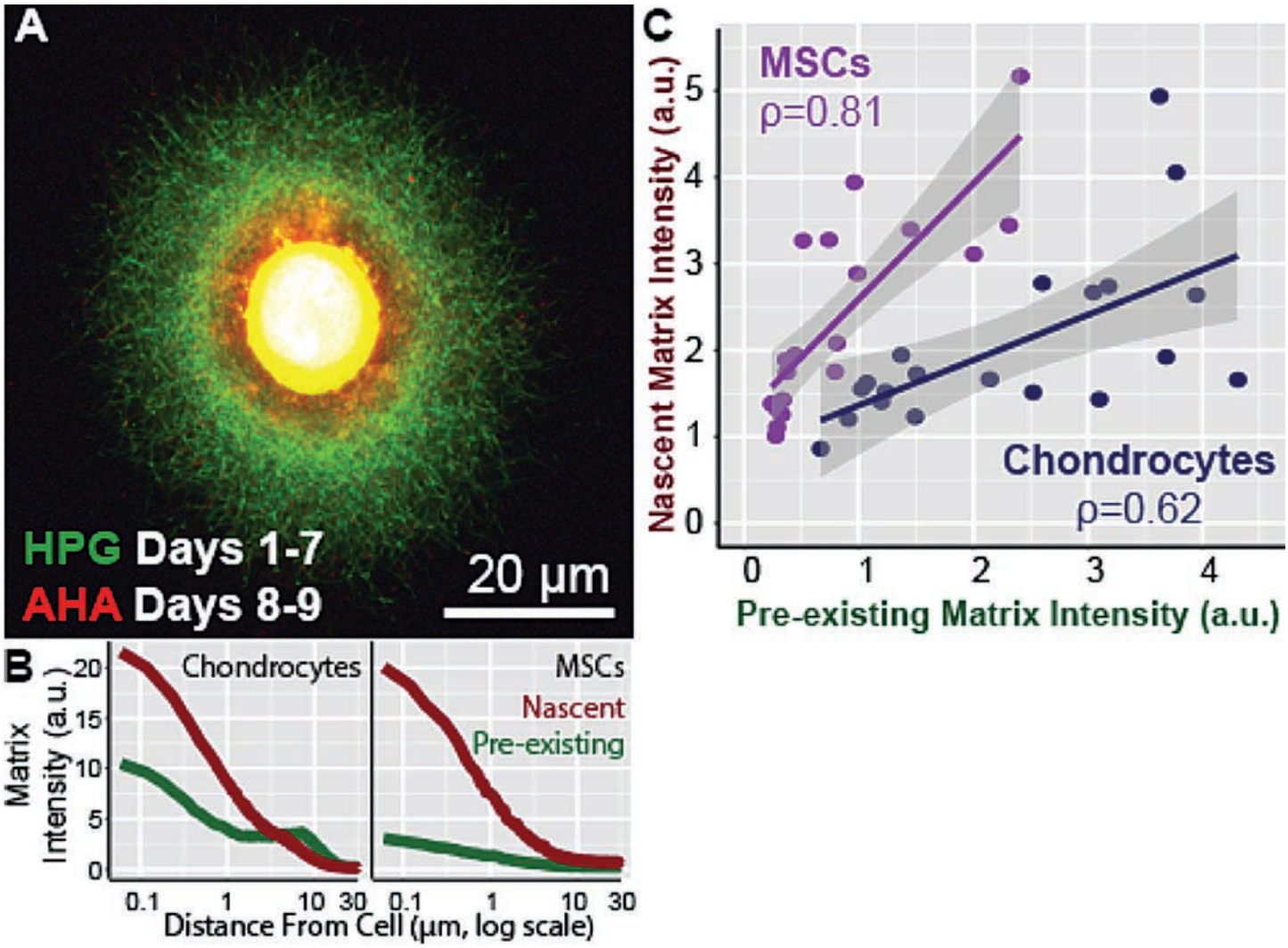

Figure 1. Metabolic labeling of ECM. (A) Day 7 chondrocyte labeled with HPG. (B) Radial profiles and (C) morphology of chondrocyte ECM with time. (D) Individual (grey) & average (blue/purple) profiles.

was primarily restricted to the pericellular space. Intensity profiles suggest chondrocyte nascent and pre-existing protein fractions segregated spatially (Figure 2B). Conversely, in MSCs, nascent matrix appeared to form discrete fibers that emanated from the cell and intermingled with pre-existing matrix. At day 9, the intensity of pre-existing matrix differed between cell types (chondrocyte = 116 a.u., MSC = 40 a.u., $p < 0.01$). However, the intensity of nascent matrix was similar between MSCs and chondrocytes. Because nascent and pre-existing matrix intensity were measured simultaneously, it was possible to examine the correlation between nascent and pre-existing matrix at the single cell level (Figure 2C). Nascent matrix was better correlated with pre-existing matrix in MSCs than in chondrocytes ($\rho_{MSC} = 0.81$, $\rho_{CH} = 0.62$, $p = 0.12$ via Fisher Z transformation).

Discussion

AHA and HPG incorporation into methionine-containing proteins (e.g. Col2, aggrecan) enabled the fluorescent labeling of temporally distinct protein fractions deposited in the extracellular space. One color labeling results were consistent with the expectation that ECM accumulates with culture time, and that chondrocytes produce more ECM than chondrogenically-induced MSCs.[1] Consistent with the notion that MSCs are comprised of multiple clonal sub-populations, ECM production by MSCs at the single cell level was more heterogeneous than chondrocytes. Sequential two color labeling showed that the matrix produced by chondrocytes at early time points differs in structure and organization from the nascent matrix produced later in culture. In contrast, the nascent and pre-existing matrix fractions were similar in MSCs, reflecting differences in the trajectory and rate of matrix accumulation between these cell types. Nascent matrix positively correlated with pre-existing matrix, suggesting that cells that initially produced extensive matrix continued to do so later in culture. The strong nascent-to-pre-existing correlation in MSCs may indicate that matrix accumulation from days 7-9 is a temporally steady process in MSCs. In contrast, the weaker correlation in chondrocytes

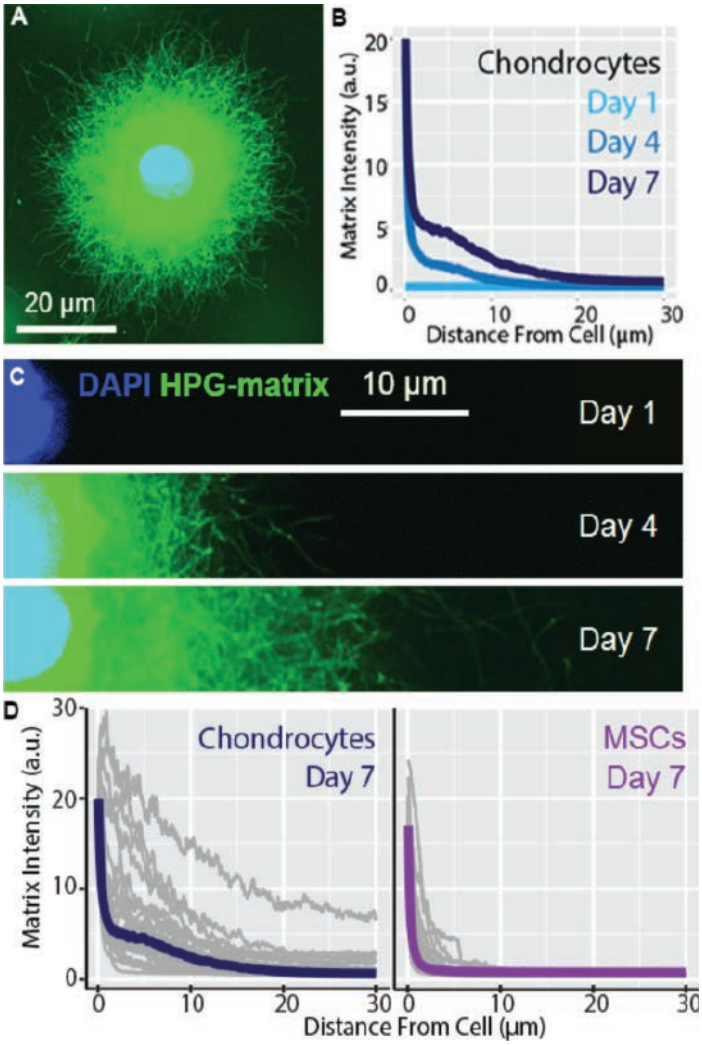

Figure 2. Sequential labeling. (A) Day 9 chondrocyte. (B) Matrix intensity profiles. (C) Correlation between nascent & pre-existing matrix.

may indicate a temporally variable (potentially switch-like) process. This difference could be inherent to cell type (MSC vs. chondrocyte), or could reflect the influence of the existing matrix on nascent matrix formation: as a cell accumulates a biologically 'sufficient' matrix, ECM production may undergo a shift in dynamics. To distinguish between these possibilities, future work will utilize two-color labeling over a time course to better ascertain matrix dynamics and heterogeneity at the single cell level.

Significance

Matrix dynamics are crucial for cartilage development and maintenance *in vivo* and *in vitro*. This technique enables interrogation of these dynamics at the single cell level.

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This work was supported by NIH R01 EB008722 & T32 HL007954.

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Matrix Degradation Enhances Cell Mobility in Dense Connective Tissues

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Introduction

Cell migration plays a pivotal role during tissue repair, where cells must first proliferate and colonize the wound site. Both high extracellular matrix (ECM) stiffness and density have been implicated as barriers to 3D migration,[1,2] especially when cell-mediated proteolysis via matrix metalloproteinases (MMPs) is inhibited. Although the highly organized ECM of mature dense connective tissues enables mechanical function,[3] these densely packed collagen fibers may inhibit cell migration after injury, resulting in poor healing in adults. We hypothesized that the adult ECM is a biophysical impediment to cell mobility during repair, and that reduction of this steric constraint would expedite cell migration to the wound site. Using the adult knee meniscus as a platform, we show that modulating the ECM microstructure via an exogenous matrix-degrading enzyme enhances interstitial cell mobility, and that this acts synergistically with endogenous MMPs to promote cell migration through the dense ECM.

Methods

Tissue Microstructure Analysis: Tissue explants (8 mm ø) were excised from adult bovine meniscal bodies and cryosectioned onto glass slides (~35 µm thick). Devitalized sections were UV sterilized and hydrated. Three substrates were tested: untreated adult ECM (Control), and adult ECM pre-treated with 0.05 or 0.1 mg/mL collagenase in basal media (BM) for 1 hour (LowC or HighC). To visualize fibrillar collagen, second harmonic generation (SHG) imaging was performed.[4] Maximum z-stack projections were used to identify discrete areas of positive and negative signal, representing aligned fibers parallel to the substrate and inter-fibrillar material that constitutes the remaining ECM (n = 10 stacks/group). The average diameter of discrete inter-fibrillar regions was quantified using Fiji's Local Thickness plugin (n = 10 stacks/group). *Interstitial Migration Analysis:* To visualize cell invasion, adult meniscal explants (4 mm ø) were incubated in CellTracker™ Green for 1 hour and then placed atop sections to allow for cell egress onto the section. Two media conditions were tested for each substrate group (n = 3 samples/group): BM with or without 1 µg/mL of the broad spectrum MMP inhibitor GM6001 (MMPi). After 48 hours, explants were removed and the nuclei of egressed cells were stained with 4', 6-diamidino-2-phenylindole (DAPI). Confocal z-stacks were obtained in the FITC and DAPI channels to visualize cells, nuclei, and devitalized matrix (autofluorescent in the DAPI channel). Cell area and aspect ratio (elongation) were determined from maximum z-stacks projections (n=100 cells/group). Cell infiltration depth was measured as the distance between the apical tissue surface and the basal cell surface (n = 100 cells/group). *Statistics:* Significance was assessed by one- or two-way ANOVA with Tukey's HSD post hoc tests to compare substrate and media conditions between groups ($p \leq 0.05$). A cumulative distribution plot, coupled with the Kolmogorov-Smirnov test, was used to determine whether the distribution of

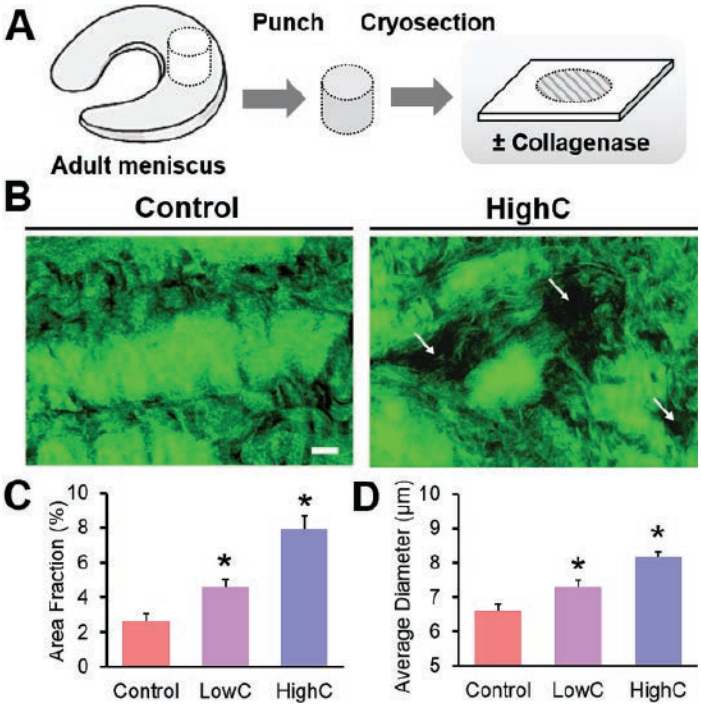

Figure 1. Matrix degradation decreases ECM density. (A) Experimental schematic. (B) SHG signal (green) of substrates. Arrows indicate inter-fibrillar regions. Scale = 20 µm. (C) Inter-fibrillar area fraction and (D) Diameter. * = $p \leq 0.05$ vs. all other groups.

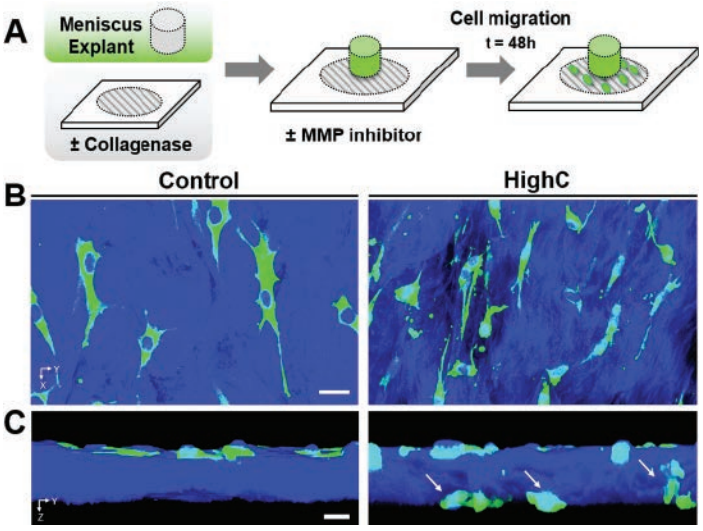

Figure 2. Matrix degradation expedites interstitial cell migration. (A) Experimental schematic. (B) Confocal reconstructions of adult meniscal cells (green) on tissue substrates (blue). Scale = 20 μm. (C) Cross-sectional view of (B). Arrows point to infiltrating cells. Scale = 10 μm.

infiltration was different between groups ($p \leq 0.05$). Data are presented as mean ± SEM.

Results

SHG imaging of adult meniscal sections pre-treated with various levels of collagenase revealed distinct microenvironments (Figure 1B). Qualitatively, the untreated Control substrate had thicker and more organized collagen bundles than the low-dose (LowC) and high-dose (HighC) collagenase groups. The area fraction of inter-fibrillar ECM, indicated by the absence of SHG signal, increased with collagenase dose (Figure 1C, $p \leq 0.05$). The average diameter of discrete inter-fibrillar regions also increased with collagenase dose, suggesting local disruption of the native collagen network (Figure 1D, $p \leq 0.05$). Adult meniscal cells from explants adhered to and infiltrated the devitalized tissue substrates within 48 hours (Figure 2B). Cells in the untreated Control group remained spread on the tissue surface, whereas cells in the collagenase groups were found within or below the tissue surface (Figure 2C). Cell infiltration depth was significantly greater for the HighC group (10.9 ± 0.6 μm) compared to the LowC (5.1 ± 0.2 μm) and Control (3.6 ± 0.1 μm) groups (Figures 3A and 3B, $p \leq 0.05$). Inhibition of cell-produced MMPs (MMPi) decreased infiltration depth for the HighC group only (7.5 ± 0.6 μm). While cells on all substrates aligned in the fiber direction of the underlying tissue, cell morphology was dependent on the substrate and media condition. Cell area decreased with increased substrate degradation, but was not affected by the addition of MMPi (not shown, $p \leq 0.05$). On the other hand, cell aspect ratio (elongation) remained constant with substrate degradation, but increased with MMPi for LowC and HighC substrates (Figure 3C, $p \leq 0.05$).

Discussion

Restricted interstitial cell migration may prevent proper healing of the adult meniscus and other dense connective tissues. An innovative strategy to promote repair may be to first free native cells from the matrix so as to facilitate migration to the wound site. Our findings suggest that interstitial cell mobility increases with matrix degradation. Adult meniscal cells on devitalized adult tissue sections pre-treated with collagenase were smaller and more invasive than the same cells on untreated tissue. In the untreated condition, migrating cells must navigate through narrow spaces between rigid, aligned collagen fibers, analogous to the constraints imposed by a decreasing pore size in transwell assays.[1] Partial enzymatic digestion improved cell mobility by increasing the area fraction and size of inter-fibrillar regions and also by decreasing the local ECM stiffness.[4] Blocking cellular MMPs increased cell elongation and decreased infiltration depth, suggesting that exogenous and endogenous MMPs act synergistically to remodel the ECM during migration. However, this effect was only observed in the HighC group, indicating that when the biophysical barrier is too great, endogenous MMPs play

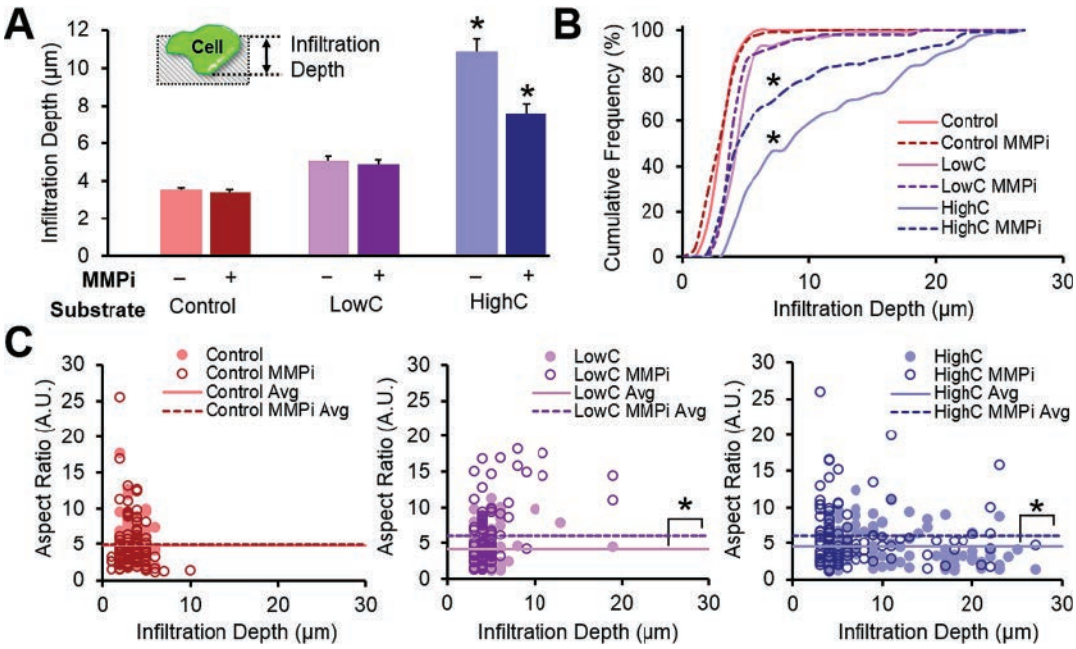

Figure 3. Exogenous and endogenous MMPs work synergistically to enhance migration. (A) Average and (B) Cumulative frequency distribution of cell infiltration depth. *= $p \leq 0.05$ vs. all other groups. (C) Aspect ratio of individual cells (circles) as a function of infiltration depth for different substrate and media conditions. Lines indicate averages. *= $p \leq 0.05$ between groups.

a limited role and migration relies more on cell deformation through the inter-fibrillar regions. Taken together, our data indicate that providing the proper microenvironment for interstitial migration ultimately results in enhanced cellularity and integration at the wound interface.[4] By addressing the inherent limitations to repair imposed by the mature ECM, these studies may define a new clinical paradigm for repairing damaged dense connective tissues

Significance

Partial enzymatic digestion of the ECM expedites interstitial cell migration, which may promote dense connective tissue repair.

Acknowledgments

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Platelet-Derived Growth Factor Promotes Interstitial Cell Migration in the Knee Meniscus

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Introduction:

Few regenerative approaches exist for injuries to adult dense connective tissues. Compared to fetal tissues, adult tissues are hypocellular and lack a sufficient population of endogenous cells at the wound site to affect repair.[1] We hypothesized that this deficiency is exacerbated by the dense and stiff adult extracellular matrix (ECM), a biophysical barrier that restricts cell mobility.[2] We also hypothesized that chemotactic cues[3] might help overcome this barrier and promote cell migration through small pores. Using the knee meniscus as a test platform, we investigated the age-dependent response of cells to physical migratory barriers and developed a novel 'tissue Boyden chamber' system to determine whether interstitial cell migration through native tissue is enhanced by a local platelet derived growth factor AB (PDGF-AB) gradient.

Methods:

Transwell Migration Assay: Menisci were isolated from fetal and adult cows and minced to isolate cells. To assess migration in the presence of physical barriers, 96-well transwell migration assays with pore diameters of 3, 5, or 8 μm were used (Millipore). Cells (Passage 1) in DMEM + 1% FBS were seeded at 50k cells per top chamber and incubated for 16 hours before being fluorometrically quantified. Three media conditions in the bottom chamber were tested (n = 3 wells/group): DMEM + 1% FBS (Control) and DMEM + 1% FBS with either 50 or 100 ng/mL human recombinant PDGF-AB. Fluorescence signal intensity was normalized to the 3 μm pore group for each condition. To visualize cell migration, fetal and adult tissue explants (8 mm ø) were incubated in CellTracker™ Green for 1 hour and then placed atop microporous membranes. After 48 hours in DMEM + 1% FBS, the egressed cell nuclei were stained with 4', 6-diamidino-2-phenylindole (DAPI). Membranes were mounted onto glass slides, and confocal z-stacks obtained at 20X in the FITC, DAPI, and TRITC channels to visualize cells, corresponding nuclei, and membranes (autofluorescent in the TRITC channel). Migration Through Native Tissue: To generate physiologic microenvironments, fetal meniscal bodies were cryotomed into transverse sections (~35 μm thick) and mounted onto glass slides to cover four holes (1 mm ø). To fabricate a 'tissue Boyden chamber' that allows interstitial migration of cells towards a chemotactic gradient, the tissue-mounted slide was set atop a concave slide containing 140 μL of either DMEM + 1% FBS (Control) or 200 ng/mL PDGF-AB. The top and bottom slides were sealed, and fetal or adult tissue explants incubated in CellTracker™ Green were placed atop the tissue sections (n = 3/group). After 48 hours in DMEM + 1% FBS, slides were stained as above. Confocal z-stacks were obtained at 10× in the FITC and DAPI channels to visualize cells and nuclei engaging with the devitalized tissue spanning the slide holes (autofluorescent in the DAPI channel). To assess the quantity of egressed cells around and within the holes, cell signal area was determined using maximum z-stack projections in Fiji (n = 9 holes/group). To quantify interstitial migration, cells from five tissue cross-sections were counted for each hole (n = 3 holes/group) and categorized as either Surface or Migrated, where a Migrated cell was entirely embedded within the tissue or had emerged onto the other side. Statistics: Significance was assessed by one- or two-way ANOVA with Tukey's HSD post hoc tests to determine the impact of pore size, media condition, and/or age ($p \leq 0.05$). Data are presented as the mean ± SD unless otherwise noted.

Results:

Transwell migration of fetal and adult meniscal cells was dependent on pore size and PDGF dose. Confocal images revealed that for both age groups, nuclei were able to deform and pass through the 5 and 8 μm pores, but could not pass through 3 μm pores (Figures 1A and 1B). In the absence of a PDGF gradient (Control), there was no difference between pore sizes for either age group (Figures 1C and 1D). With the addition of PDGF to the bottom chamber, pore size had a significant effect, with cell migration increasing as a function of pore size for both ages ($p \leq 0.05$). Addition of 100 ng/mL PDGF-AB (High PDGF) to the bottom chamber significantly increased migration through both the 5 and 8 μm pores compared to the 3 μm pores. A similar trend was seen for the 50 ng/mL PDGF-AB (Low PDGF) group, though migration was increased for only the 8 μm pores. While

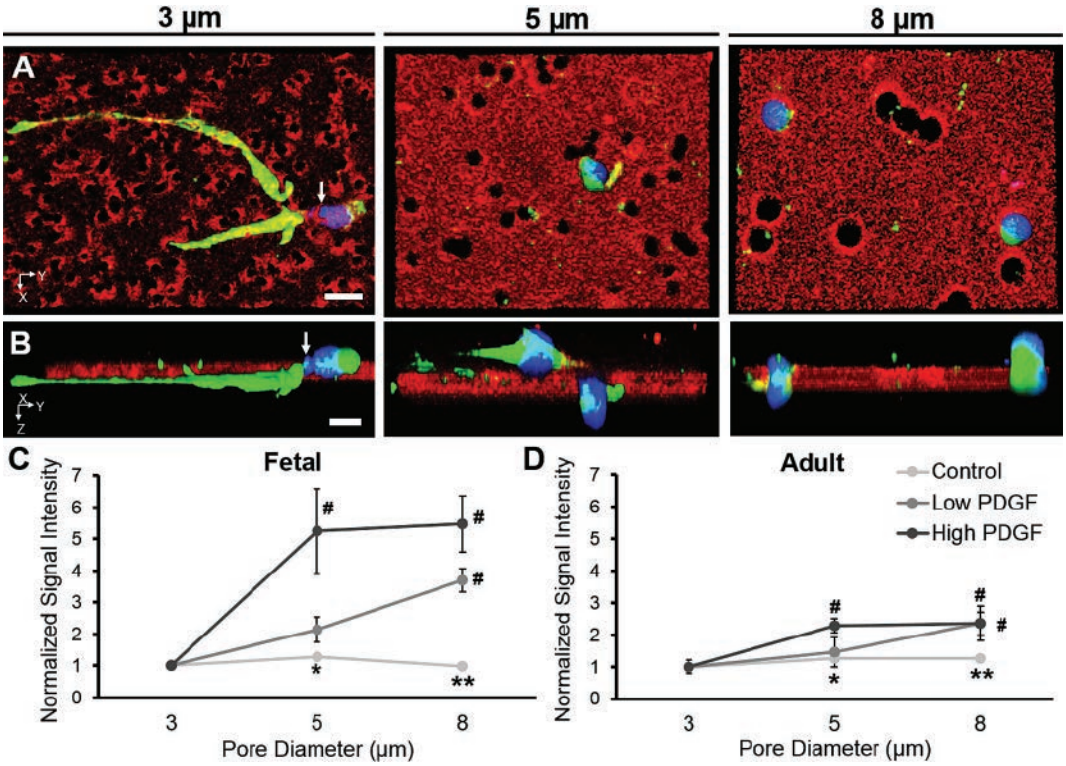

Figure 1. PDGF-AB enhances cell migration through intermediate size pores. (A) Confocal reconstructions of adult cells (green) passing through microporous membranes (red) of 3, 5, and 8 μm diameter. Arrow points to constricted nucleus (blue). Scale = 10 μm. (B) Cross-section of the cells in (A). Scale = 10 μm. (C) Migrated fetal and (D) Adult cell signal intensity normalized to 3 μm pore for each media condition. = $p \leq 0.05$ vs. High PDGF. **= $p \leq 0.05$ vs. all other media conditions. #= $p \leq 0.05$ vs. 3 μm pore.

types,[2] meniscus cell migration declines with decreasing pore size, and eventually cells are rendered immobile. Notably, the nucleus is the rate-limiting organelle in migration due to its large size and stiffness.[2] Since 3D migration depends on deformability through interstitial space, age-related changes in cell and/or nuclear mechanics may play a role in the differential mobility seen between fetal and adult cells. While low interstitial cell mobility may contribute to the lack of cell-mediated repair in the adult meniscus, this limitation may be partly overcome via the provision of a soluble chemotactic gradient that promotes migration to the injury site. Here, we show that a PDGF-AB gradient enhances cell infiltration through membranes of intermediate pore sizes, as well as through migration increased with PDGF dose for both age groups, fetal cells were more mobile than adult cells. To study interstitial migration towards a PDGF gradient, a 'tissue Boyden chamber' was fabricated (Figure 2). When fetal and adult tissue explants were placed atop the devitalized meniscal tissue, egressing cells adhered, spread, and began migrating into the substrate within 48 hours. In all conditions, the cell signal area (% of total area) was greater for fetal cells than adult cells (Fig. 2D, $p \leq 0.05$). Without PDGF in the bottom chamber (Control), few cells from either age migrated through the tissue (Figure 2E). Addition of 200 ng/mL PDGF-AB to the bottom chamber increased the adult cell signal area as well as the number of Migrated cells for both age groups compared to Controls ($p \leq 0.05$).

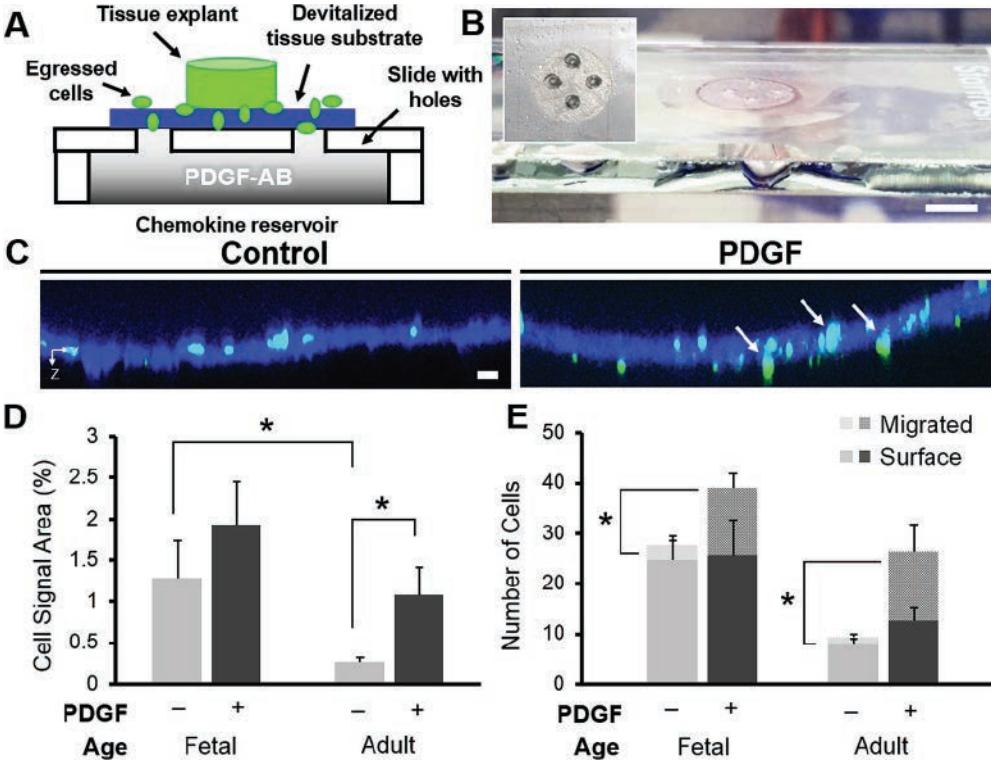

Figure 2. PDGF-AB enhances cell migration through native tissue. (A) Schematic of a 'tissue Boyden chamber.' (B) Tissue slide atop concave slide containing media. Inset shows tissue cryosection. Scale = 5 mm. (C) Confocal cross-section of cells migrating through the tissue section. Arrows point to Migrated cells. Scale = 20 μm. (D) Fetal and adult cell signal area for each media condition (mean ± SEM). *= $p \leq 0.05$ between groups. (E) Fetal and adult cells within the slide hole. *= $p \leq 0.05$ comparing Migrated cells between groups.

Discussion:

The ECM of dense connective tissues is a physically restrictive microenvironment. As with other cell

a physiologic microenvironment using a novel 'tissue Boyden chamber.' Given the potent chemotactic effect of PDGF-AB, we are currently investigating whether this factor can expedite cell migration to the wound site after localized matrix degradation, which decreases ECM density at the wound interface.[4] By combining these diverse but complementary approaches to augment interstitial migration, we aim to recapitulate the robust healing response of fetal tissues.

Significance

Platelet-derived growth factor-AB stimulates interstitial migration, which may facilitate cell-mediated repair of dense connective tissues.

Acknowledgments

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Aberrant Glycosaminoglycan Accumulation and Sulfation in Epiphyseal Cartilage in Mucopolysaccharidosis VII

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Introduction

The mucopolysaccharidoses (MPS) are a family of genetic, lysosomal storage diseases that are characterized by deficient activity of one of the 11 acid hydrolases responsible for degradation of glycosaminoglycans (GAGs). MPS VII is characterized by impaired β-glucuronidase activity, leading to the incomplete digestion and progressive accumulation of heparan, chondroitin, and dermatan sulfate GAG byproducts.[1] MPS VII presents with severe skeletal manifestations, which are particularly prevalent in the spine and include scoliosis, kyphosis, and spinal cord compression.[2-4] Previously, our lab established the presence of cartilaginous lesions in the vertebral bodies of MPS VII patients and dogs, which represent failed epiphyseal bone formation during postnatal development.[5] Using the naturally-occurring canine model, we identified the developmental window (between 9 and 14 days-of-age) when failed bone formation first manifests (Figure 1A), and showed that resident chondrocytes fail to undergo hypertrophic maturation. However, the links between chondrocyte dysfunction and aberrant GAG accumulation in MPS VII remain to be established. GAGs perform crucial roles in controlling the distribution and availability of many growth factors that regulate cell differentiation during endochondral ossification. The biological function of these GAGs, including binding to specific growth factors, is a function not only of their concentration, but also fine structure, including critical dependence on sulfation.[6-8] The objectives of this study were to 1) identify defects in GAG sulfation pathways in MPS VII epiphyseal cartilage using whole-transcriptome sequencing (RNA-Seq), 2) define the nature of GAG accumulation in MPS VII epiphyseal cartilage, and 3) validate an in vitro explant culture model for measuring GAG accumulation for future mechanistic studies of cellular dysfunction.

Methods

For this study, we used the naturally-occurring MPS VII canine model that mimics both the progression and pathological phenotype of the skeletal abnormalities found in human patients.[9] With IACUC approval, unaffected control and MPS VII dogs were euthanized at 9 and 14 days-of-age, and T12, L1, and L2 vertebrae were excised for analyses. Whole-Transcriptome Sequencing: Vertebral epiphyseal cartilage from T12 vertebrae of control and MPS VII dogs ($n = 5$, all groups) was collected, total RNA extracted, and RNA-Seq libraries prepared (Illumina TruSeq mRNA stranded kit). Paired-end, 100-base pair sequencing was performed (Illumina HiSeq 2500) and results mapped to the canine genome. Differential gene expression for GAG metabolic pathways was determined with DESeq2[10] (significance, $p < 0.05$). GAG Content and Disaccharide Composition: Cranial and caudal vertebral epiphyseal cartilage from L1 vertebrae of control and MPS VII animals at 9 days-of-age ($n = 3$, both groups) was excised, combined, and digested with collagenase until cells were released from the extracellular matrix. Digests were centrifuged to separate the supernatant (extracellular fraction) from the cell pellet (intracellular fraction). Total GAG content in each fraction was measured using the dimethylmethylene blue (DMMB) assay and normalized to total cell count. Disaccharide composition of chondroitin and dermatan sulfate (CS/DS) extracellular GAGs isolated as above from 9-day control and MPS VII epiphyseal cartilage ($n = 3$, both groups) was determined using UPLC. Significant differences in GAG composition ($p < 0.05$) were determined using unpaired t-tests. Explant Culture: Epiphyseal cartilage from L2 control ($n = 4$) and MPS VII ($n = 2$) vertebrae was cultured as explants for 5 days in serum-free medium (α-mem, 0.1% BSA, 1% PSF) and total GAG content in intracellular and extracellular fractions, and in media, was measured using the DMMB assay and normalized to total cell count, with significant differences between groups ($p < 0.05$) established using unpaired t-tests.

Results

Whole-Transcriptome Sequencing: RNA-Seq principal component analysis (PCA) of global gene expression showed distinct clustering (Figure 1B), indicating clear effects of both age and disease state between all groups. At 9 days-

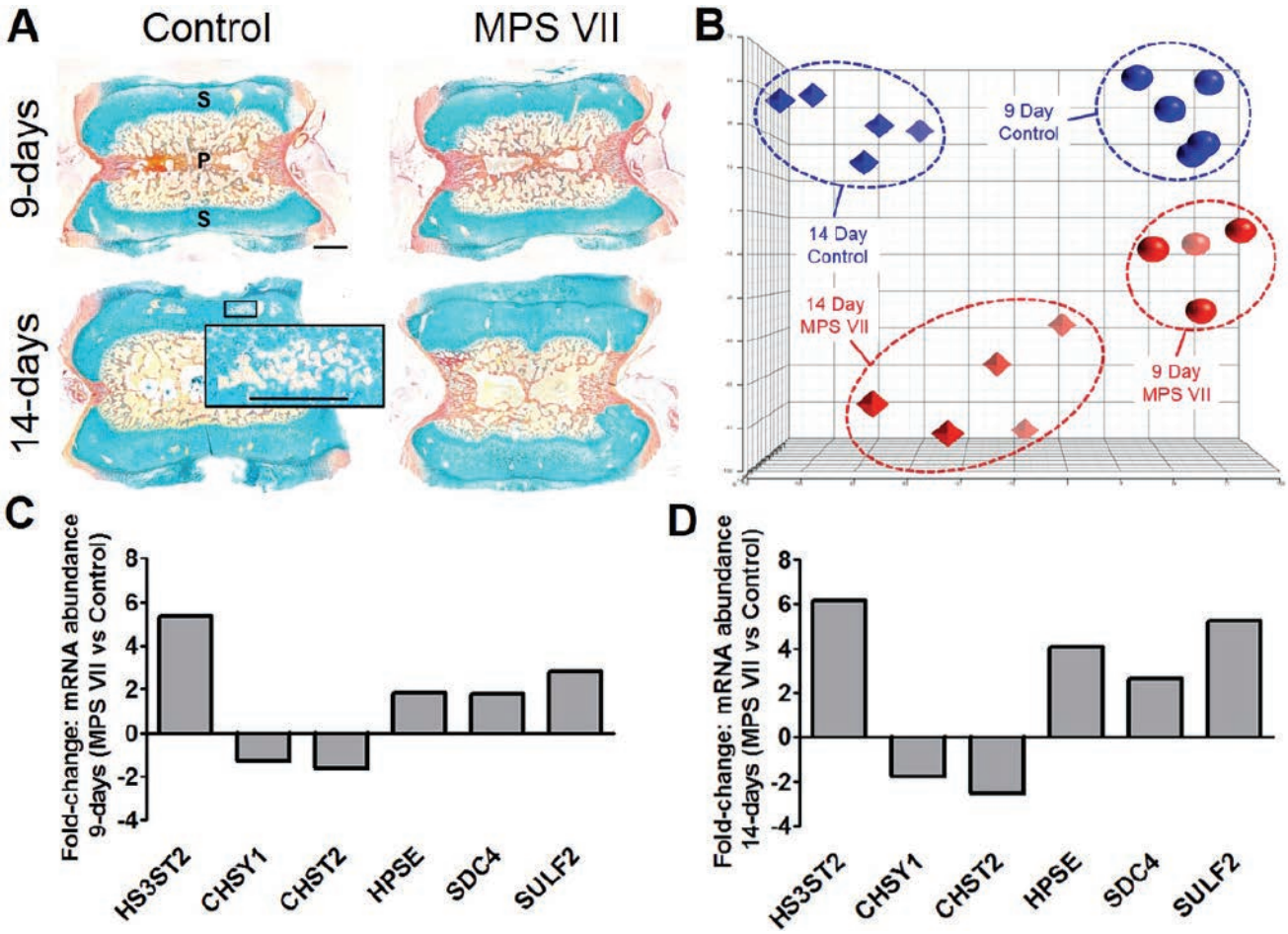

Figure 1. A. Representative mid-coronal ABPR-stained images of T11 vertebrae at 9 and 14 days-of-age. Inset: bone formation in secondary ossification centers in 14-day control animals. S: Secondary and P: Primary ossification center. B. RNA-Seq PCA plot. C. Fold-change of mRNA expression in MPS VII vertebral epiphyseal cartilage vs control at 9 days-of-age. D. Fold-change of mRNA expression in MPS VII vertebral epiphyseal cartilage vs control at 14 days-of-age. Scale = 1mm; n = 5; all $p < 0.05$.

of-age, there was significant differential mRNA abundance of key genes involved in GAG sulfation, and differences were even greater at 14 days-of-age (Figure 1C, D). GAG Content and Disaccharide Composition: Both intracellular and extracellular GAG content were significantly higher in MPS VII cartilage at 9 days (Figure 2A, B). There were also significant differences

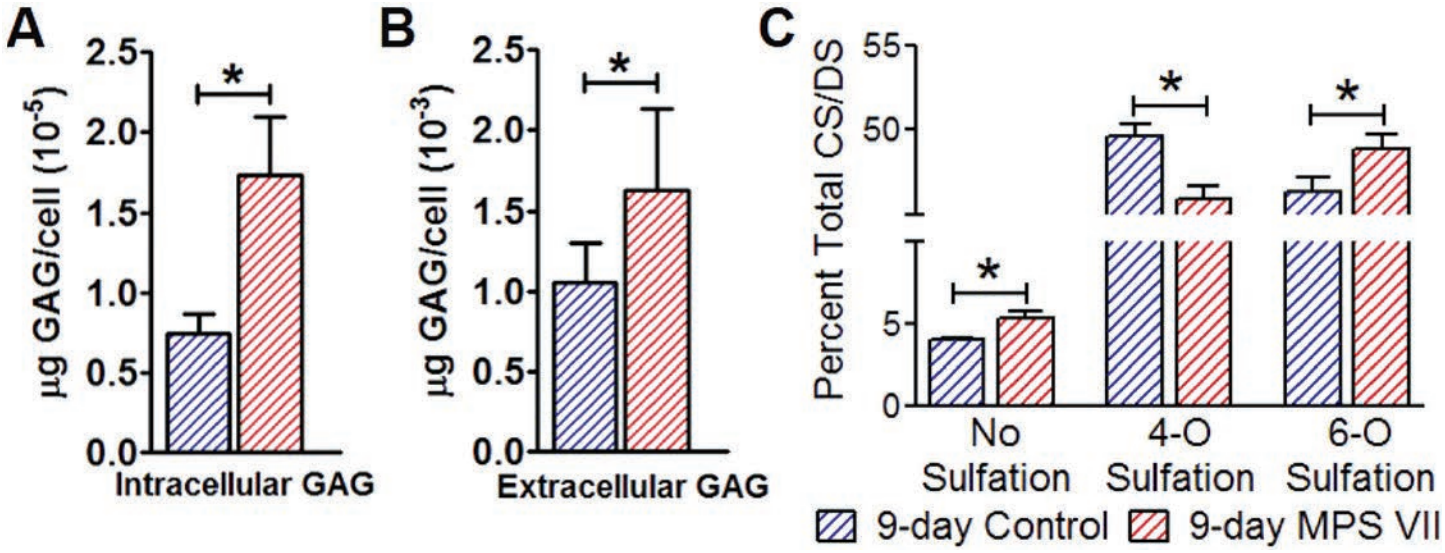

Figure 2. GAG analysis of 9-day vertebral epiphyseal cartilage. A. Intracellular GAG content. B. Extracellular GAG content. C. Extracellular GAG chondroitin and dermatan sulfate disaccharide composition. N = 3; *$p < 0.05$.

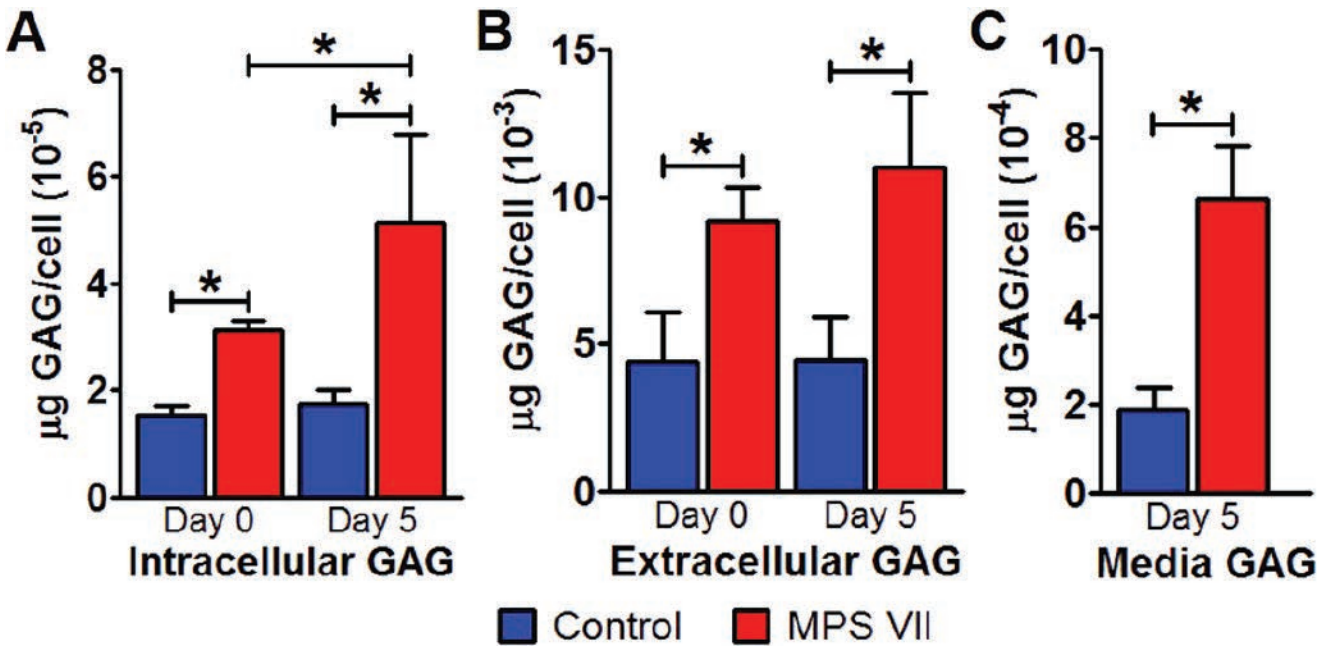

Figure 3. GAG content in epiphyseal cartilage explant model. A. Intracellular fraction. B. Extracellular fraction. C. Culture media, normalized to total cell count. Control (n=4), MPS VII (n = 2); *p < 0.05.

in CS/DS disaccharide composition between control and MPS VII vertebral epiphyseal extracellular GAGs, in both extent and position of sulfation (Figure 2C). Explant Culture: After 5 days in culture, MPS VII cartilage explants exhibited increased GAG content in both intracellular and extracellular fractions compared to controls, with intracellular GAG content significantly higher in MPS VII after 5 days of culture, while GAG content in controls remained stable over time (Figure 3A, B). Media GAG content after 5 days was also significantly higher for MPS VII compared to controls (Figure 3C).

Discussion

Results demonstrate that while tissue-level differences are not yet evident between control and MPS VII epiphyses at 9-days-age, molecular level abnormalities are present that may impact cell function and initiation of ossification. Differential expression of genes involved GAG sulfation indicates that there is broad dysregulation of GAG metabolic pathways in MPS VII that occurs secondary to the primary GUSB mutation. Elevated extracellular GAG content and abnormal GAG sulfation patterns in MPS VII cartilage may disrupt the signaling pathways that are necessary to initiate and sustain chondrocyte hypertrophic differentiation through altered growth factor binding and distribution. Elevated intracellular GAG content also likely contributes to cellular dysfunction by increasing cellular stress. Continued accumulation of GAGs in MPS VII explants over 5 days of in vitro culture suggest that resident cells remain metabolically active, validating this model for future mechanistic studies of abnormal GAG metabolism.

Significance

MPS VII is associated with severe skeletal disease for which there are no treatments. This study establishes the nature of aberrant GAG accumulation in MPS VII epiphyseal cartilage and identifies defects in GAG sulfation pathways, which likely contribute to cellular dysfunction and failed bone formation.

Acknowledgments

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Impaired Wnt Signaling Contributes to Delayed Chondrocyte Differentiation in Mucopolysaccharidosis VII Dogs

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Introduction

Mucopolysaccharidosis (MPS) VII is a lysosomal storage disorder characterized by mutations in the β-glucuronidase (GUSB) gene. Impaired GUSB enzyme activity leads to the incomplete digestion and progressive accumulation of heparan, chondroitin, and dermatan sulfate glycosaminoglycan (GAG) byproducts.[1] MPS VII presents with severe skeletal manifestations, which are particularly prevalent in the spine. Vertebral dysplasia due to failed cartilage-to-bone conversion during postnatal development leads to kyphoscoliosis and spinal cord compression, significantly reducing patient quality of life and life expectancy.[2,3] Using the naturally-occurring canine model, we previously identified the developmental stage (between 9 and 14 days-of-age) when failed vertebral bone formation first manifests in MPS VII[4] and subsequently found that resident chondrocytes in the vertebral epiphyseal cartilage fail to undergo hypertrophic differentiation (Figure 1). GAGs perform crucial roles in the distribution and availability of many secreted signaling molecules that regulate chondrocyte differentiation.[5,6] We hypothesized that aberrant GAG accumulation in MPS VII epiphyseal cartilage disrupts these signaling pathways, preventing initiation of chondrocyte differentiation at the appropriate developmental stage. Our objectives in this study were to 1) establish pathways that fail to activate in MPS VII epiphyseal cartilage using whole-transcriptome sequencing (RNA-Seq) and 2) examine cellular responses to related secreted growth factors using a cartilage explant model.

Methods:

With IACUC approval, vertebral epiphyseal cartilage from unaffected control and MPS VII dogs was collected postmortem at 9 and 14 days (n = 5 all groups, schematic, Figure 1B). High quality total RNA (RIN > 7) was extracted from each sample, and RNA-Seq libraries were prepared using the TruSeq mRNA stranded kit (Illumina; San Diego, CA). Paired-end, 100-base pair sequencing was performed (Illumina HiSeq 2500) and results mapped to the canine genome. Differential gene expression between all groups was determined with DESeq2,[7] with litter as a covariate and adjusted for false discovery rate (significance, $p < 0.05$). Differential mRNA expression was confirmed using qPCR, and nuclear β-catenin protein levels were measured via Western blots and densitometry. For explant culture studies, vertebral epiphyseal cartilage explants from 9 day control (n = 4) and MPS VII (n = 2) animals were cultured for 1, 3, or 7

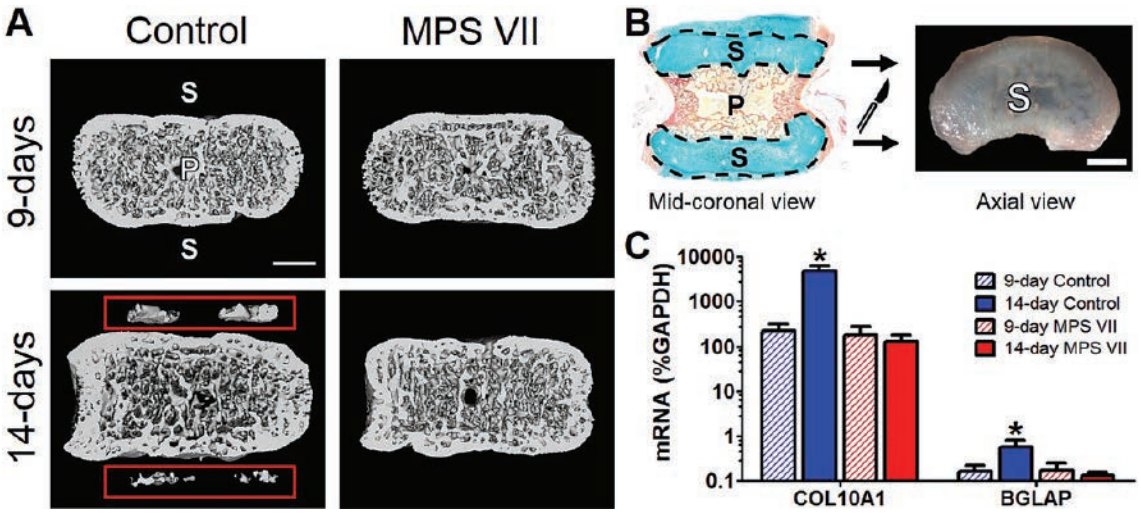

Figure 1. Representative mid-coronal microCT images of T7 vertebrae. Red boxes: bone formation in secondary ossification centers in 14-day control animals. (B) Schematic of vertebral epiphyseal cartilage excision (mid-coronal ABPR-stained histological section). (C) mRNA levels of COL10A1 (hypertrophic marker) and BGLAP (bone marker) in vertebral epiphyseal cartilage at 9 and 14 days-of-age. N = 5; scale = 1mm; *p < 0.05 vs all. S: Secondary and P: Primary ossification center.

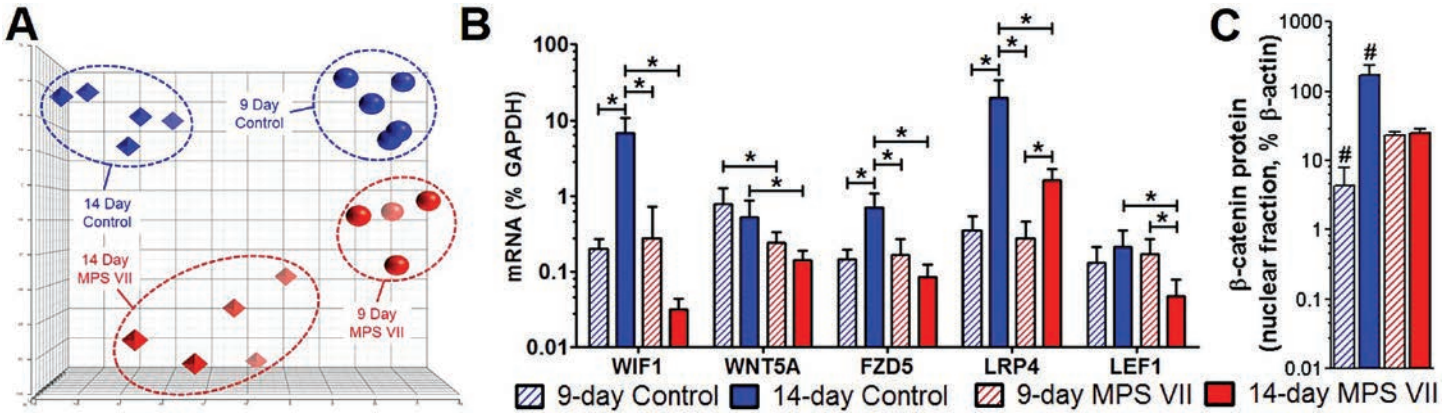

Figure 2. (A) RNA-Seq PCA plot. (B) mRNA levels of Wnt/β-catenin signaling pathway genes measured by qPCR. (C) Nuclear β-catenin protein expression in control and MPS VII vertebral epiphyseal chondrocytes. N = 5; *p < 0.05; #p < 0.05 vs all.

days in serum-free medium (α-mem, 0.1% BSA, 1% PSF) in the presence or absence of 500 ng/mL Wnt3a (R&D, Minneapolis, MN). Following culture, RNA was isolated from explants and SOX9 mRNA expression was determined using qPCR. Significance ($p < 0.05$) was established with 2-way analyses of variance and post-hoc Tukey's tests (RNA-Seq confirmatory mRNA and protein expression) or unpaired t-tests (cell culture mRNA expression).

Results

Principal component analysis (PCA) of global gene expression from RNA-Seq showed distinct clustering of each sample group (Figure 2A), indicating clear effects of both age and disease state between all groups. A total of 411 and 1104 genes were significantly differentially expressed with a fold-change greater than 2 between control and MPS VII at 9 and 14 days, respectively. The Wnt/β-catenin pathway was identified as the top dysregulated bone formation pathway at both ages with 14 and 54 pathway-associated genes differentially expressed at 9 and 14 days, respectively. Specifically, there was significantly lower expression of both key inhibitory elements and activating molecules in MPS VII compared to controls, verified by qPCR (Figure 2B). Immunoblots showed significantly higher levels of nuclear β-catenin protein at 14 compared to 9 days in controls but no change in MPS VII (Figure 2C). Control explants treated with Wnt3a exhibited a significant decrease in SOX9 mRNA after 1 and 3 days. After 3 and 7 days, untreated control explants also exhibited a significant decrease in SOX9 expression (Figure 3). In contrast, MPS VII explants exhibited no significant response to Wnt3a at day 1, but did exhibit significant decreases in SOX9 mRNA after 3 and 7 days of treatment. Unlike untreated controls, untreated MPS VII explants did not show decreased SOX9 expression after 3 or 7 days.

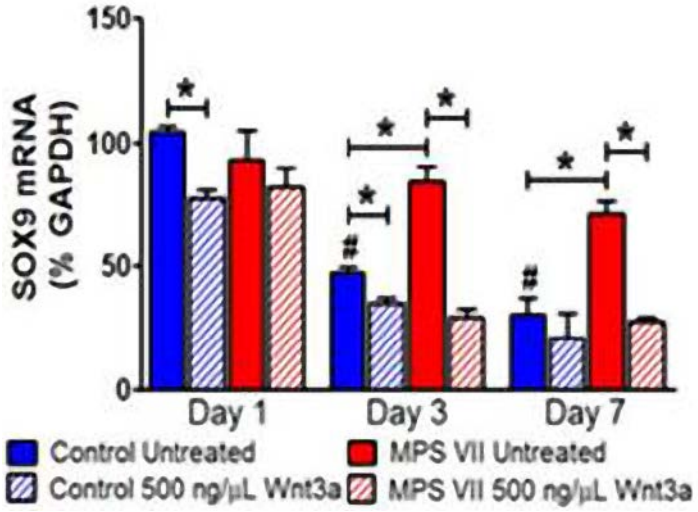

Figure 3. SOX9 mRNA for control (n = 4) and MPS VII (n = 2) explants with Wnt3A treatment after 1, 3 and 7 days. *p < 0.05, #p < 0.05 vs day 1.

Discussion

Wnt/β-catenin signaling regulates both the timing and rate of chondrocyte differentiation during endochondral ossification.[8,9] Our results demonstrate that in MPS VII epiphyseal cartilage, Wnt/β-catenin signaling does not activate at the appropriate developmental stage to initiate and sustain chondrocyte differentiation. Lower expression of both inhibitory and activating molecules in the Wnt pathway suggests that MPS VII chondrocytes experience decreased Wnt signaling but are unable to upregulate compensatory responses. Sustained low levels of nuclear β-catenin protein expression from 9 to 14 days in MPS VII is consistent with this mechanism. Wnt3a is the prototypical activator of Wnt/β-catenin signaling, potentiating chondrocyte maturation and subsequent bone formation.[10] Downregulation of SOX9 expression is necessary for chondrocytes to proceed from proliferation to hypertrophy.[11] Control explants (with healthy chondrocytes) treated with Wnt3a exhibited an immediate decrease in SOX9; further, with increasing culture time, untreated control explants exhibited decreased SOX9, suggesting an intrinsic propensity of healthy cells to mature towards hypertrophy even in the absence of exogenous signals. In contrast, MPS VII explants exhibited a delayed response to Wnt3a treatment, and in the absence of Wnt3a

continued to express high levels of SOX9 after 3 and 7 days, suggesting an intrinsic inability of diseased cells to both respond to exogenous signals and transition from proliferation to hypertrophy. These results provide the basis for further mechanistic investigations of skeletal disease in MPS VII and identify the Wnt/β-catenin pathway as a potential therapeutic target.

Significance

MPS VII is associated with severe skeletal disease for which there are currently no effective treatments. This study identifies the Wnt/β-catenin pathway as a promising target for therapeutic intervention in MPS VII.

Acknowledgments

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Engineered Nanofiber Crimp Alters Scaffold Mechanics and Mesenchymal Stem Cell Mechanotransduction

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Introduction

Tendons and ligaments are composed of highly aligned collagen fibers that, at the micron-scale, have an intrinsically crimped micro-architecture.[1] This crimping results in a non-linear mechanical response, which provides low-force deformation at small strains (in the so-called 'toe' region) and resists excessive deformation via the higher 'linear region' modulus that follows.[2] While existing biomaterials can reproduce features of this non-linear response,[3] these materials fail to provide the micro-scale topography that cells within these tissues encounter, which may be important for proper mechanobiological signaling. Recently, we developed electrospun nanofibrous scaffolds that exhibit non-linear mechanics and mimic the native crimped fibrous tissue environment.[4] In this study, we used a fiber-reinforced structural constitutive model to characterize the mechanics of these scaffolds as a function of fiber crimp and investigated the effect of crimping on mesenchymal stem cell (MSC) mechanotransduction.

Methods

Composite aligned nanofibrous scaffolds were generated by co-electrospinning poly-L-lactide (PLLA, 8.5% w/v in HFP) and poly(ethylene oxide) (PEO, 10% w/v in 90% EtOH) onto a common rotating mandrel. Composite PLLA/PEO scaffolds were washed (to remove the PEO fiber fraction) or washed and heated to 65° between two glass plates (to induce fiber crimp). Scaffolds were separated into three groups: washed (DW), heated and then washed (DHW), or washed and then heated (DWH). Scanning electron microscopy (SEM) was used to calculate the ratio between the fiber contour and end-to-end lengths, which defines the strain required to uncrimp the fibers. Scaffolds (40x10 mm2) were tested in uniaxial tension using a Bose 5500 (n = 3-4/grp) either parallel or perpendicular to the fiber direction. The linear modulus was calculated using a bilinear fit and the mechanical data prior to sample yield was fit with a hyperelastic constitutive model incorporating crimped fibers embedded in a neo-Hookean matrix.[5,6] Additional scaffolds (70x5mm2) were coated with fibronectin (20ug/ml), seeded with passage 1 bovine MSCs (100k cells), and cultured for 2 days in chemically defined media. Scaffolds were stained with Hoechst (nuclei) in DMEM (20 min; 37° C) and then stretched (n = 4/grp) in 1% increments to 8% strain using a microscope-mounted tensile device. Microscale Lagrangian strains were calculated from nuclear triads and a Poisson's ratio was calculated for each triad. In parallel unstained samples, stretch was applied as above and protein was collected for Western blotting with p44/p42 MAPK (ERK) or phospho-p44/p42 MAPK (pERK) antibodies to determine MAPK/ERK activation. Differences were evaluated via one-way ANOVA and Bonferroni post-hoc tests with $p < 0.05$.

Results

Scaffolds that were heat-processed to induce crimp (DHW and DWH) exhibited markedly different mechanical properties compared to the non-heated DW group, with the greatest change observed for the DWH samples (Figure 1A). The model successfully fit all scaffold groups; however, a non-zero matrix term was required to fit only the DWH samples, given their extensive toe-region (Figure 1B). Conversely, the DW and DHW groups could also be fit by the crimped fibers alone (data not shown). While SEM measurements showed that fiber crimping increased for the DHW and DWH scaffolds, the fiber uncrimping indicated by the model fits increased only for the DWH group (Figure 1C). The fiber modulus predicted by the model agreed with the measured linear modulus, which significantly decreased for both heat-processed groups (Figure 1D). The matrix modulus determined from testing perpendicular to the fiber direction was similar across all groups; however, the matrix modulus determined from testing parallel to the fibers in the DWH group was significantly higher (Figure 1E). Finally, the Poisson's ratio was generally comparable between scaffold types (Figure 1 F). Cells stretched on these scaffolds showed increased ERK phosphorylation with tensile stretch on both the DHW and DWH samples, but not on the DW scaffolds (Figure 2).

Discussion

This study aimed to determine whether the micron-scale crimping produced in nanofibrous scaffolds via heat-processing produced commensurate changes in scaffold mechanics

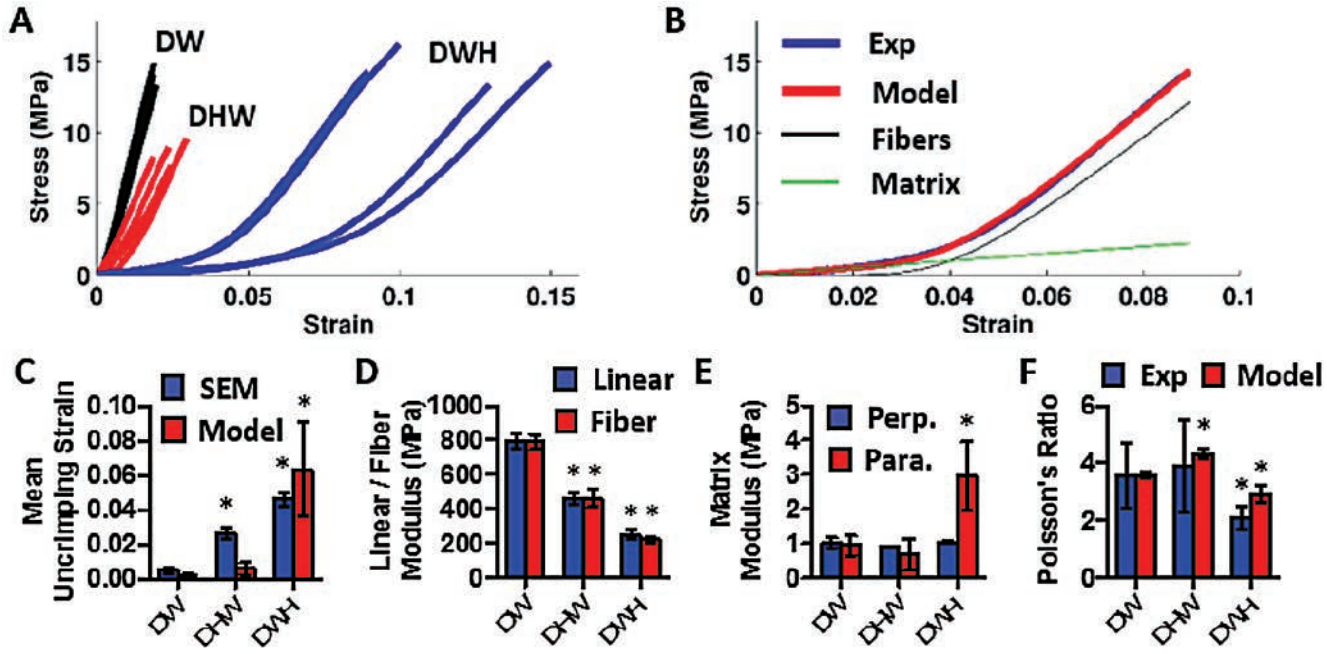

Figure 1. (A) Stress-strain response for each scaffold. (B) Representative model fit of DWH sample (R2=0.999). (C) Mean uncrimping strain increased with heat treatment. (D) Linear-region modulus and model fiber modulus decreased with treatment. (E) Testing perpendicular to fibers produced similar matrix moduli across all groups but the DWH group exhibited a higher value when tested parallel to fibers. F) Poisson's ratios were consistent between model and experiments. Mean ± SD. * $p < 0.05$ compared to DW.

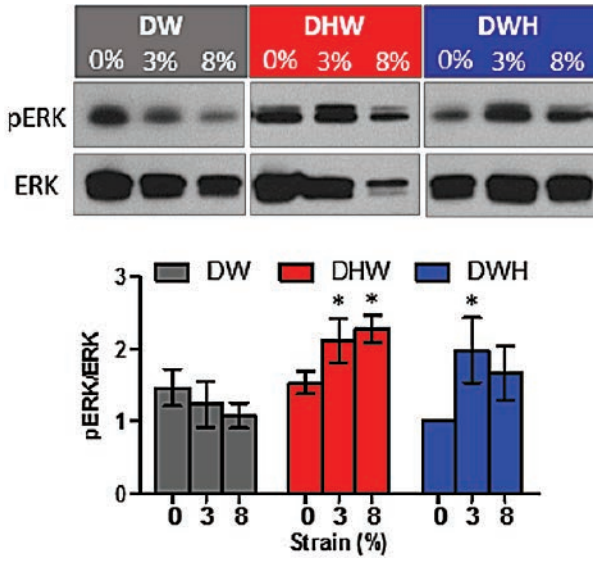

Figure 2. The level of phosphorylated ERK (normalized to total ERK) did not change with strain for the DW group. However, strain applied to both DHW and DWH scaffolds increased ERK activation. Mean ± SD. * $p < 0.05$ compared to 0%.

and altered cell mechanotransduction with applied uniaxial stretch. We found that the greatest amount of fiber crimping was generated in the DWH scaffolds, which also exhibited the greatest changes in tensile mechanics, with a large toe-region and significantly reduced fiber modulus. Interestingly, this was the only scaffold group whose mechanical behavior included resistance generated within the non-fibrous matrix (e.g., fiber-fiber interactions). In contrast, the DHW scaffolds, which also exhibited crimped fibers under SEM, had a minimal toe-region and mechanical contribution from the matrix term. This suggests that washing and then heating the scaffolds not only generates greater fiber crimping, but also more fiber-fiber interactions that influence the uncrimping process in response to load. Despite these differences in mechanics with scaffold treatment, cell mechanosensing appeared be more dependent on local fiber topology and interaction than bulk scaffold mechanics, as increased ERK activation was evident for both the DHW and DWH scaffolds in response to applied stretch. Future work will investigate additional mechanotransduction mechanisms that may lead to functional changes in scaffold maturation during culture as a result of this crimped micro-architecture.

Significance

Micron-scale crimp produced within nanofibrous electrospun scaffolds successfully reproduced the non-linear mechanics of native tissue and provided topological cues that influence cell mechanotransduction. As such, these engineered materials may provide better replication of native tissue structure and biological function.

Acknowledgments

This work was supported by the National Institutes of Health (T32 053461 and R01 EB02425).

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Electrospun PLGA Nanofiber Scaffolds Release Ibuprofen Faster and Degrade Slower after In Vivo Implantation

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Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are often prescribed, and are effective, for pain relief following tendon repairs. While improved healing after delayed delivery of NSAIDs has been demonstrated,1 these drugs have also been shown to impair healing in early stages of tendon repair by inhibiting inflammatory responses.[1,2] Therefore, to support the use of NSAIDs after tendon repair, it is imperative to identify a dose, timing, and mode of delivery that provides pain relief but does not impede tendon healing. Recent tissue engineering work has demonstrated that nanofibrous electrospun scaffolds may be useful in this realm.[3] Release of factors that are directly incorporated into nanofibers can be modulated by selecting polymers with appropriate degradation properties.[3] Therefore, the objective of this study was to develop a scaffold that would allow for local controlled release of NSAIDs during tendon healing. We further aimed to characterize the release profile and scaffold degradation properties both in vitro and in vivo.

Methods

Scaffold Fabrication: Poly(lactic-co-glycolic acid) (PLGA) scaffolds were fabricated with and without the incorporation of ibuprofen (IBP) using standard electrospinning techniques.[4] Solutions of 35% w/v 75:25 PLGA, with 5% w/w ibuprofen (IBP) or without IBP (blank), were dissolved in 1:1 tetrahydrofuran and N,N-dimethylformamide and then electrospun on a rotating mandrel to create aligned nanofibrous scaffolds. In Vitro IBP Release in PBS: IBP scaffolds were placed in phosphate buffered saline (PBS) at 37°C on a shaker. At designated time points, the PBS solution was removed and centrifuged and the IBP concentration in the supernatant quantified by measuring optical absorbance and normalized to scaffold weight. In Vivo IBP Release: With IACUC approval, 8mm diameter PLGA scaffolds were implanted subcutaneously in Sprague Dawley rats (4/animal). Rats were sacrificed at 0.5, 3, 7, and 14 days after implantation, and scaffolds harvested for subsequent ibuprofen quantification, continued in vitro release in PBS, histological analysis, and SEM imaging. IBP remaining within the scaffolds was determined by dissolving them in dimethyl sulfoxide (DMSO) and measuring absorbance. In Vitro Release in Serum: To more accurately replicate in vivo conditions in vitro, 10 IBP-containing and 5 blank scaffolds per time point were incubated in either PBS or rat serum for 0.5, 3, 7, 14, and 21 days at 37°C on a shaker. Scaffolds were removed after these incubation times for SEM (n = 1), mechanics (n = 3, retained for future analysis), and IBP quantification (n = 3, IBP only). For three IBP and one blank scaffold, the serum was replaced with PBS for continued release and analysis of degradation. Histology: Scaffolds explanted from rats were immediately placed in formalin, soaked in sucrose, flash frozen in embedding compound, cryosectioned at 10 μm, and stained with hematoxylin and eosin. SEM: Samples were flash frozen immediately after in vitro or in vivo incubation, lyophilized, mounted, and imaged at 1000× (not shown) and 5000x. Mechanical Testing: As-spun IBP and blank scaffolds (day 0) were cut into 60 × 5 mm strips with the fibers oriented along the long axis and mechanically evaluated using a ramp to failure test (0.5%/second).

Results

In Vitro Response in PBS: A distinct and reproducible release profile of IBP was observed in PBS: a burst phase over the first 3 days (releasing ~10% of the total IBP), followed by a lag phase from days 3-10, and then a linear phase during which roughly 1 g/mg scaffold/day was released (Figure 1—green plot). Macroscopically, the scaffold began to degrade and lose its shape after ~20 days (Figure 2A). SEM demonstrated that the fibers swelled immediately upon hydration, coalesced by day 14, and then disintegrated by day 21 (Figure 2A). In Vivo Response: After removal from subcutaneous implantation for 0.5, 3, 7, and 14 days, all scaffolds retained only 10-20% of the original IBP load (Figure 1—red plot). Macroscopically, there was no degradation apparent and little to no tissue adherence. Histologically, there was only scant cellular infiltration into the scaffold by day 14 (Figure 2D). After further incubation in PBS, the scaffold showed a rapid but small burst release of the remaining 10% of IBP, and failed to degrade even after nearly 3 months of incubation in PBS (data not shown). SEM demonstrated the maintenance of a fibrous structure throughout, with minimal fiber swelling compared to the PBS incubated

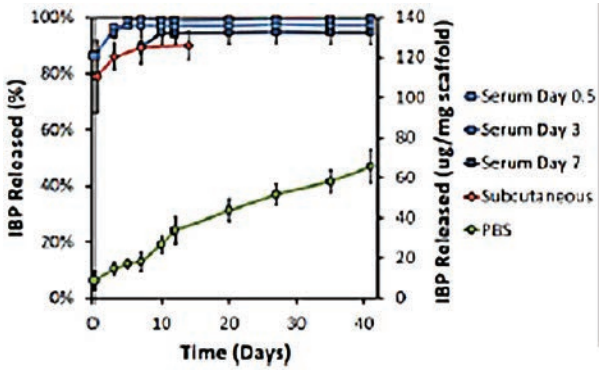

Figure 1. IBP release data represented as % release from the total (left y-axis) and ug/mg scaffold (right y-axis) after incubations in serum (blue) for 0.5, 3, and 7 days and PBS (green), as well as in vivo subcutaneous incubation (red).

scaffolds (Figure 2C). In Vitro Response in Serum: Scaffolds placed in serum for 0.5, 3, 7, 14, and 21 days demonstrated a pattern of release similar to those implanted in vivo, namely, an immediate burst release of nearly 100% of the IBP (Figure 1A—blue plots). Macroscopically, the scaffolds did not show any signs of degradation, even after almost 2 months of incubation in PBS (Figure 2B). SEM demonstrated that the scaffolds maintained their fibrous structure with very little fiber swelling over 21 days (Figure 2B). Blank vs. IBP Scaffolds: Incubation in PBS steadily degraded both the IBP and blank scaffolds, though the IBP scaffold degraded at a faster rate (data not shown). Interestingly, neither scaffold showed signs of degradation when incubated in serum for up to 40 days (data not shown). Additionally, mechanical testing demonstrated that as-spun IBP scaffolds had no change in stiffness, failure load, and yield strain properties (data not shown) compared to blank scaffolds. However, the IBP scaffolds showed a decreased modulus (Figure 3B), yield load, yield stress, and failure stress (data not shown),

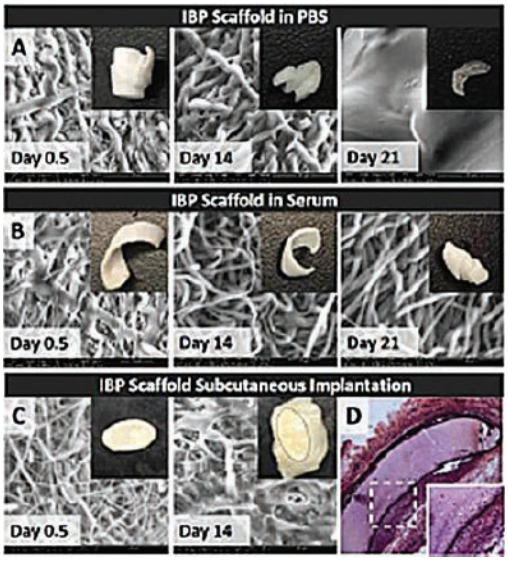

Figure 2. SEM (5000x) and macroscopic images of IBP scaffolds at 0.5, 14, and 21 days of incubation in (A) PBS, (B) rat serum, and (C) in vivo subcutaneous implantation. The macroscopic view at 14 days includes surrounding tissue, with the scaffold location outlined. (D) Histological image demonstrating sparse cellular infiltration into the scaffold at 14 days.

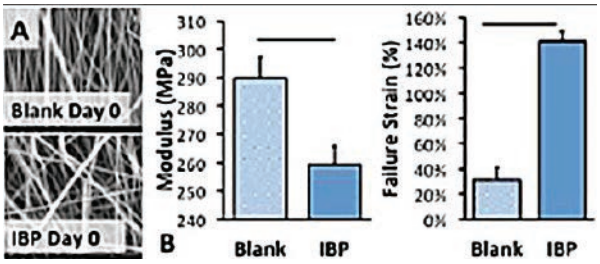

Figure 3. (A) SEM images of blank and IBP scaffolds prior to incubation. (B) Modulus and failure strain of IBP and blank scaffolds prior to incubation ($p < 0.01$).

as well as an increased failure strain (Figure 3B) compared to blank scaffolds ($p < 0.01$).

Discussion

This study demonstrated that a controlled linear release profile of IBP can be created using PLGA nanofiber scaffolds when maintained in vitro in PBS. Although this release profile is highly desirable for our clinical application, the release profile of scaffolds placed in serum or in vivo was not (i.e., we observed a burst release). This may be due to IBP's tendency to bind to serum albumin, as it does within the vascular system.[5] Additionally, serum and in vivo conditions seem to inhibit degradation of the scaffold, the reason for which remains unknown. It is known that biomaterials perform differently in vitro vs. in vivo, as these data confirm, and this study demonstrates the necessity to fully evaluate biomaterials in environments similar to the intended application. Additionally, this study supports a method for more accurately mimicking the in vivo environment, allowing a more thorough in vitro investigation prior to progressing to in vivo animal studies. Ongoing work is focused on determining the polymer molecular weight and dispersion, and evaluating the mechanical properties of these scaffolds during in vitro, in vivo, and simulated-in vivo degradation. Additionally, we are working to create a scaffold whose release profile following in vivo implantation better mirrors the more desirable in vitro results.

Significance

This work demonstrates that electrospun nanofibrous scaffolds can deliver NSAIDs, though the in vivo release profile has not yet matched the more desirable in vitro behavior. Additionally, this study supports the use of serum over saline for in vitro evaluation, to more accurately represent in vivo conditions, and thereby, reduce the number of animal subjects.

Acknowledgments

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Injured Tendons Increase Lactate Synthesis and Pharmacological Inhibition of Lactate Synthesis Improves Tendon Repair.

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Introduction

Incomplete tendon healing leads to significant mobility restriction, pain and substantial health care costs. To develop novel targeted therapies for tendon injury, it is necessary to define the molecular changes and mechanisms governing the tendon healing process. Up-regulation of glycolysis and lactate synthesis occurs in wound, inflammation and cancer. Recently, we have found that IL-1 inhibits tenogenic differentiation of injured-tendon derived progenitors and increases their lactate synthesis and that inhibition of lactate synthesis competed against the IL-1 action on tenogenic differentiation.[1] We also know that inflammatory cytokines are up-regulated in injured tendons.[2] Taken together, we hypothesize that tendons increases lactate synthesis in response to injury and pharmacological inhibition of this alteration is beneficial for tendon repair. We analyzed activities of glycolysis and lactate synthesis in injured tendons with ^{13}C-glucose labeling and examined the effects of dichloroacetat (DCA), an inhibitor of lactate synthesis, on recovery of collagen fiber formation and biomechanical properties in the mouse achilles tendon injury model.

Methods

All animal experiment procedures were approved by the Institutional Animal Care and Use Committee of the Children's Hospital of Philadelphia. *Tendon surgery:* A complete transverse incision was made at the midpoint of the right Achilles tendon in 8-week-old female C57/BL6 mice and the gap was left open.[3] Animals were returned to cage activity and euthanized 1 or 4 weeks after surgery, representing the inflammation/proliferation and repair phases, respectively. Harvested tendons were subjected to metabolomics, histological and biomechanical analyses. *^{13}C-glucose labeling*: 13C-glucose (400mg/kg) was peritoneally injected 1 h prior to euthanization. The uninjured or injured tendons ($n = 4$) were snap-frozen in liquid nitrogen and subsequently ground to powder for perchloric acid extraction. ^{13}C-metabolites and intermediates were analyzed by combination of LC-MS and MC-MS.[4] *DCA administration:* Dichloroacetate (DCA) (100 mg/kg, daily) was given to C57/BL6 mice from 1 day to 4 weeks post-surgery. *Mechanical testing:* Achilles tendons with attached calcanei ($n = 10$) were fine dissected and hydrated in PBS. Tendon cross-sectional areas were calculated with a custom laser-based device.[5] For tensile testing to failure, tendons were placed in a custom fixture that grips the calcaneus and tendon ends. Fiber alignment measures were collected during mechanical testing with a cross-polarizing technique.[5] *Statistics:* Student's t-tests or two-way factorial ANOVA followed by Boneferroni post-hoc multiple comparison tests were used to identify differences between groups. Significance for all tests was set as $p < 0.05$.

Results

The molar percent enrichment of ^{13}C-latate was strongly increased at 1 week post-injury and remained high after 4 weeks (Figure 1). In addition, enrichment of ^{13}C-glyceraldehyde, a metabolite in glycolysis pathway, was significantly higher in injured tendons in the 1week group compared to uninjured tendons. Four weeks after injury, ^{13}C-glyceraldehyde enrichment decreased, but was still higher than the uninjured tendon (Figure 1). DCA-treated samples had smaller cross sectional areas (Figure 2A Analysis of axial view of collagen fibers by electron microscopy revealed that the DCA-treated tendon contained thicker fibers. Finally biomechanical assessments demonstrated that modulus and maximum strength were significantly higher in the DCA-treated tendons than the vehicle-treated tendons (Figure 2B and C). In addition, the DCA-treated tendons had a lower circular variance, which is indicative of better alignment (Figure 2D).

Discussion

The results indicate that injured tendon acutely increases glycolysis and lactate synthesis and that inhibition of lactate synthesis improves recovery of collagen fiber structure and biomechanical properties. Alterations of glucose metabolism were found not only in an inflammation phase but also in the repair phase, indicating that the responsible cells for the alterations are not only in inflammatory cells and vessels but also the tendon cells and tendon progenitors that contribute to tendon regeneration. Thus, the findings indicate that

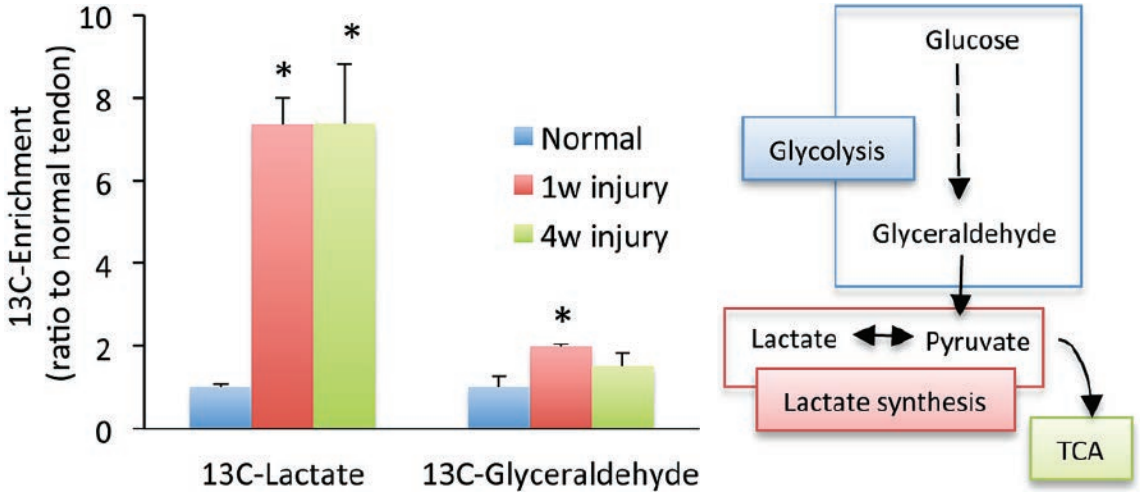

Figure 1. Injured tendons increased an influx of glucose to glycolysis and lactate synthesis pathway. 13C-glucose was injected 1 h in prior to euthanization. The uninjured or injured tendons (1 or 4 weeks postinjury) were harvested and subjected to metaboromics analysis to measure the molar percent enrichment of 13C-metabolites. *, $p < 0.05$.

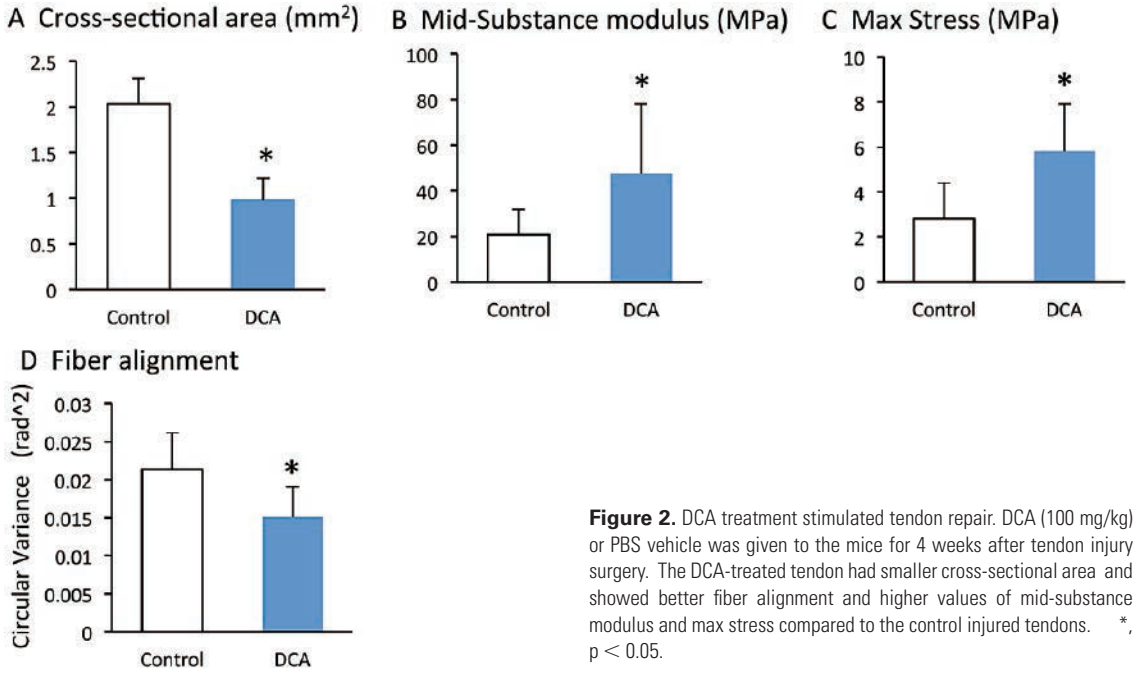

Figure 2. DCA treatment stimulated tendon repair. DCA (100 mg/kg) or PBS vehicle was given to the mice for 4 weeks after tendon injury surgery. The DCA-treated tendon had smaller cross-sectional area and showed better fiber alignment and higher values of mid-substance modulus and max stress compared to the control injured tendons. *, $p < 0.05$.

injured tendons reprogram glucose metabolism and that metabolic drugs can modify this alteration and improve tendon healing. In wounds, lactate accumulates regardless of oxygen concentration and stimulates VEGF in macrophages and collagen synthesis in fibroblasts.[6] Lactate my mediate angiogenesis and fibrous tissue formation (scar) in injured tendons.

Significance

While a large number of clinical and preclinical approaches have been attempted, none result in complete recovery of mechanical structure and function in injured tendons. This study provides direct evidence that glycolysis and lactate synthesis can be novel therapeutic targets for tendon repair.

Acknowledgments

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Structure and Composition in Tendons with Altered Collagen V Expression are Location-Dependent

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Introduction

Classic (type I) Ehlers-Danlos Syndrome (EDS) is a rare genetic disease associated with mutations in collagen V.[1] Patients with classic EDS exhibit connective tissue hyperelasticity and laxity. While collagen V is a quantitatively minor component of collagen fibrils in tendons, it plays a critical role during fibrillogenesis.[2] Recent studies have found that the regulatory role of collagen V in establishing the mechanical properties of tendons is tissue-dependent, with major alterations occurring in the supraspinatus tendon.[3] However, the role of collagen V in determining the regional structure and composition of this particular tendon is still unknown. Therefore, the objective of this study was to investigate structural and compositional changes associated with decreased expression of collagen V at both the insertion site and midsubstance of the supraspinatus tendon. We hypothesized that the fibril structure would be altered (i.e., increased diameter, reduced density) in both locations with decreasing collagen V expression, but that no changes would be present in the cell morphology or extracellular matrix composition at either location.

Methods

Male mice of three genotypes, *Col5a1*$^{+/+}$ (WT), *Col5a1*$^{+/-}$ (HET), and a tendon/ligament-targeted conditional knockout, *ScxCre+Col5a1*$^{-/-}$ (NULL) were sacrificed at P120 (IACUC approved). *Electron Microscopy:* Samples for TEM (n=10/group) were prepared as described.[4] Ten non-overlapping cross-sectioned digital images were obtained from the central areas of each specimen. Diameters were measured along the minor axis of cross sections. Fibril density was obtained as the fibril number per unit area. A measure of fibril roundness, fibril irregularity factor (FIF), was defined as the ratio of the radius as determined from a circle with the fibril's perimeter to the radius as determined from a circle with the fibril's area, where increasing values would define an increasing number of folds along the surface of the fibril. *Histology:* Histological samples (n = 8/group) were harvested from the shoulder and processed for paraffin-embedding. Coronal sections (7 μm) were stained with H&E. Each sample was then evaluated for cellularity and cell shape (tendon proper only).[5] *Biochemistry:* Biochemistry samples (n = 20/group) were prepared for analysis (tendon + surrounding sheaths) as described.[6] DNA content and glycosaminoglycan (GAG) content were quantified using the PicoGreen and dimethylmethylene blue assays, respectively. The remaining digest was hydrolyzed, resuspended, and used to quantify total collagen (COL) using the hydroxyproline assay and pyridinoline crosslinks (PYD) using the MicroVue PYD ELISA kit. GAG and COL content were normalized to DNA content and PYD was normalized to total COL. *Statistics:* Statistical comparisons were made using one-way ANOVAs with post-hoc Bonferroni tests. Statistical significance was set at $p < 0.05$ and a trend at $p < 0.10$.

Results

At the midsubstance, fibril diameter was significantly increased and fibril density was decreased in the heterozygous and null groups (Figure 1). The distribution of fibril diameters broadened from wild type to null to include fewer small diameter fibrils and increased large diameter fibrils (not shown). At the insertion site, fibril diameter was increased in the heterozygous group and fibril density was slightly decreased in the null group (Figure 1). The insertion site fibril diameter distribution displayed an increased number of small and large diameter fibrils in the experimental groups (not shown). In addition, irregular fibril shapes were present at the insertion site of the tendon in the null tendons only (Figure 1C). Cell density was slightly decreased in the null group at the insertion site compared to wild type (Figure 2A). Cell shape was not different between the groups in either region (Figure 2B). There also appeared to be pockets of hypercellular non-fibrillar tissue in between fibers or fascicles in the null group that were not present in the other groups (not shown). At the midsubstance, there were no differences between groups in DNA, GAG, or COL content (Figure 3). At the insertion site, DNA was increased while GAG and COL content were decreased in the null group (Figure 3A-C). Additionally, PYD crosslinks were increased in the heterozygous group at the insertion site and in the null group at both locations (Figure 3D).

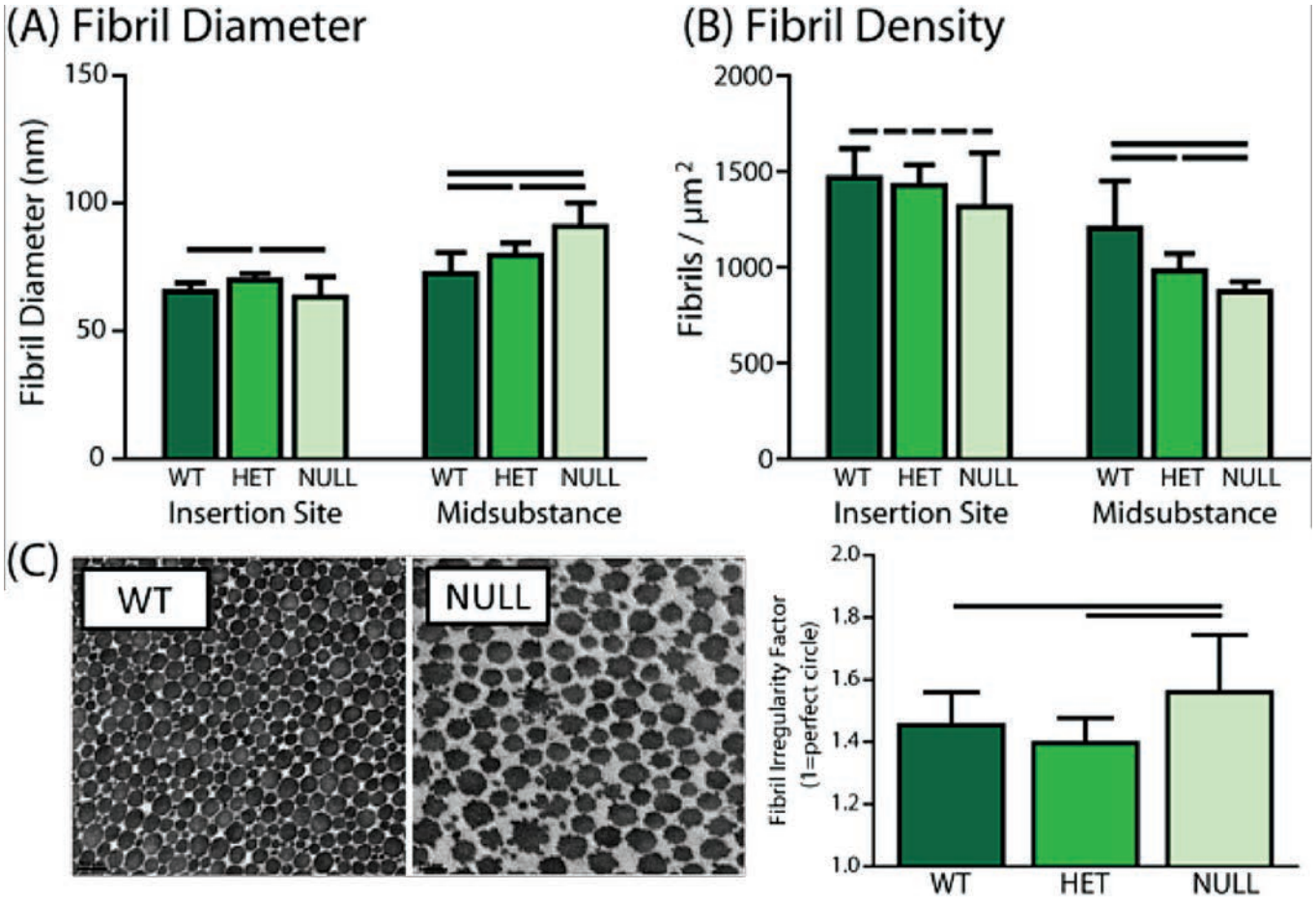

Figure 1. (A) Fibril diameter was increased in the heterozygous groups at the insertion site and midsubstance and in the null group at the midsubstance (significant). (B) Fibril number was decreased in the experimental groups at the midsubstance (significant). (C) Irregular fibril morphology was present in the null tendon insertion site both qualitatively and quantitatively.

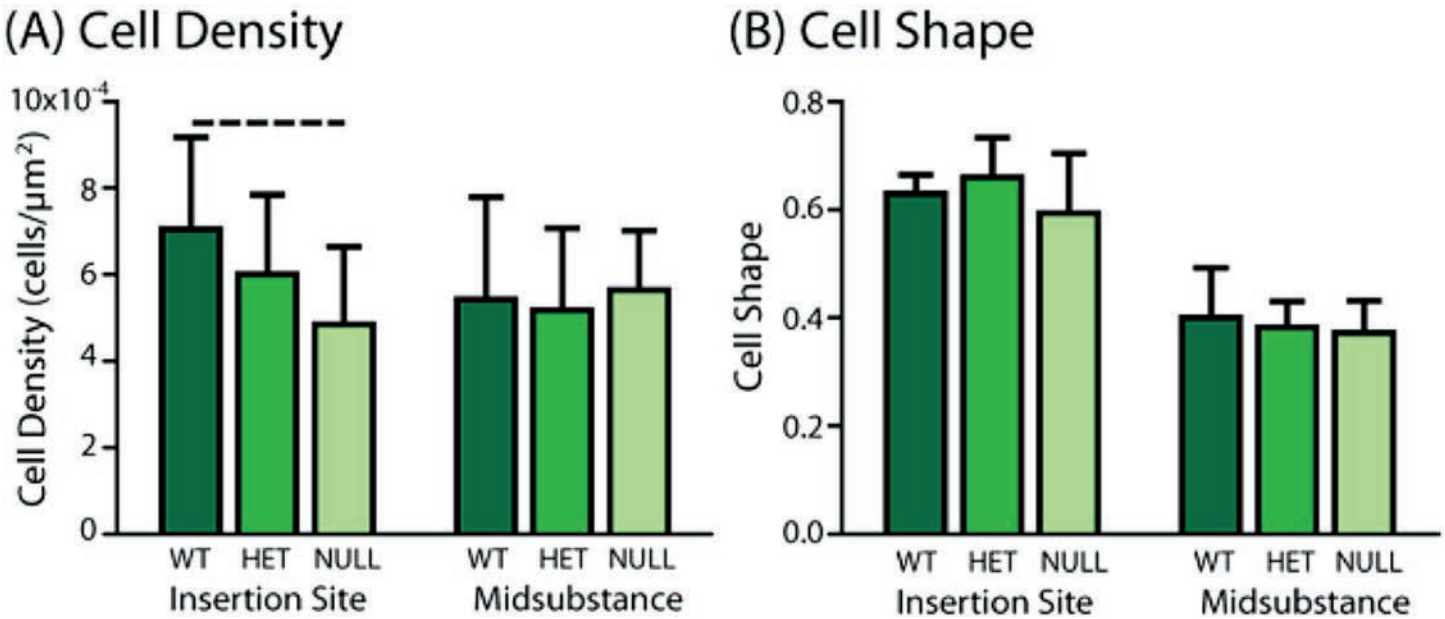

Figure 2. (A) Cell density was decreased in the null group at the insertion site only (trend, $p < 0.1$). (B) Cell shape was not different between groups in either region (significant, $p < 0.05$). Data presented as mean ± SD.

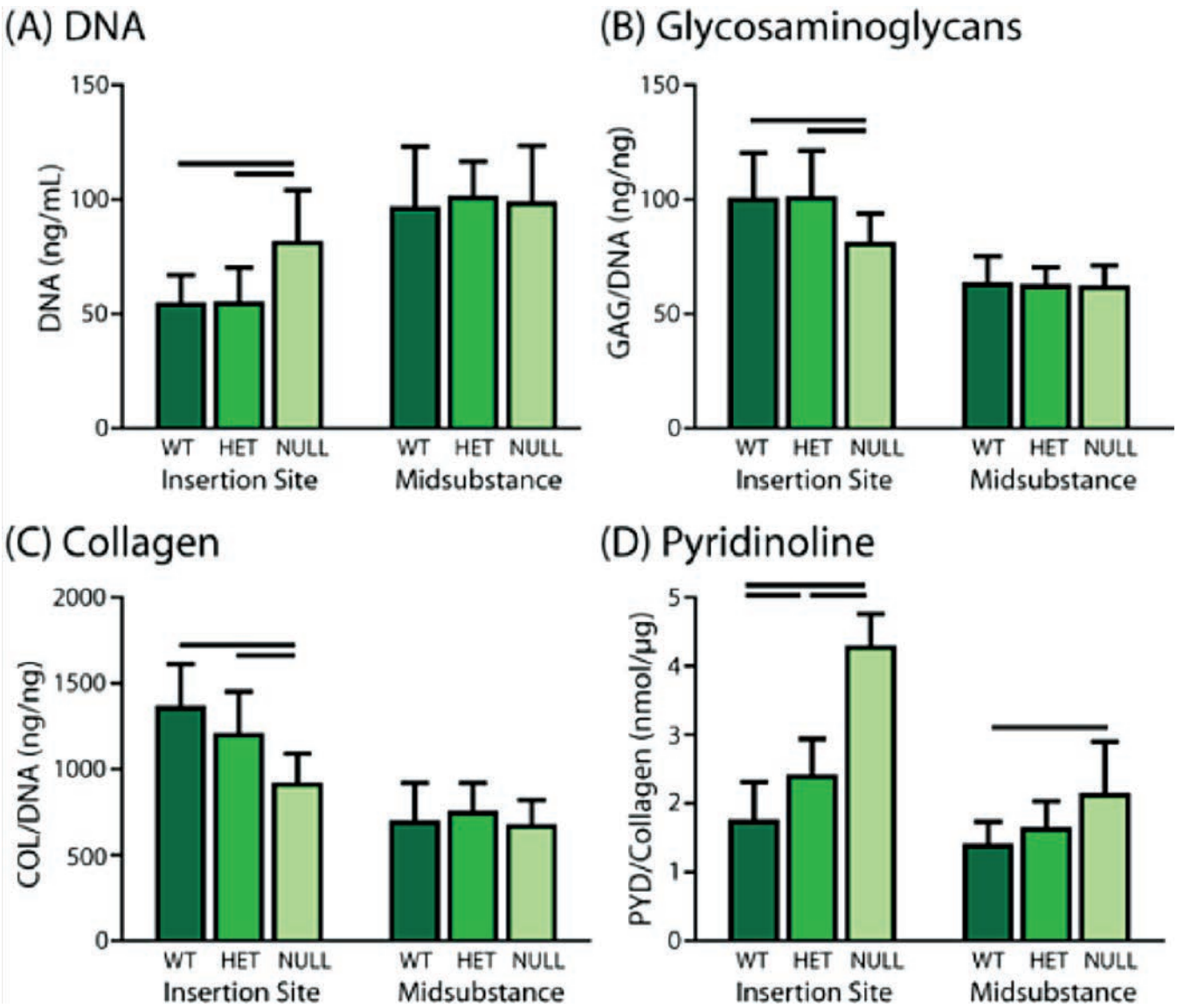

Figure 3. (A) DNA content was increased in the null group at this insertion site (significant). (B) GAG content and (C) COL content was decreased at the insertion site (significant). (D) PYD density was increased in the null group at both regions and in the heterozygous group at the insertion site (significant).

Discussion

Fewer, but larger, collagen fibrils were found with reduced collagen V expression in both the heterozygous and null groups at the midsubstance, consistent with previous literature.[7,8] These changes were present with no other alterations in cell density, cell morphology, collagen content or GAG content, as expected. However, these structural changes were not mimicked at the insertion site. At the insertion site, slightly reduced fibril density was quantified that was not accompanied by an increase in fibril diameter. Instead, increases in both small and large diameter fibrils were present in the null group, demonstrating differences in the distribution of fibril diameters (data not shown). Furthermore, many null tendons had irregularly shaped fibrils, suggesting the packing of collagen molecules inside the fibril may have been disrupted. This finding is currently being investigated further. Although cell density was slightly decreased at the insertion site in the tendon proper, DNA content of the tendon and associated sheaths was significantly increased due to the increased amount of hypercellular tissue between collagen fibers in the null tendons. The insertion site also had decreased collagen and GAG content, alluding to alterations in the viscoelasticity of the tissue shown recently.[9] Finally, pyridinoline, a mature collagen crosslink, was significantly increased with the reduction of collagen V expression in both regions, suggesting an alteration in collagen fibril processing that has not been previously described. This result could explain alterations in the shape of collagen fibrils and will be the subject of further study.

Significance

These studies define a crucial location-dependent role for collagen V in developing supraspinatus tendon structure and composition.

Acknowledgements

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Collagen V-Deficient Tendons Exhibit Altered Dynamic Mechanical Behavior at Multiple Hierarchical Scales

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Introduction

Tendon's mechanical behavior is exhibited by a stress-strain curve with an initial, non-linear "toe-region", thought to be attributed largely to collagen I fiber and fibril re-alignment, uncrimping, sliding and deformation,[1-3] followed by the "linear-region". Recent studies with collagen V deficient mouse tendons have demonstrated altered fibril structure and reduced macroscale quasi-static and dynamic mechanical function.[4-6] However, the role of collagen V in developing the dynamic microstructural response has not yet been established. Therefore, the objective of this study was to investigate the microstructural response of collagen V deficient tendons to load at the fiber (re-alignment) and fibril (deformation, sliding) scale. We hypothesized that collagen V-deficient tendons would have impaired dynamic mechanical function at all length scales, with a stronger phenotype in the null than heterozygous mice. We also hypothesized that changes in dynamic responses at fibril and fiber levels would be more apparent than those we found at the tissue-level.

Methods

Male mice with three genotypes, *Col5a1*$^{+/+}$ (WT), *Col5a1*$^{+/-}$ (HET), and a tendon/ligament-targeted conditional knockout, *ScxCre+Col5a1*$^{-/-}$ (NULL) were sacrificed at P120 (IACUC approved). The supraspinatus tendon-bone complex was dissected from the shoulder and prepared for mechanical testing as described.[4] For collagen fiber re-alignment, tendons were subjected to a viscoelastic testing protocol as described and re-alignment was quantified using our established cross-polarizer technique.[7-8] Re-alignment during toe and linear region strain levels were analyzed by comparing the change in circular standard deviation of fiber angles between 0-4% strain (toe region) and 4-8% strain (linear region). In addition, the strain required to reach the plateau and the amount of re-alignment that occurred during that time was measured for each specimen using a bilinear curve fit. Statistical comparisons were made between these measures using one-way ANOVAs with post-hoc Bonferroni tests. To investigate collagen fibril deformation and sliding, samples were stretched to a randomly assigned grip-to-grip strain value (0, 1, 3, 5, or 7%) following preconditioning. Tendons were then immediately flash frozen, sectioned, and fixed. AFM imaging of 2μm × 2μm regions was performed in tapping mode using a modified protocol as described.[9] Fibril D-period was determined for ~20 fibrils for each image, across the width of each section, and across multiple sections.[9] An increase in the fibril D-period with increasing strain is indicative of fibril stretch, while a change in the variance of the distribution is indicative of strain heterogeneity between fibrils, or fibril sliding.[9] Statistical comparisons were made using non-parametric Kruskal-Wallis tests followed by post-hoc Dunn's tests. Comparisons of variance were performed using a Bartlett's test for unequal variances with post-hoc F tests. For all statistical comparisons in this study, $p < 0.05$ was considered significant.

Results

Re-Alignment: There were no differences in the amount of re-alignment during toe region strain levels between groups (Figure 1A). However, re-alignment during linear region strain levels was reduced in both groups (Figure 1B). All of the groups returned to a more disorganized state with the removal of strain and then re-aligned again during the ramp to failure. However, the null group re-aligned fully with less strain at the midsubstance (Figure 1C) and exhibited less re-alignment at the insertion site (Figure 1D). *Fibril Deformation*: At the insertion site of the wild type tendons, collagen fibril D-period showed a bimodal response, with an initial hold at 3% strain, followed by an increase at 5% strain (Figure 2A). At the midsubstance of the tissue, the fibril D-period increased monotonically, peaking at 3% strain. In the heterozygous group, the insertion site showed a similar trend to the wild type group, but with a large decrease at 3% strain (Figure 2B). At the midsubstance, the D-period in the heterozygous group peaked at 1% strain. At the insertion site in the null group, there was an initial decrease in D-period at 1% strain followed by an increase at 3% strain (Figure 2C). At the midsubstance in null tendons, the D-period initially decreased at 1% and increased thereafter. *Fibril Sliding:* At the insertion site of the wild type group, fibril sliding occurred at the insertion site between 1% and 3% applied strain (Figure 2A). Fibril sliding occurred in the heterozygous

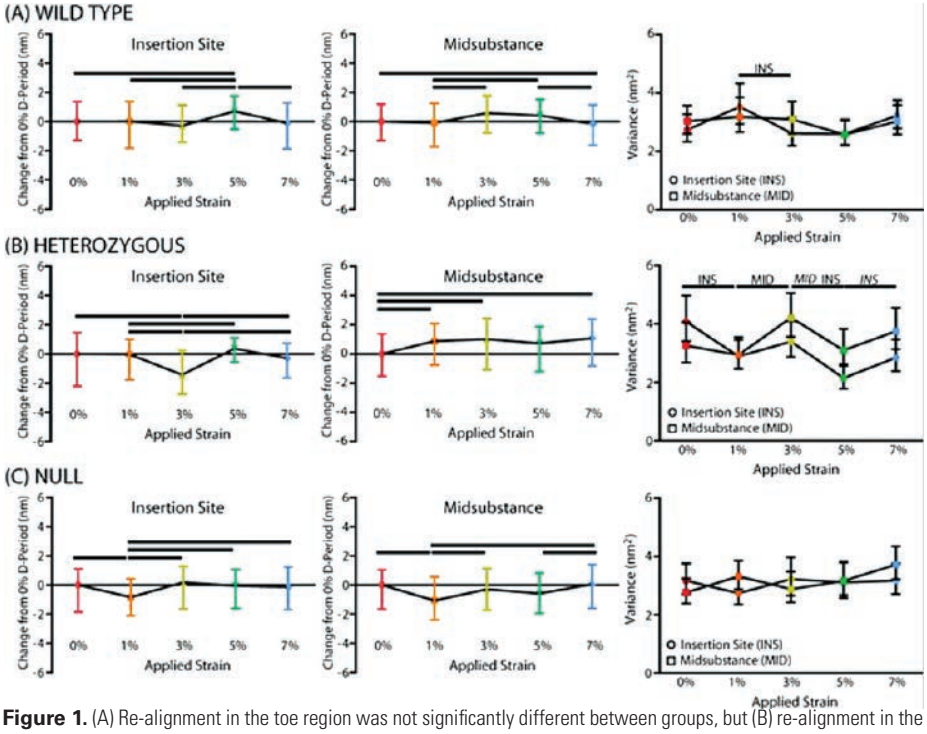

Figure 1. (A) Re-alignment in the toe region was not significantly different between groups, but (B) re-alignment in the linear region was reduced in the experimental groups. (C) The null group required less strain overall to full re-align and (D) re-aligned less overall. Data is reported at mean ± standard deviation.

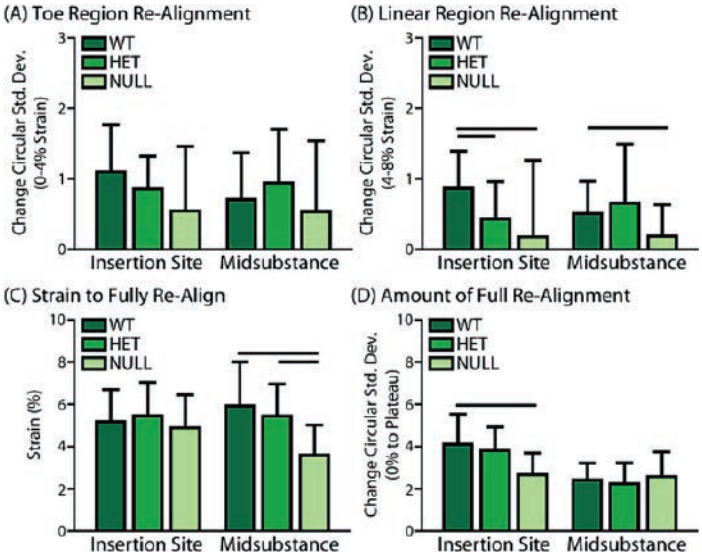

Figure 2. Results from fibril stretch at the insertion site (left) and midsubstance (middle) and fibril sliding at both location (right) are shown for each group. Data is reported at median ± interquartile range.

group continuously throughout the test, while the null group did not exhibit significant fibril sliding (Figure 2B,C).

Discussion:

Collagen V has a major role in regulating tendon fibrillogenesis. As hypothesized, a severely diminished response at the microscale and nanoscale was revealed as collagen V expression was decreased and therefore regulation of fibril assembly was further disrupted (in the heterozygous and null groups compared to the wild type group). With all of the multi-scale results taken together, these results suggest different mechanisms whereby tendons with reduced collagen V (50 to100%) expression attempt to ameliorate their altered fibril assembly at maturity. The wild type group is able to reduce stress at the lower hierarchical scales (fibers, fibrils) through a series of coordinated dynamic responses, specifically collagen re-alignment and sliding. The heterozygous group compensates for the lack of fibril strength via earlier re-alignment and a large amount of fibril sliding. The sliding reduces strain on the collagen fibrils initially, and thus, prevents early fibril failure, but with a large amount of repeated fibril sliding, the fibrils eventually pull away from each other and fail in shear. This allows the heterozygous group to respond elastically, but only at low strain levels, which is consistent with clinical observations. In contrast, the null group also responds early to load, but is incapable of producing significant fibril sliding, and therefore, the tendons fail earlier and with lower maximum loads. These studies highlight the hierarchical relationships that exist in tendon and suggest that this unique set of dynamic processes provide normal tendons with a series of protective measures to prevent early failure.

Significance

These studies deepen our understanding of the multi-scale response of tendons to loading and define a key role for collagen V in developing the structure responsible for this response.

Acknowledgements

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Ground Reaction Forces Are More Sensitive Gait Measures Than Temporal Parameters in Rodents Following Rotator Cuff Injury

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Introduction

Musculoskeletal conditions affect 1 out of every 7 Americans, costing our society an estimated $254 billion yearly.[1] While tissue level animal studies provide valuable insight into the causes and treatment of musculoskeletal injuries and diseases, interpretation of the clinical and translational importance of such studies can be aided with a quantitative measure of resulting limb/joint function in live animals.[2,3] Several human and animal gait analysis studies have observed decreases in temporal parameters (e.g., stance time—how long the foot remains in contact with the ground in a single step) that seem to correspond with decreases in kinetic gait parameters (e.g., vertical ground reaction force). However, the relationship between temporal properties and ground reaction forces during locomotion has not been investigated.[2,4,5] This information is critical given the relative ease and low cost of measuring temporal parameters compared to the more complicated kinetic parameters. Therefore, the objective of this study was to compare the sensitivity of temporal versus kinetic parameters in response to functional changes using a validated gait and force-plate analysis system in rodent models following rotator cuff injury.[2] We hypothesized that temporal and kinetic parameters would correlate and be equally sensitive measures of rodent gait.

Methods

The data used in this study were obtained from our previous studies using rat rotator cuff injury models.[3,6,7] In one study, 28 animals underwent unilateral detachment of the supraspinatus only (SO) or supraspinatus and infraspinatus (SI) rotator cuff tendons and ambulatory measurements were collected preoperatively and at 3, 7, 14, 28, 42, and 56 days post-operatively. To create a more comprehensive and general model, data from the SO and SI groups were combined and used for the correlation and regression analysis in the current study as these groups demonstrated significant differences in shoulder function measured by kinetic gait parameters, as well as tendon and cartilage mechanical and histological properties.[3] Data from two other studies were used to assess the efficacy of the regression model created from the SO and SI data. In one study, 18 animals underwent unilateral detachment of the supraspinatus, infraspinatus, and biceps (SIB) tendons and ambulatory measures were collected identically to the SO and SI groups. In the other study, 20 animals underwent unilateral detachment of the supraspinatus tendon and repair with (RW) or repair without (RWO) post-operative analgesics and ambulatory measurements were collected at 2, 4, 6, 14, and 28 days following surgery. *Correlation analysis:* Pearson's correlation coefficients were calculated between temporal parameters (i.e., braking, propulsion, and stance times) and kinetic parameters (i.e., vertical, braking, and propulsion forces). *Kinetic and temporal parameter sensitivity:* The total number of significant differences in kinetic parameters alone between SO & SI, SIB & baseline, and RW & RWO was compared to the subset of significant differences identified simultaneously in corresponding temporal-kinetic pairs (i.e., vertical force & stance time, braking force & braking time, propulsion force & propulsion time) for each pair of experimental groups. Comparisons between kinetic and temporal parameters were also performed for uninjured and injured (SO and SI, combined) animals at 3, 7, and 14 days. *Regression modeling:* Step-wise backward elimination linear regression analysis was performed on the combined SO/SI data set to select the best temporal variables for predicting kinetic gait parameters. Resulting equations were then used to predict vertical, braking, and propulsion forces for all groups. The total number of known significant differences in experimental ground reaction forces between groups was compared to the number of significant differences identified by predicted kinetic parameters for these groups. *Statistical analysis:* Correlation coefficients and two-tailed p-values were calculated using bivariate Pearson correlation. Comparisons between groups for temporal and kinetic parameters were made using two-tailed t-tests. Significance was set at $p < 0.05$ for all tests (SPSS v20.0).

Results

Correlation analysis: Numerous significant correlations between kinetic, spatial, and temporal parameters were identified (Table 1). As expected, corresponding temporal-kinetic pairs were

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Table 1. Kinetic and Temporal Parameter Correlation Coefficients. Significance key: * ($p < 0.05$), ** ($p < 0.01$), * ($p < 0.001$)**

	Stance Time	Braking Time	Propulsion Time
Vertical Force	0.85***	0.91***	0.53*
Braking Force	0.55*	0.84***	0.84***
Propulsion Force	0.41*	0.77**	0.76**

significantly correlated. *Kinetic and temporal parameter sensitivity*: Known functional differences between groups were successfully identified by temporal parameters at rates of 20%, 29%, and 0% for SO vs. SI, SIB vs. baseline, and RW vs. RWO, respectively (data not shown). Overall, temporal parameters were better able to identify significant functional differences at early time points when the percent difference in kinetic parameters was greatest between experimental groups (Figure 1). *Regression modeling*: Stance and propulsion time were identified as the best variables for predicting vertical, braking, and propulsion force through backward elimination (data not shown). When the regression equations were used to predict ground reaction forces, they successfully predicted 70% of the known significant differences in kinetic parameters between SI and SO (Figure 2). When predicting data sets not used to create the model, the regression equations were less successful at predicting differences in ground reaction forces between groups as expected, especially when these differences were smaller in magnitude. Specifically, the model was able to predict 57% of known significant differences in kinetic parameters between uninjured animals and animals with a three-tendon tear (SIB), whereas it was able to predict 0% of known significant differences in ground reaction forces between animals with or without post-surgical pain relief (RW, RWO).

Discussion

Temporal parameters were consistently less sensitive than ground reaction forces in detecting functional differences in rat gait despite the significant correlations observed between these metrics. While the regression model developed to predict kinetic parameters did aid in identifying significant functional changes compared to temporal parameters alone, only differences that were large in magnitude, such as between injured and uninjured animals, were able to be detected with greater than 50% probability. Therefore, while gait analysis systems without force plates can be efficient and often feature fully automated analyses, they may only be adequate for use when large changes are expected. This agrees with previous rodent studies that found more pronounced differences in ground reaction forces than temporal parameters when evaluating changes in gait.[8,9] Future work may include analyzing other commonly injured joints such as the ankle.

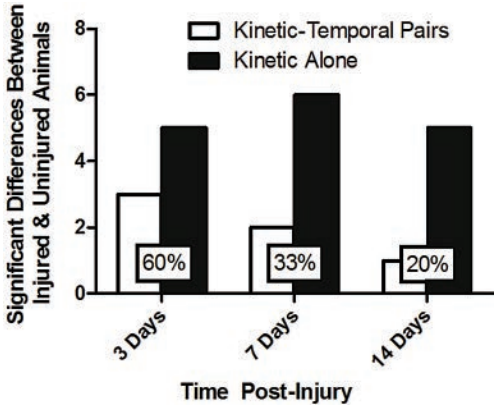

Figure 1. Sensitivity of Kinetic vs. Temporal Gait Parameters. The total number of significant differences observed between injured (SI, SO) and control animals in kinetic parameters (ground reaction forces) (black bars) was compared to the subset of differences identified simultaneously in corresponding kinetic-temporal pairs (e.g., braking force & time) (white bars) at 3, 7, and 14 days post-op

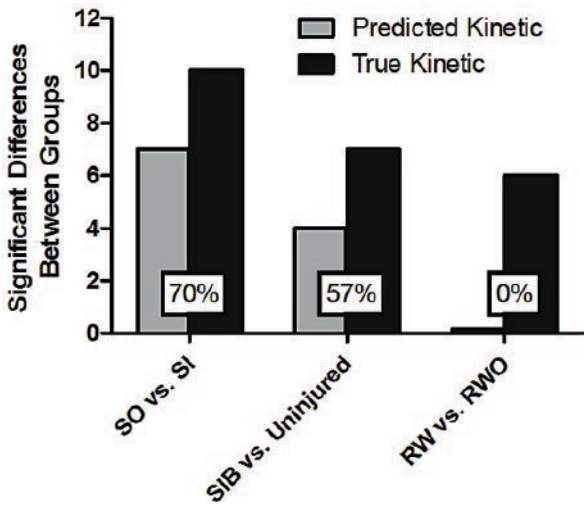

Figure 2. Kinetic Parameter Predictability. Regression modeling was used to predict ground reaction forces from stance & propulsion times for in-model (SI, SO), and out-of-model (SIB, RW, RWO) data sets. Significant differences between groups in predicted kinetic parameters (gray bars) were compared to known significant differences in true kinetic parameters (black bars).

Significance

This study supports the use of ground reaction force quantification as a more sensitive metric of limb/joint function than temporal gait parameters.

Acknowledgements

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Immunohistochemical Analysis of Muscle Tissue Following Blunt Achilles Tendon Transection in a Rat Model

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Introduction

Achilles tendon tears are common musculoskeletal injuries that often require surgical repair.[1] An abundance of evidence exists that suggests tendon loading, to a certain limit, is beneficial to healing and may promote more mature and robust cellular architecture.[2] However, there is debate regarding whether surgical or non-surgical intervention is the optimum treatment strategy.[3] There is a paucity of evidence regarding the effect of surgical treatment, the length of immobilization, and rehabilitation protocols on *muscle* tissue of the Achilles musculotendinous unit.[4,5] A better understanding of how treatment of Achilles tendon tears affect the muscle tissue may result in improvements in immobilization protocols, rehabilitation and injury prevention.[6] Therefore, the objective of this study was to elucidate the biological and structural effects of surgical and immobilization protocols on muscle tissue during early healing using immunohistochemistry. We hypothesized that prolonged immobilization, as well as non-repair, would result in immunohistochemical evidence of muscle remodeling compared to shorter immobilization lengths and repaired groups.

Methods

30 adult male Sprague-Dawley rats (400-450g) were used in this IACUC approved study. Animals received 2-weeks of treadmill training (up to 60 minutes at 10m/min) prior to surgical removal of the right central plantaris longus tendon and blunt transection of their right Achilles tendon. Animals were then divided into 4 groups: 1) surgical repair, using a modified Kessler technique, followed by 1 week of cast immobilization, 1 week of cage activity, and 1 week of treadmill exercise 2) surgical repair followed by 3 weeks of immobilization; 3) no surgical repair followed by 1 week of cast immobilization, 1 week of cage activity, and 1 week of treadmill exercise; and 4) no surgical repair followed by 3 weeks of cast immobilization. All animals were sacrificed at 3 weeks post-injury. Animals undergoing only 1 week of cast immobilization were considered "aggressive" rehabilitation (Agg), while those that underwent 3 weeks of immobilization were termed "conservative" rehabilitation (Cons). At sacrifice, the right Achilles musculotendinous units were dissected and processed for histological analysis. Transverse muscle tissue samples were then harvested, embedded in optimal cutting temperature (OCT) compound, frozen in N-methyl butane, and sectioned at 20mm on a cryotome. Samples were stained with Laminin-DAPI for immunofluorescence and analyzed using a custom computer program to quantitatively elucidate cellularity, fiber size and shape, and nuclear centrality.[7] Immunohistochemical protocols were developed to test for Collagens 1 & 3 (denoting structural properties of muscle tissue), IL-1α & b, IL-6, and TNF-α (corresponding to remodeling and inflammatory pathways seen in muscle repair models).[8] Samples were incubated with primary and secondary antibodies and incubated according to manufacturer specifications. DAB was used to label activity and analyzed using a quantitative software program denoting activity by percent area stained (imageJ, NIH). MMP activity (a marker of muscle fiber remodeling) was quantitatively measured using the Sensolyte 520 Generic MMP assay kit. All parameters assessed were compared between groups using one-tailed Student's t-tests, with significance set at $p \leq 0.05$.

Results

Results show that the rats in the non-surgical cohort treated with conservative rehabilitation had increased MMP activity compared to repair. Conversely, rats in the surgical cohort with conservative rehabilitation showed decreased Collagen 3, increased Collagen 1:3 ratio, and decreased MMP activity (Figure 1). No difference was seen between groups with regard to physical fiber properties, including fiber size, shape, and nuclear number or percent centrality (Figure 2). Immunohisochemical analysis showed conservatively treated animals had greater amounts of IL-1α and IL-6 in non-repair groups, while TNF-α was decreased in repair rats treated conservatively compared to repair treated aggressively (Figure 3).

Discussion

Results suggest that non-repair of Achilles tendon transection results in higher levels of IL-1α and IL-6, markers of muscle remodeling. These findings are consistent with previous data showing that non-repair results in significantly longer tendons, likely causing a decreased muscle tension and force output.[4] Additionally, longer

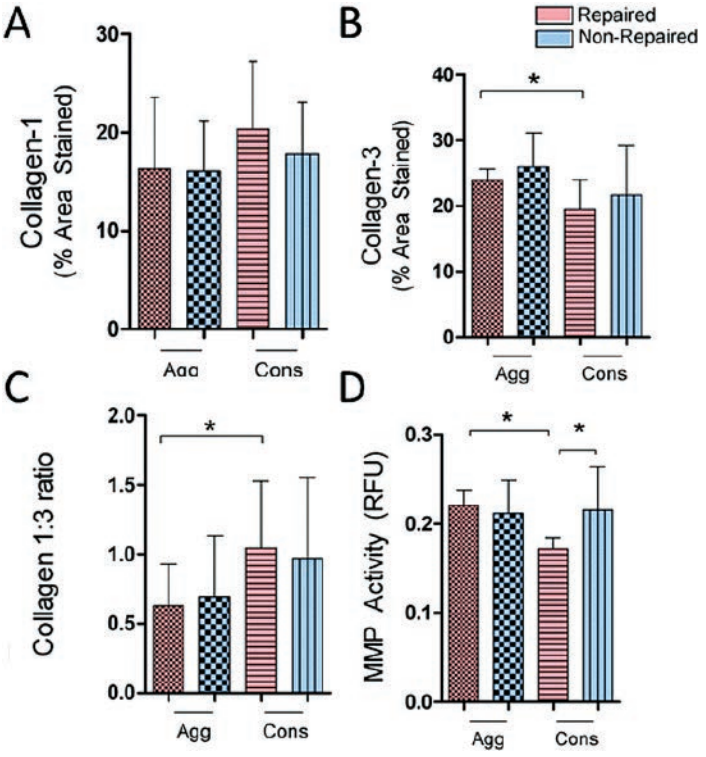

Figure 1. Muscle Structural Properties During Healing A) Collagen I B) Collagen III C) Collagen I:III ratio D) MMP Activity. Error bars: standard deviation, *: $p < 0.05$.

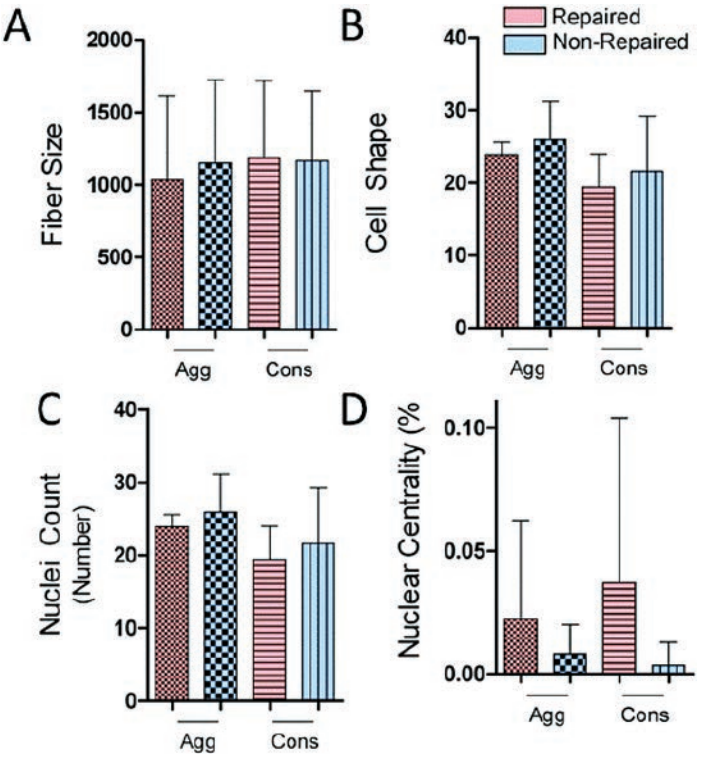

Figure 2. Muscle Physical Properties during Healing A) Fiber size B) Cell shape C)Nuclear count D)Nuclear Centrality. Error bars: standard deviation, *: $p < 0.05$.

periods of immobilization showed decreased TNF-α and a greater proportion of collagen 1, rejecting our hypothesis that increased immobilization would correspond to increasing muscle damage. This is in opposition to tendon findings, which

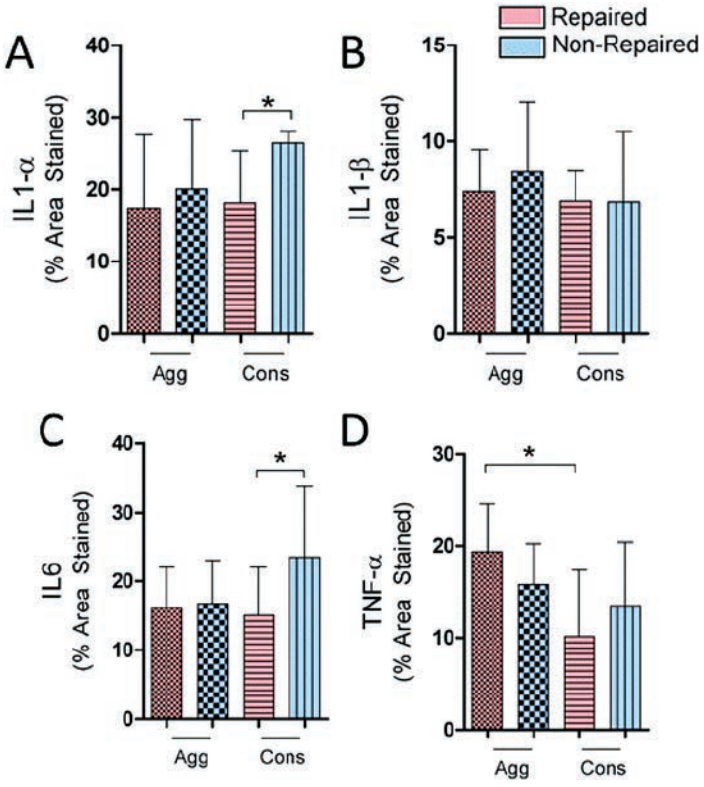

Figure 3. Muscle Remodeling and Repair during Healing A)IL 1-alpha B) IL 1-beta C)IL-6 D)TNF-alpha. Error bars: standard deviation, *: $p < 0.05$.

show increasing time of immobilization resulting in decreased range of motion at the ankle and worse functional properties.[9] Taken together, this suggests that muscle and tendon tissue may have antagonistic responses to treatment modalities. Overall, findings show that surgical repair improves outcomes in muscle tissue, while increasing immobilization is beneficial to muscle tissue and may be detrimental to tendon.

Significance

Surgical repair and conservative rehabilitation, corresponding to increasing immobilization times, is beneficial to muscle tissue following Achilles tendon injuries in an animal model.

Acknowledgments

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Sex Differences Are Present in Uninjured Achilles Tendon and Muscle

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Introduction

Achilles tendon ruptures are common and devastating injuries. Although men and women both experience Achilles tendon ruptures at similar ages and sport participation times, it is surprising that 84% of all ruptures occur in men.[1] Differences in hormone physiology between sexes may influence Achilles tendon and gastrocnemius muscle homeostasis and injury risk.[2,3] However, it is unknown whether the primary effects of hormone differences act specifically on tendon or on its origin muscle.[4,5] Therefore, the objective of this study was to determine mechanical, structural and histological properties of uninjured Achilles tendon and muscle in female, ovariectomized (OVX) female, and male rats. We hypothesized that female and OVX rats would exhibit equal tendon mechanical, structural, and histological properties but decreased muscle fiber size compared to male rats.

Methods

Experimental design and sample preparation: Achilles tendon-foot complexes from 54 age-matched adult male (n = 16), female (n = 16), and OVX (6-weeks after OVX) (n = 16) Sprague Dawley rats were harvested (IACUC approved). Tendons were then either fixed for histological analysis or frozen until preparation for high-frequency ultrasound (HFUS) analysis and mechanical testing as described.[6] All tendons were randomized to blind a single dissector at time of fine dissection and subsequent testing and analysis. Additionally, gastrocnemius muscle tissue was excised, embedded in optimal cutting temperature (OCT) compound, and flash frozen for histological analysis. *Tendon histology:* Samples (n = 8/group) were processed using standard techniques, sectioned sagitally at 7 μm, and stained with hematoxylin & eosin or safranin-o/fast green. Images were graded on a scale of 1-3 by three blinded investigators for cell shape, cellularity, and proteoglycan staining intensity. *Muscle histology:* Samples (n = 8/group) were sectioned axially at 10μm, co-stained with DAPI and laminin, imaged, and analyzed for average fiber cross sectional area (CSA) using the SMASH application.[7] *HFUS:* Tendons (n = 12/group) were loaded at 1N in a PBS bath while a series of sagittal images were acquired using a 40MHz scanner (Vevo 2100, MS550D; VisualSonics) and analyzed for fiber alignment. *Mechanical testing:* Tendons (n = 8/group) were measured for CSA and underwent a ramp to failure at 0.1%/sec. Tendons used for HFUS were subjected to a separate mechanical testing protocol consisting of stress relaxation at 6% strain, a low-load dynamic frequency sweep (ranging from 0.1 to 10 Hz), and fatigue testing at 2 Hz using a sinusoidal waveform (from ~10-40% of ultimate strength) until failure. From these tests, quasi-static mechanical properties (toe and linear modulus, and percent relaxation) and dynamic mechanical properties (modulus, tan(δ), hysteresis, and laxity) were computed. *Statistics:* One-way ANOVAs were used to compare between groups for all assays except tendon histology. Significant relationships were evaluated using post-hoc tests with Bonferroni corrections ($\alpha = 0.05/3$). Kruskal-Wallis tests were used to evaluate differences in tendon histological properties between groups ($\alpha = 0.05$).

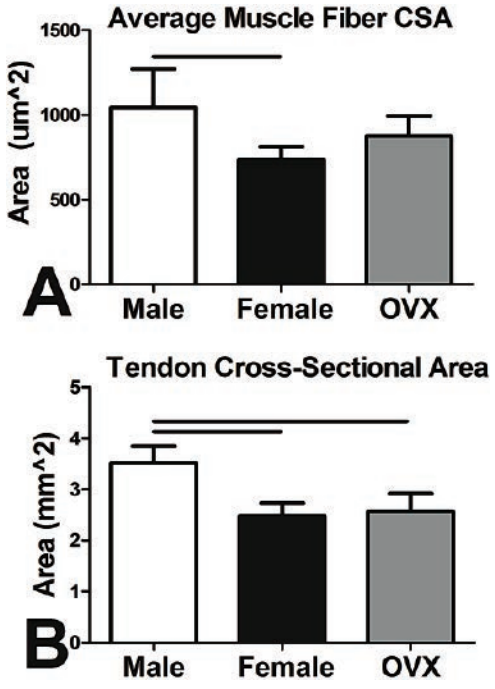

Figure 1. (A) Achilles muscle fiber area and (B) tendon area were both increased in males. Solid lines indicate significant differences ($p<0.017$). Data presented as mean and standard deviation.

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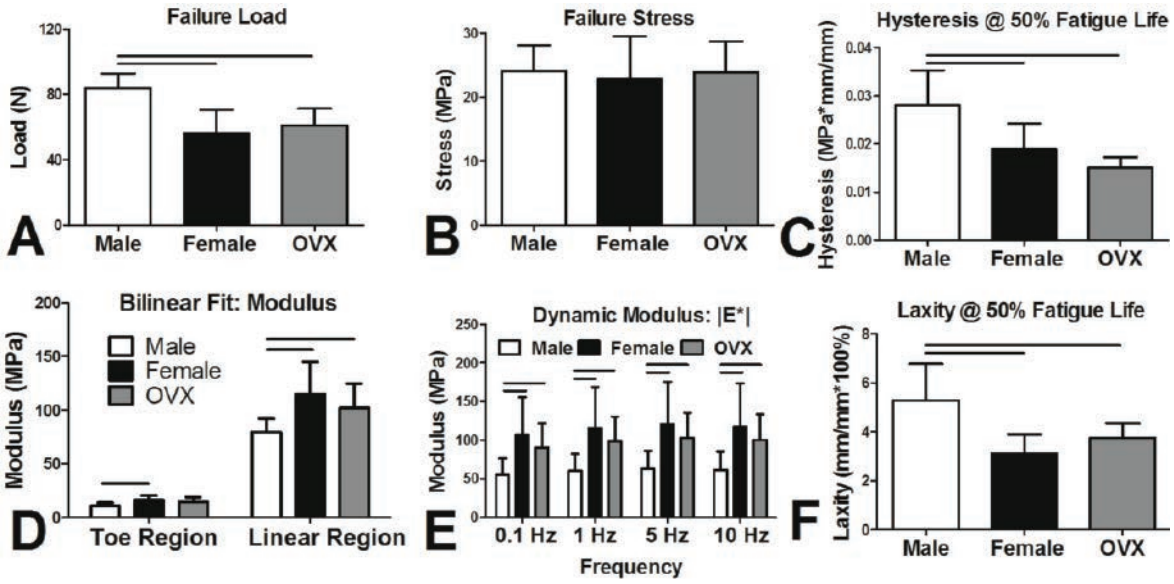

Figure 2. Achilles tendon mechanical properties. (A) Failure load was increased in males, (B) failure stress was not different between groups, (C) hysteresis was increased in males, (D) toe and linear modulus were both increased in females and OVX, (E) dynamic modulus was increased in females and OVX at 0.1, 1, 5, and 10 Hz, and (F) laxity was increased in males. Solid lines indicate significant differences ($p<0.017$). Data presented as mean and standard deviation.

Results

Tendon histology: There were no differences between groups in cell shape, cellularity, or proteoglycan content (not shown). *Muscle histology:* The average fiber CSA (Figure 1A) was significantly increased in male compared to female muscle samples. There were no differences between OVX muscle samples and the other two groups. *HFUS:* Circular standard deviation of the mean fiber orientation (a measure of collagen alignment) did not differ between groups (not shown). *Mechanical testing:* Tendon CSA (Figure 1B) and failure load (Figure 2A) were significantly greater in male tendons compared to female and OVX tendons. Failure stress (Figure 2B) was not different between groups. Percent relaxation (not shown) and hysteresis (Figure 2C) were significantly increased in male compared to female and OVX tendons but there were no differences in tan(δ) between groups (not shown). Toe and linear stiffness, as well as transition strain, were not different between groups (not shown). Conversely, toe modulus was significantly decreased in male compared to female tendons and nearly significant compared to OVX tendons ($p = 0.02$) (Figure 2D). Similarly, the linear modulus was significantly decreased in male compared to female and OVX tendons. Dynamic modulus (Figure 2E) was significantly decreased in male tendons at all frequencies tested and during fatigue testing (not shown), Tendon laxity (Figure 2F) was significantly increased in males.

Discussion

Contrary to our hypothesis, sex differences were found in both uninjured Achilles tendon and muscle. Female and OVX tendons exhibited increased moduli (quasi-static and dynamic) compared to male tendons, which suggests that female and OVX tendons have superior material properties. Further, the decreased percent relaxation and hysteresis in female tendons suggest that they may be more efficient at returning stored elastic energy following deformation. Taken together with the decreased muscle fiber size compared to males, these mechanical differences may, in part, explain the apparent decreased susceptibility to tendon rupture that is observed clinically in women despite their lower failure load. Interestingly, there may be a dose-dependent response with respect to estrogen levels for various mechanical and histological properties (e.g., muscle fiber CSA, tendon modulus, laxity), given that mean values for OVX rats generally fall between those of males and females. Further investigation is required to elucidate the mechanism governing the superior mechanical properties observed in female tendons, especially given that several previous studies implicate estrogen as an inhibitor of collagen production.[5] Future work will study the effect of tendon matrix composition and analysis of protein markers for muscle strength and fiber type, and investigate sex differences in the context of tendon healing.

Significance

This study identified inferior tendon mechanical properties and increased muscle fiber size as a potential explanation for the increased susceptibility for Achilles tendon damage observed clinically in men compared to women.

Acknowledgements

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Aggressive Rehabilitation with Nonsurgical Treatment Demonstrates Improved Fatigue Mechanics and Functional Outcomes Following Achilles Tendon Rupture in an Animal Model

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Introduction

Achilles tendon ruptures are devastating injuries that affect 8.3 in 100,000 people yearly.[1] Despite the higher risk for complications and increased costs, operative treatment has historically been believed to provide superior outcomes compared to non-operative treatment in terms of function and re-rupture rates.[2] However, recent studies have suggested that surgical treatment for Achilles ruptures is not necessarily superior.[3,4] To elucidate the basic mechanical and structural mechanisms governing these clinical outcomes, it is necessary to evaluate the role of various surgical and rehabilitation strategies on tendon quality and function in a controlled model system. Therefore, the objective of this study was to investigate the effects of surgical repair and limb immobilization on Achilles healing and ankle joint function following complete tendon transection in a rat model. We hypothesized that surgical treatment and aggressive rehabilitation would result in superior Achilles tendon mechanical, structural, and functional properties following injury.

Methods

Study Design: Sprague Dawley rats (n = 108) received 2 weeks of treadmill exercise training (up to 60 minutes at 10m/min)5 (IACUC approved) prior to surgical removal of the right central plantaris longus tendon and blunt mid-substance transection of the right Achilles tendon. Animals were then randomized into repaired (R) (Modified Kessler approach) (n = 54) or non-repaired (NR) (n = 54) groups, and all hind limbs were immobilized in plantar flexion. These groups were further divided into aggressive (Agg), moderate (Mod), or conservative (Con) rehabilitation (Figure 1). Functional evaluation (n = 18/group) of passive ankle joint range of motion (ROM) and stiffness was completed using a custom torque cell and accelerometer-based device on anesthetized animals.[5] All assays were performed after 6 weeks of healing. Ex vivo Assays: After sacrifice, the Achilles tendon-foot complex was carefully removed en bloc, fine dissected, measured for cross sectional area, and secured in fixtures. Tendons were then loaded at 1N in a PBS bath while a series of sagittal B-mode high frequency ultrasound images (HFUS) were acquired using a 40MHz scanner (Vevo 2100, MS550D; VisualSonics) (n = 10-11/group).[6] Tendons were then mechanically tested and imaged (n = 10-11/group) with a protocol consisting of stress relaxation (6% strain), a low-load dynamic frequency sweep (0.1 to 10 Hz), and fatigue testing (~10-75% of ultimate failure load) at 2Hz using a sinusoidal waveform until failure (Instron Electropuls 3000). Analysis: Functional ankle joint properties (i.e., ankle ROM and stiffness) for both dorsiflexion and plantar flexion were evaluated. Achilles tendon percent relaxation, dynamic modulus, tanδ, toe and linear modulus, hysteresis, cycles to failure, and laxity were computed from mechanical and optical testing data. Echogenicity and collagen fiber alignment were evaluated from the HFUS images for the injury region.[6] Two-way ANOVAs with post hoc Fisher's tests were used to evaluate the

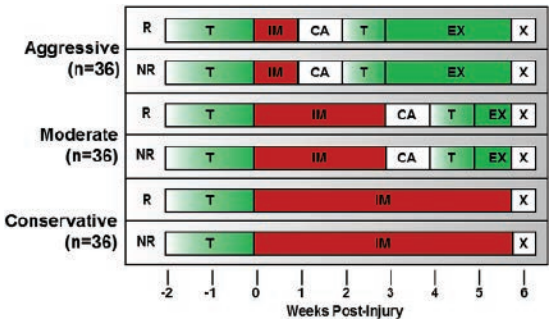

Figure 1. Study Design. Animals were trained for two weeks prior to injury. Following injury, animals were divided into aggressive, moderate, and conservative rehabilitation groups. Definitions: CA- cage activity; T- treadmill training; IM- immobilization; EX- exercise; R- repaired; X- Sacrifice.

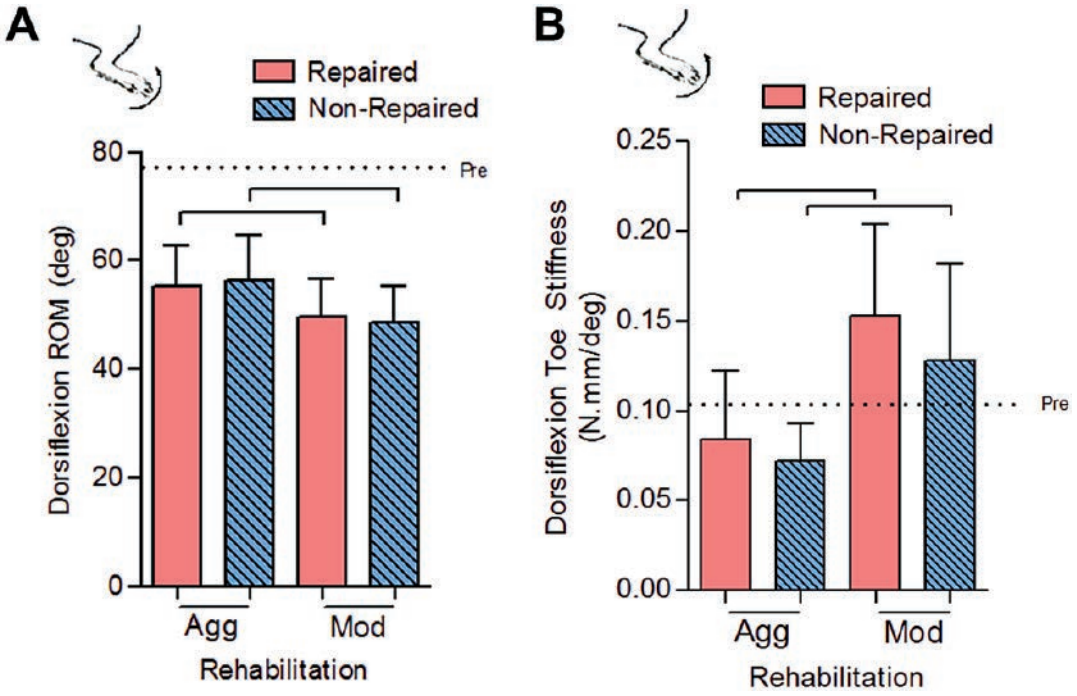

Figure 2. Ankle Joint Range of Motion (ROM) and Stiffness Functional Outcomes. Rehabilitation was a significant factor ($p < 0.05$) on dorsiflexion ROM (A) and toe stiffness (B) after 6-weeks of healing. Data shown as mean ± standard deviation. Lines indicate significant difference ($p < 0.05$). Pre-Surgery values.

effects of surgical treatment and rehabilitation on mechanical, functional, and structural properties.

Results

After 6 weeks of healing, the plantar- (data not shown) and dorsi-flexion ROM was superior in aggressively rehabilitated animals, closer to pre-injury values (Figure 2). Aggressively rehabilitated animals had dorsiflexion toe stiffness values closer to pre-injury values compared to the moderate rehabilitation group. No changes in plantarflexion toe or linear stiffness, or dorsiflexion linear stiffness were observed. Tendon cross sectional area was higher in repaired tendons, and this effect was exacerbated in animals with aggressive rehabilitation. Mechanical property evaluation revealed an increase in the toe modulus in non-repaired aggressively rehabilitated tendons, but no changes in the percent relaxation or dynamic properties. Marked differences in quasi-static linear modulus and fatigue properties were observed (Figure 3) ($p < 0.05$). Specifically, non-repaired tendons with aggressive and moderate rehabilitation had an increased linear modulus and number of cycles to failure (Figure 3) ($p < 0.05$). Additionally, the number of cycles to failure was greatest in the aggressively rehabilitated group. Non-repaired tendons experienced more

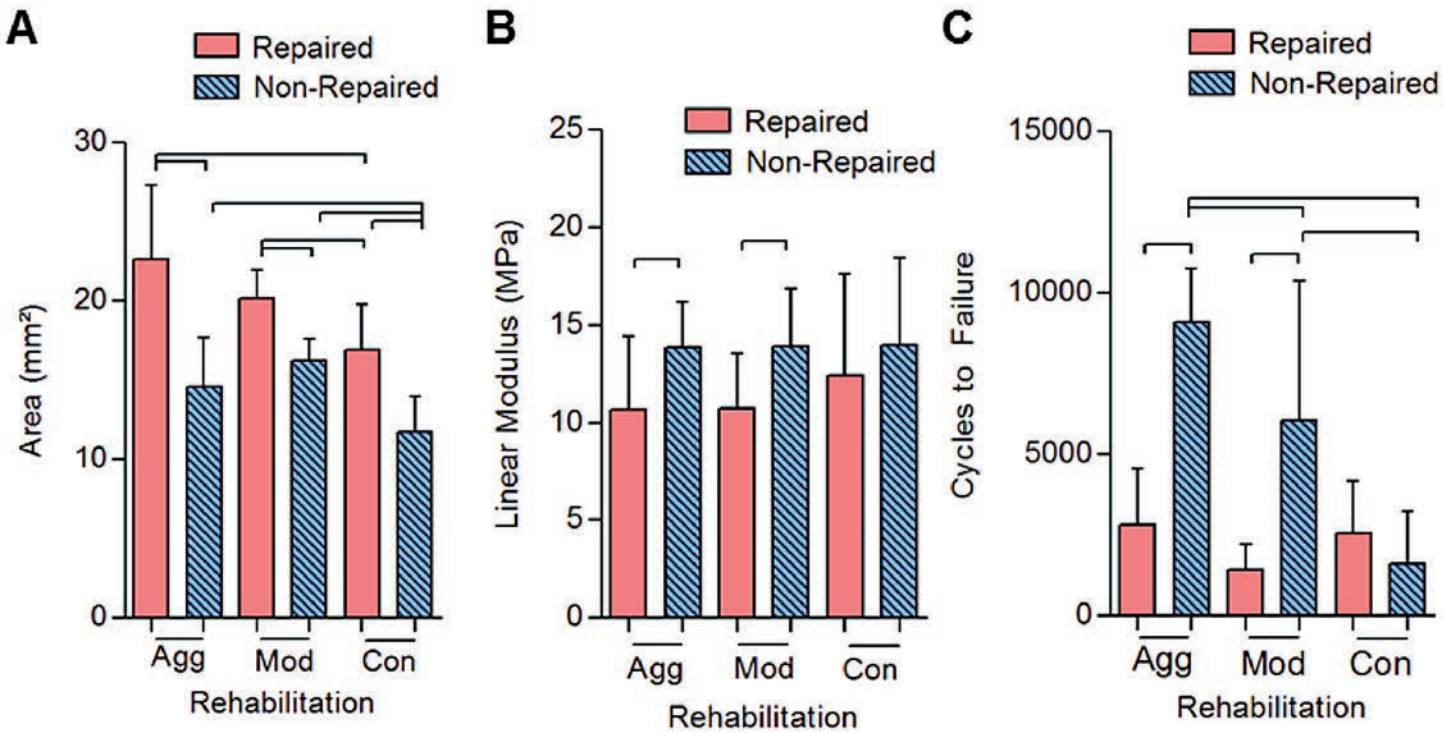

Figure 3. Mechanical Testing Results. Surgery was a significant factor in tendon cross sectional area (A), linear modulus (B), and cycles to failure (C) ($p < 0.05$). Rahabilitation was a significant factor I ntendon cross sectional area and cycles to failure (A,C) ($p < 0.05$). Data shown as mean ± standard deviation. Lines indicate significant differences ($p < 0.05$).

laxity through 5% fatigue life compared to repaired tendons in aggressive and moderate rehabilitation, and transitioned to the secondary phase of fatigue life earlier ($p < 0.05$) (data not shown). Ultrasound evaluation revealed an effect of rehabilitation, but not surgical treatment type, on increased matrix echogenicity, a surrogate measure of fiber density, and alignment ($p < 0.05$) (data not shown).

Discussion

Achilles tendon healing following a variety of common clinical treatment methods was evaluated after 6 weeks of healing in a rat model. We discovered a mechanism whereby non-repaired tendon fatigue properties had marked improvements in the number of cycles to failure. This work suggests the functional and mechanical benefits of aggressive rehabilitation on Achilles tendon healing following a variety of treatment paradigms.[7] Ultrasound evaluation showed promise to detect changes in healing capacity between groups with different rehabilitation strategies. Although the conservative rehabilitated tendons had higher echogenicity and alignment compared to other groups, they also had lower cross sectional area, which likely limited the capacity of the more aligned tendon to withstand fatigue loading. Future work will relate organizational measures from HFUS to tendon fatigue mechanical properties. Additional ongoing studies will evaluate the long-term effects of these treatment and rehabilitation paradigms.

Significance

This study demonstrates that aggressive rehabilitation with nonsurgical management leads to improved tendon fatigue mechanics and ankle function after 6 weeks of healing in this rat Achilles tendon injury model. Ultrasound evaluation showed promise to detect changes in healing capacity between groups.

Acknowledgements

We acknowledge financial support from NIH/NIAMS R01AR064216, NIH/NIAMS P30AR050950, and the NSF GRFP. We thank Adam Pardes, Jennica Tucker, Bob Zanes, James Cirone, and Joanna Fried for assistance.

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U·P·O·J

Whole-Transcriptome Profiling of Notochord-Derived Cells during Embryonic Nucleus Pulposus Formation

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Introduction

Intervertebral disc degeneration is implicated as a major cause of low back pain,[1] necessitating new therapeutic strategies that alleviate symptoms as well as restore disc structure and mechanical function. The earliest degenerative changes occur in the central nucleus pulposus (NP), where altered composition initiates a cascade that compromises mechanical function and culminates in structural failure. An impediment to the development of cell-based strategies for NP repair is the unique developmental origin of the NP, as NP cells are derived from the notochord and not the mesenchyme.[2-4] Improved understanding of embryonic NP formation may enable recapitulation of developmental signals that might drive therapeutic cell types, such as mesenchymal stem cells, towards an NP cell-like phenotype to optimize adult disc regeneration. The objective of this study was to establish changes in global mRNA expression profiles of resident cells as the notochord transforms into the NP using whole-transcriptome sequencing (RNA-Seq), focusing on signaling pathways that regulate patterning, growth, differentiation, structural extracellular matrix (ECM) molecules, and putative NP cell-specific markers.

Methods

For these studies (IACUC approved), we used the Shh-cre;ROSA:YFP mouse model,[3] which takes advantage of the fact that all notochordal cells express the morphogen Sonic Hedgehog (SHH), while the cells of the surrounding mesenchyme do not. In this model, SHH-expressing cells also express YFP, enabling isolation of pure populations of notochord-derived cells at any developmental stage. Two key developmental stages were examined: embryonic day 12.5 (E12.5, immediately prior to initiation of the notochord to NP transformation) and postnatal day 0 (P0, fully formed NP) (Figures 1A, B). Each biological replicate ($n = 4$, both groups) consisted of pooled embryos or pups (~6) from one litter. E12.5 RNA was extracted from isolated notochords, and P0 RNA was extracted from YFP-positive cells isolated using fluorescence-assisted cell sorting (Figure 1C). High quality total RNA (RIN > 7) was isolated from each sample and RNA-Seq libraries prepared using the TotalScript Kit (Illumina; San Diego, CA). Single-end, 100-base sequencing was performed (Illumina HiSeq 2500) and results aligned to the mouse genome. Differential gene expression was established using DESeq2[5] with significance as $p < 0.05$.

Results

Principal component analysis (PCA) revealed clear differences in global mRNA abundance between E12.5 and P0 (Figure 2). There were > 4600 genes significantly differentially expressed with fold-changes greater than 2. There was significantly higher mRNA abundance of ECM structural genes, including proteoglycans (aggrecan (ACAN); brevican (BCAN); biglycan (BGN); decorin (DCN)) and collagens (COL1A1, COL6A1, COL10A1), at P0 compared to E12.5 (Figure 3A). Examining signaling pathways known to regulate skeletal patterning, growth, and differentiation, there was significantly lower mRNA abundance of Shh pathway activators including ligand (SHH), receptors (Patched1 (PTCH1); Smoothened (SMO)), and transcription factors (GLI1, 2, 3) (Figure 3B). A large number of genes associated with the TGF-β pathway were

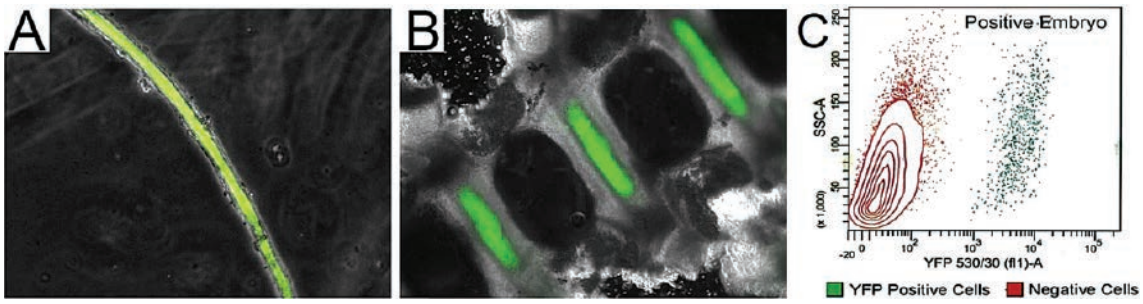

Figure 1. (A) E12.5 YFP-positive notochord. (B) P0 spine with YFP-positive NP. (C) FACS plot of isolated YFP-positive notochord-derived cells.

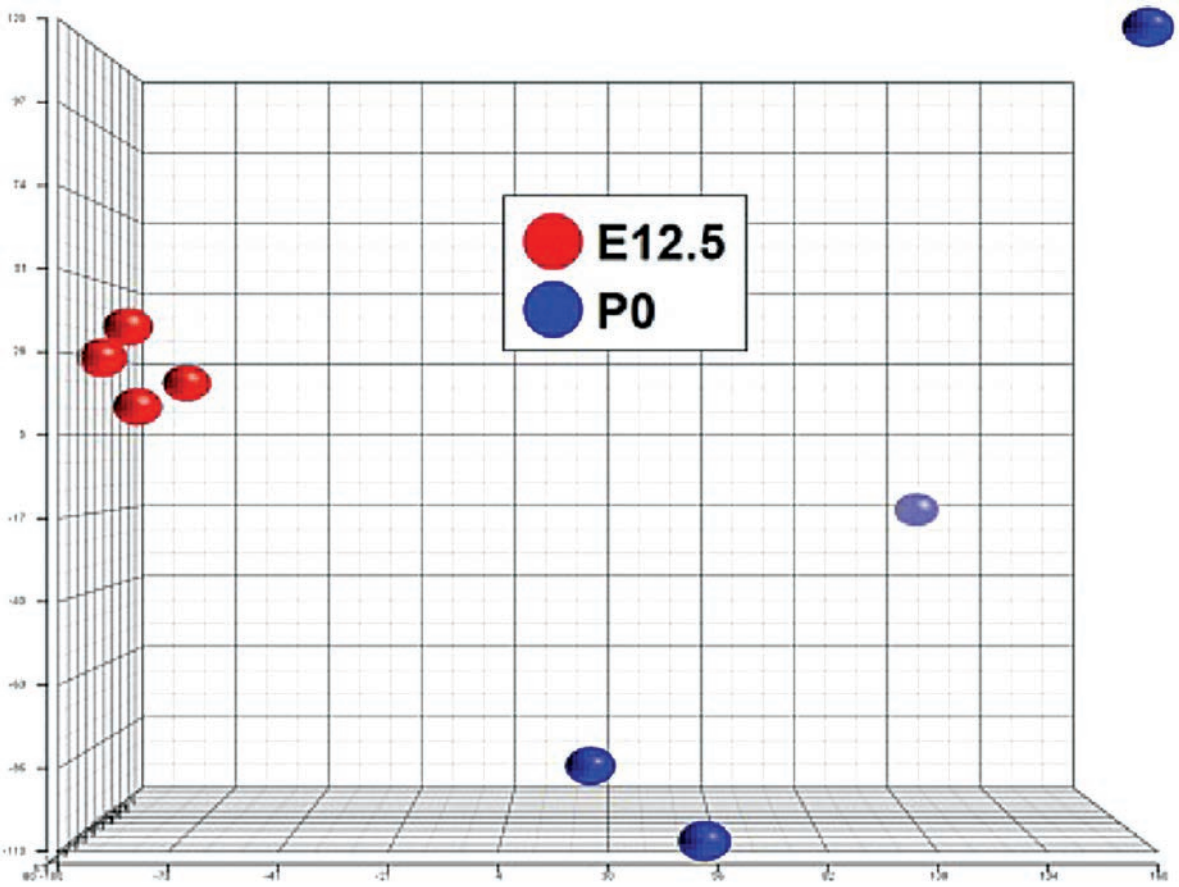

Figure 2. RNA-Seq PCA plot. N = 4.

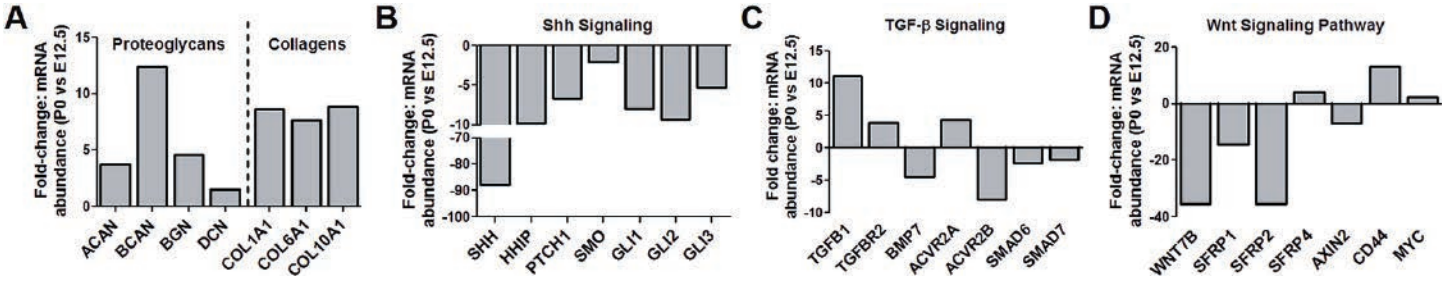

Figure 3. Fold-change in mRNA expression of P0 vs E12.5 cells. A. ECM structural genes. B. Shh pathway genes. C. TGF-β pathway genes. D. Wnt pathway genes. N = 4; all $p < 0.05$.

also differentially expressed at P0 compared to E12.5, including 11.2 and 3.9-fold increases in TGFB1 and TGFBR2, respectively (Figure 3C). Furthermore, many genes of the Wnt signaling pathway were differentially expressed at P0, including pathway ligands such as WNT7B, modulators such as SFRPs (secreted frizzled-related proteins), and downstream target genes, AXIN2, CD44, and MYC (Figure 3D). Finally, we examined differential expression of molecules considered to be specific markers of the NP cell phenotype.[6] Many such markers exhibited stable expression across the E12.5 to P0 developmental window, including brachyury (T), keratins 8 and 18 (KRT8, KRT18), and hypoxia-induced factor (HIF1A). Others exhibited significant changes in expression from E12.5 to P0, including keratin 19 (KRT19, 2.9-fold increase), carbonic anhydrase 3 (CAR3, 8.3-fold increase), carbonic anhydrase 12 (CAR12, 4.6-fold decrease), and vimentin (VIM, 5.7-fold increase).

Discussion

The large number of differentially expressed genes at P0 compared to E12.5 is not surprising given the substantial morphological changes occurring in this developmental window. Changing expression levels of ECM and signaling molecules likely reflect a switch from patterning (altered Shh and Wnt signaling) to growth (increased TGF-β signaling and ECM) as the NP develops into a functional, load-bearing tissue. TGF-β signaling has previously been identified as critical for disc development,[7] and our findings support those results. Differential expression of many Shh and Wnt pathway genes supports the changing role of these pathways at the intersection of the embryonic and postnatal phases of development.[8] Analysis of putative NP markers[6] also showed significant differential expression at P0 when compared to

E12.5. Ongoing work will validate these RNA-Seq results and establish the effects of targeted activation or inactivation of these signaling molecules on embryonic disc formation and postnatal growth. NP markers found to exhibit stable expression throughout embryonic development may be the most faithful indicators of a cell's notochordal origin, although the importance of many of these markers in the context of adult function and regenerative therapeutics remains to be fully elucidated. Our long term goal is to establish and recapitulate the specific developmental signals required for embryonic NP formation in order to improve cell-based therapeutic strategies for disc regeneration.

Significance

Low back pain associated with intervertebral disc degeneration is a significant global health issue. The results from this study will inform the development of improved cell-based therapeutics for disc regeneration.

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Sustained Release of Ibuprofen from Labrafil-Modified PLGA Microspheres

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Introduction

Sustained delivery of non-steroidal anti-inflammatory drugs (NSAIDs) is an attractive method for long-term management of pain and inflammation after tendon repair. Delivery systems have been developed to enable the controlled or targeted delivery of drugs and biofactors, using micelles, emulsions, and micro/nanoparticles.[1-3] The rate of release from micro/nanoparticles depends on several factors, including the type of polymer used, the molecular weight of the drug, and others that can be controlled during fabrication.[4] While NSAIDs like ibuprofen (IBP) can be incorporated into poly(lactic-co-glycolic acid) (PLGA) microspheres,[5] previous studies indicate that this system exhibits a high initial burst release.[6,7] We hypothesized that this characteristic burst release could be attenuated by including excipients to the polymer solution during microparticle formation.[8,9] In this study, we employed Labrafil, a PEG derivative that is biocompatible and biodegradable, as a non-ionic amphiphilic excipient to attenuate the burst release of IBP from PLGA microspheres.[11] The objective was to investigate the effect of Labrafil concentration on the release kinetics of IBP. Furthermore, we sought to couple these IBP-loaded microparticles with a nanofibrous scaffold[12] to generate a delivery vehicle for localized and sustained release.

Methods

Four different microsphere formulations were investigated with varying Labrafil® M1944CS oil concentrations: PI 0 (PLGA-IBP-Oil free), PI 30 (PLGA-IBP-Oil 30 μL), PI 300 (PLGA-IBP-Oil 300 μL), and PI 600 (PLGA-IBP-Oil 600 μL). In each formulation, 75:25 PLGA was used (0.15 g/mL, Mw = 70 kDa) and the IBP content was maintained at 30 mg/mL. Labrafil oil was dissolved in l mL of dichloromethane and added to the polymer solution prior to microsphere formation. The external phase of the emulsion consisted of 5 mL of a 1% Poly(vinyl alcohol) aqueous solution. The emulsion was sonicated, added to distilled water, and stirred for 2 min. Formed microspheres were suspended in distilled water and stirred continuously for 4 h. Finally, the microspheres were washed and dried under vacuum for 48 h, followed by SEM imaging. To measure IBP release, microspheres (20 mg) were suspended in 20 mL of phosphate buffered saline (PBS) at 37°C on a shaker. The supernatant (5 mL) was withdrawn (and replaced with fresh PBS) at various time intervals and centrifuged. IBP content was determined by UV spectrophotometry at λ = 223 nm. To determine the biocompatibility of delivery, tendon fibroblasts (2×10^4 cells/well in a 24-well plate) were cultured in media pre-treated with or without microspheres that had released for 18 hours. Cell adhesion and proliferation over 7 days were evaluated via light microscopy and the MTT assay. Next, composite electrospun scaffolds were formed with PI 300 microspheres. For this, 0.2 g microspheres (0.05 g/mL) were dispersed in 10% poly(ethylene oxide) (PEO), which was co-electrospun (2.5 mL/hr) with 14.3 % w/v poly(ε-caprolactone) (PCL) (3 mL/hr, in 1:1 tetrahydrofuran and N,N-dimethylformamide) onto a common collecting mandrel. After fabrication, scaffolds were hydrated to remove the PEO. Scaffolds were imaged via SEM and IBP release evaluated over 21 days.

Results

SEM images revealed that Labrafil-modified PI microspheres were larger than those without Labrafil (PI 0) (Figure 1). Inclusion of Labrafil attenuated the initial burst release and resulted in release profiles that became more linear with increasing Labrafil concentration. In PI 0 microspheres, 100% of the encapsulated IBP was released within 26 days, with 63% released over the first 2 h. Conversely, Labrafil-modified microspheres had a much slower release profile, with 72% and 61% of IBP released from PI 300 and PI 600 microspheres over 102 days, respectively. Importantly, the initial burst release was greatly reduced (3% for PI 600 in the first 2 h, Figure 2A). The MTT assay showed that, compared to proliferation on TCP, cells proliferated more slowly when more IBP was released (i.e., at lower Labrafil concentrations) (Figure 2B). For composite nanofibrous scaffolds containing IBP microspheres, SEM images after PEO removal showed that microspheres remained entrapped

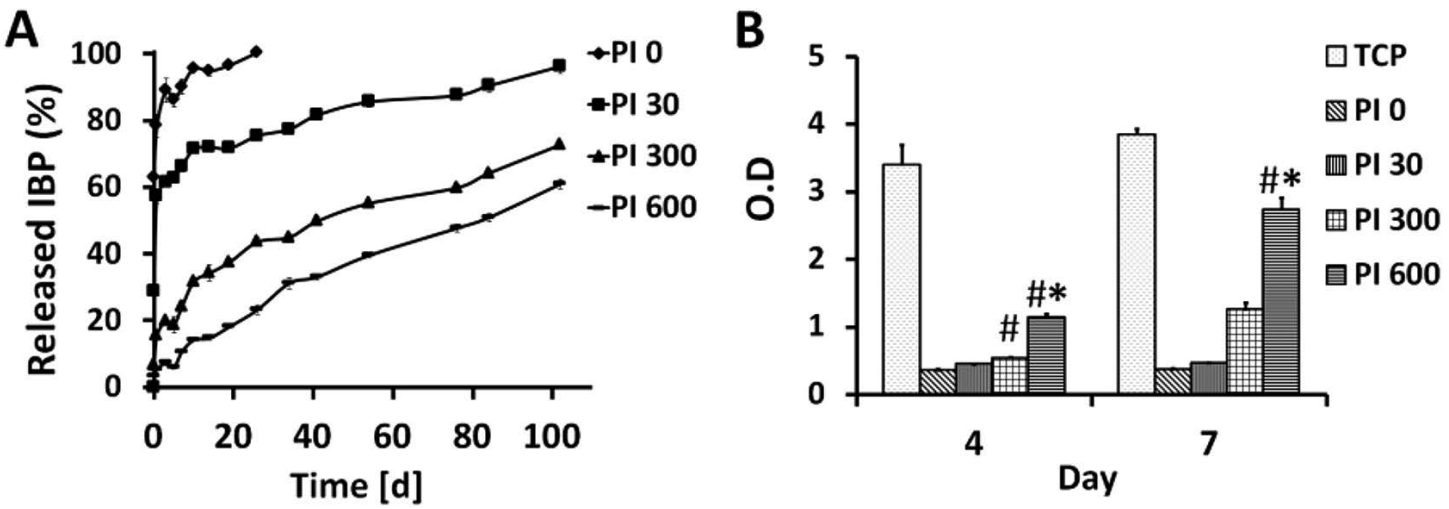

Figure 1. SEM images of IBP-loaded PLGA microspheres with/without oil. (A) PI 0 (PLGA-IBP-Oil free), (B) PI 30 (PLGA-IBP-Oil 30 µl), (C) PI 300 (PLGA-IBP-Oil 300 µl), (D) PI 600 (PLGA-IBP-Oil 600 µl) (scale bar = 10 µm, ×5000), (E) PI 300 (scale bar = 5 µm), (F) PI 600 (scale bar = 100 µm).

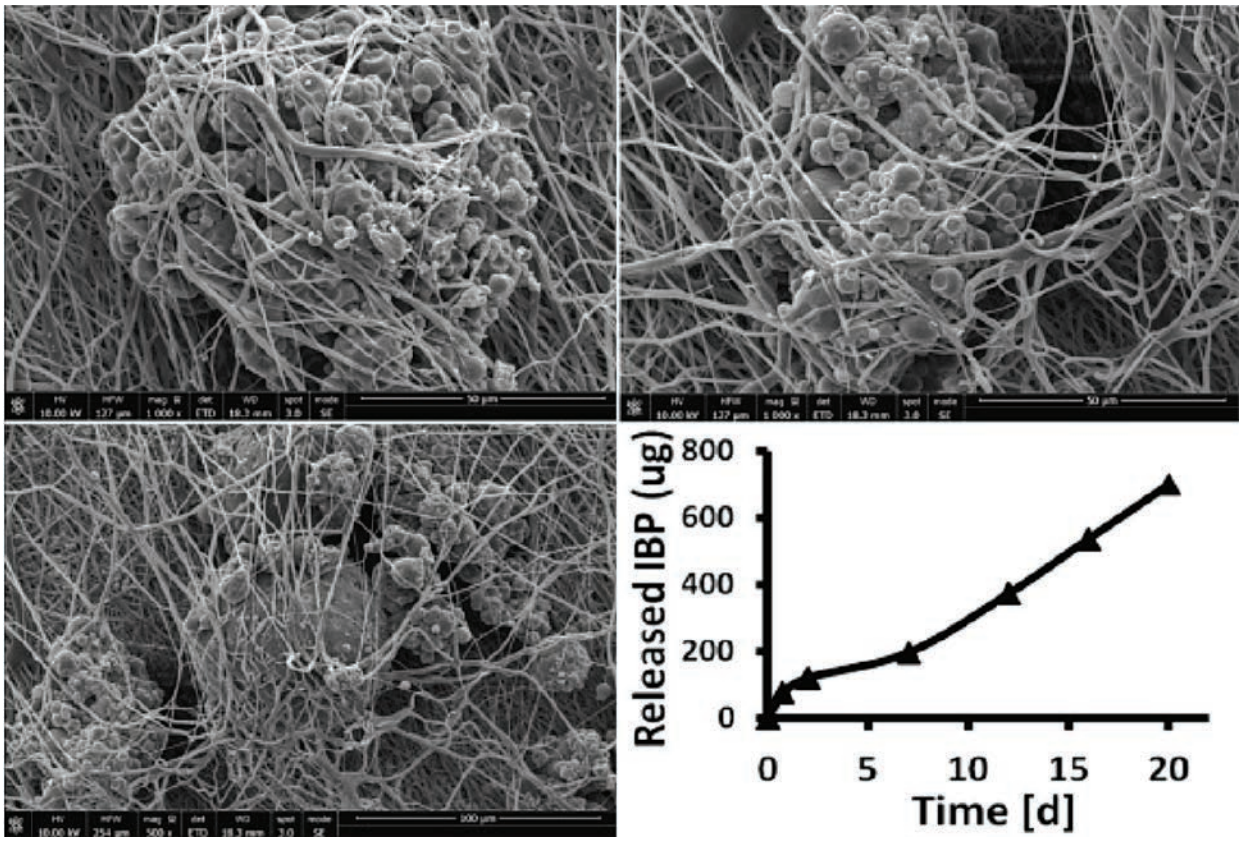

Figure 2. (A) Release profile of IBP from PLGA microspheres with/without oil in PBS at 37°C. (B) MTT assay results (*: $P < 0.05$ vs. PI 300, #: $P < 0.05$ vs. PI 0).

between the aligned fibers. These microsphere-containing scaffolds released in a continuous fashion over the first 21 days (Figure 3).

Discussion

This study demonstrated that sustained and steady release of IBP, with a reduced initial burst release, could be achieved by modifying PLGA microspheres with Labrafil oil. When a burst release was present, IBP inhibited cell adhesion and proliferation, suggesting that a more gradual release might be more effective for *in vivo* drug delivery applications. When these microspheres were embedded in a structural PCL nanofiber scaffold, microspheres remained entrapped and released at a steady rate over 21 days. Taken together, these results demonstrate that the IBP-loaded PLGA microspheres with Labrafil oil show promise as a multifunctional drug

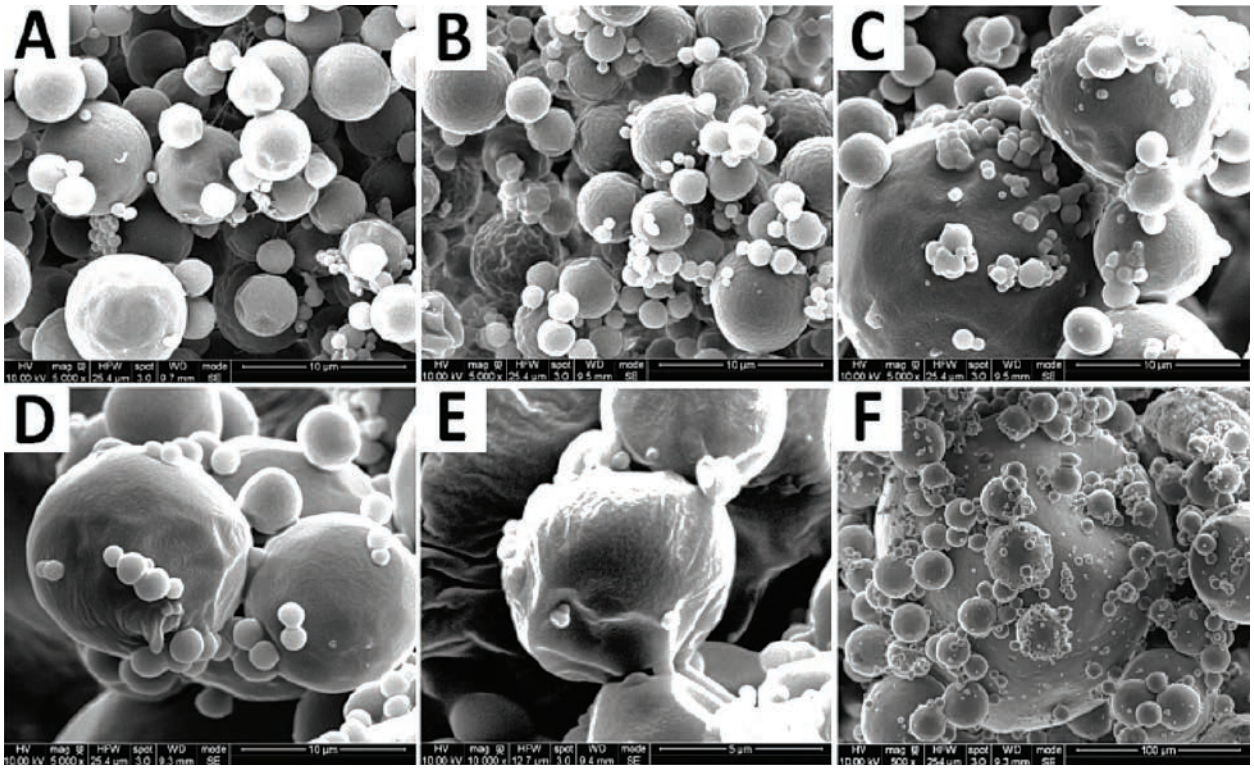

Figure 3. SEM images and release profile of IBP from PCL/PEO composite nanofiber scaffold with Labrafil-modified IBP PLGA microspheres.

delivery system. In future studies, the clinical application of these novel scaffolds will be investigated in the context of rotator cuff repair.

Significance

This work demonstrates that the controlled release of IBP can be achieved by modifying PLGA microspheres with Labrafil oil, providing a means by which the effect of prolonged release of this anti-inflammatory drug can be evaluated in tendon repair.

Acknowledgements

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A Low-Cost, Wearable Magnet-Based Detection System to Assess Joint Kinematics in Humans and Large Animals

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Introduction

Functional outcomes such as joint kinematics and gait are important indicators of efficacy in musculoskeletal research.[1] Traditional technology to objectively assess these parameters, such as visual tracking systems and/or force plates, are challenging to deploy in long-term translational and clinical studies. To address this limitation, we previously developed a low-cost, multi-sensor device that detects animal activity and joint angle, and hypothesized that it could quantify post-operative functional recovery.[2] Here, we establish longitudinal changes in animal activity after a bilateral arthrotomy, and demonstrate the feasibility of measuring joint kinematics in both large animals and humans using a magnet-based detection system.

Methods

Device Hardware: The device consists of an enclosure containing a polymer lithium ion battery, microcontroller board, radio board, data logger, and sensor board, which integrates a triple-axis accelerometer, triple-axis gyroscope, and triple-axis magnetometer.[2] *Device Validation:* To measure changes in joint angle, the device and a neodymium magnet (1" ø, 1/4" thick) were affixed 4" distal and 4" proximal to a human knee joint on the posterior surface, respectively. The knee was moved to flexion angles of 0º, 30º, 60º, and 70º. Position was held for 5 s at each angle (n = 3-4/group) and the magnetic field strength was recorded at 40 Hz. An equation relating sensor-magnet angle as a function of magnetic field magnitude was derived to predict flexion angle. To validate that the device could capture dynamic range of motion of the knee during normal gait, a human subject (n = 1) walked at a step frequency of approximately 1 Hz, and the magnetic field strength and angular velocity were recorded. Individual steps (n = 10) were used to obtain the range of motion (ROM) during the gait cycle. *Animal Monitoring:* To evaluate general activity of a large animal, the device was attached to a harness worn by a castrated male Yucatan minipig pre- and post-surgery (n = 1, 26 kg pre-op) in an unrelated study involving bilateral arthrotomy of the stifle, with analgesics given for the first 5 days after surgery. Data was collected at 8 Hz for 30 min of unsupervised activity in a 4' × 6' pen pre-operatively on Day −1 (Baseline) and post-operatively on Day 1 and weekly thereafter until euthanasia at Week 12. Angular velocity (º/s) parallel to the dorsal plane (animal turning left or right) was recorded and the absolute values binned into four activity intensity levels: 0-5 (Rest), 5-50 (Low), 50-100 (Moderate), and > (High). On Week 11, the sensor and magnet were laterally attached to the left hindlimb 4" proximal and 5" distal to the stifle joint, respectively. The stifle was manually flexed to angles of 20º (maximum extension), 30º, 60º, and 90º. Position was held for 5 s at each angle (n = 3-4/group) and the magnetic field strength was recorded at 8 Hz. The animal was allowed to freely ambulate within the pen and the magnetic field strength was recorded. Individual steps (n = 10) were used to obtain the ROM and were visually confirmed with synchronized high-speed video. *Statistics:* Significance was assessed by one-way ANOVA with Tukey's post-hoc tests to compare magnetic field strength between groups ($p \leq 0.05$). Data are presented as the mean ± SD unless otherwise noted.

Results

A wearable device capable of sensing motion and quantifying joint kinematics was fabricated with off-the-shelf electronics for <$200. Knee flexion angle was predicted via changes in the magnetic field strength, which increased exponentially with flexion (Figure 1B, $p \leq 0.05$). Using this method, we measured the range of motion (ROM) of a human knee during a normal gait cycle. Flexion angle and angular velocity of the tibia appeared as repetitive and predictable patterns during ambulation (Figures 1C and 1D). The average ROM of the human knee during the gait cycle was 54 ± 4º, with a peak flexion angle of 55 ± 3º. Next, the device was used to monitor unsupervised large animal activity pre- and post-surgery, and to quantify stifle joint kinematics. The device was worn by a Yucatan minipig and angular velocity in the dorsal plane was recorded over 12 weeks (Figure 2). On Day −1 (Baseline), the animal had full ROM and activity was characterized by Rest and Low intensity activity, with short periods of Moderate and High intensity activity (Figure 2A). Immediately post-operative on Day 1, the animal was predominately sedentary and ambulated with a stiff, limping gait. The animal regained

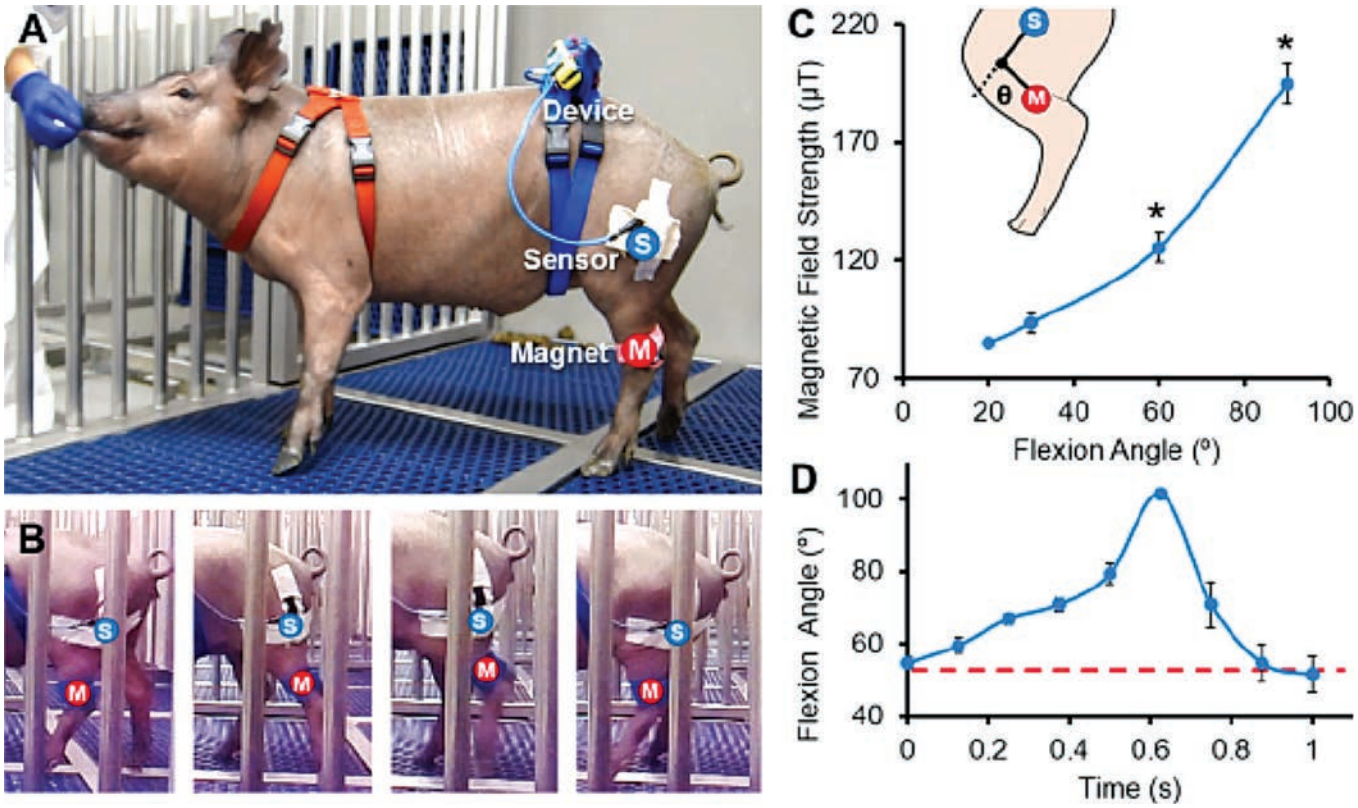

Figure 1. Magnet-based system for quantifying joint kinematics. (A) Experimental schematic. (B) Magnetic field strength as a function of flexion angle. * = $p \leq 0.05$ vs. all other angles. (C) Average flexion angle and (D) Angular velocity for a human knee during a gait cycle (mean ± SEM for 10 steps).

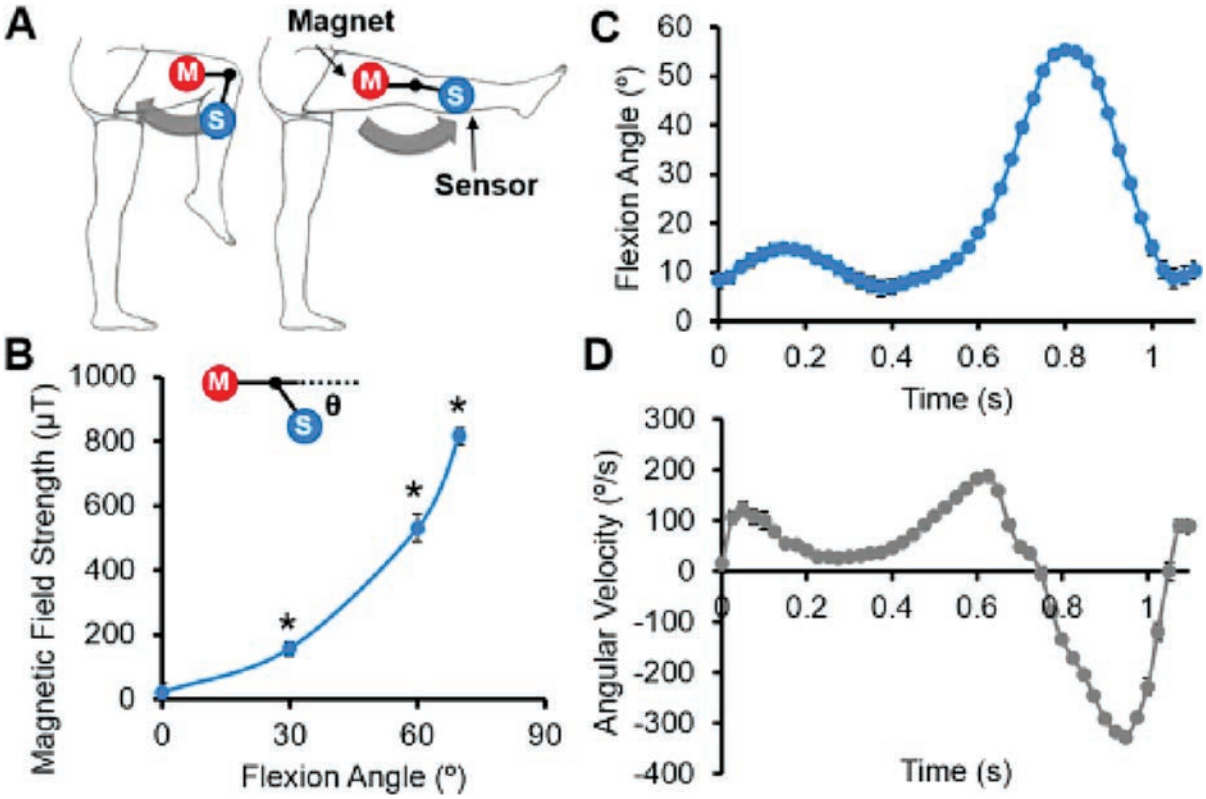

Figure 2. Unsupervised activity monitoring demonstrates time course of recovery in a porcine model. (A) Distribution of activity intensity pre- and post-surgery. (B) Non-Rest Activity normalized to the pre-operative Baseline value (red dashed line) and animal weight over 12.

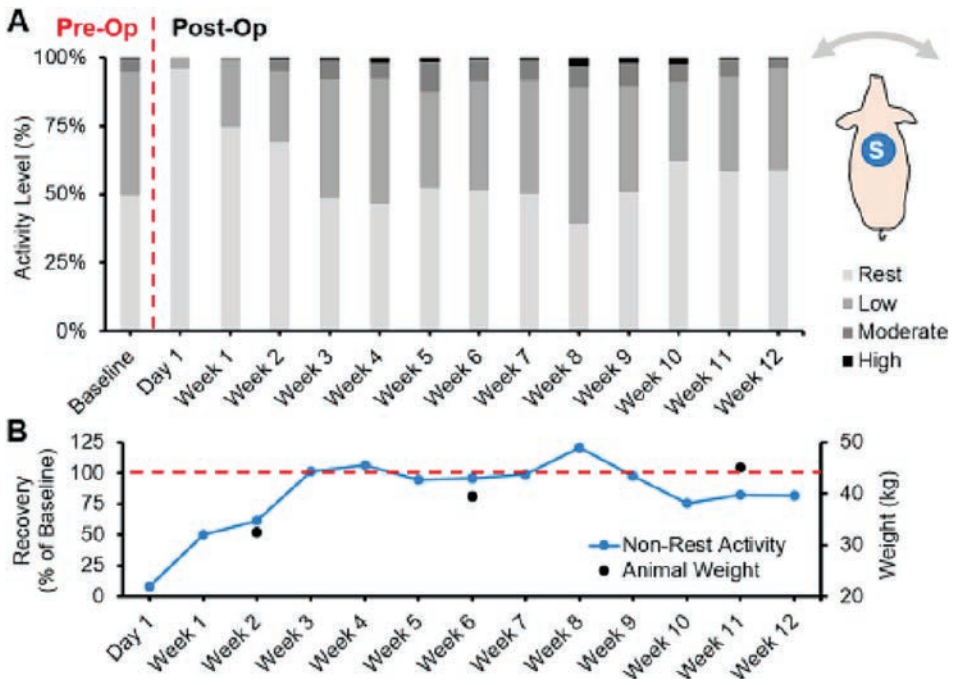

Figure 3. Dynamic range of motion during unprovoked ambulation. (A) Image of animal wearing the device. (B) Sensor and magnet positions during gait cycle. (C) Magnetic field as a function of flexion angle. *= $p \leq 0.05$ vs. all other angles. (D) Average flexion angle during a gait cycle at Week 11 (mean ± SEM for 10 steps). Red line indicates neutral stance.

50% of its pre-operative Non-Rest activity level by Week 1, and was fully recovered by Week 3. Non-Rest activity levels were maintained until Week 10, when it slightly declined (Figure 2B). The average ROM of the porcine stifle during the gait cycle was 55 ± 13° at Week 11, with a peak flexion angle of 101 ± 4° (Fig. 3).

Discussion

Wearable motion sensors have the potential to provide objective, individualized data on physical activity and locomotion for animal models. However, basic accelerometer-based monitors cannot assess the type or quality of movement,[3] whereas more complicated analytical systems are costly and often require the use of multiple sensor components in a supervised environment.[4,5] To that end, we developed a low-cost device using a single integrated sensor that quantifies both joint kinematics and activity in an unsupervised manner. Using this system, we tracked the time course of recovery of a pig after arthrotomy and found that return to baseline activity occurred 3 weeks after surgery. Monitoring also revealed a slight decline in activity in the long term, which may indicate behavioral changes due to increasing weight or age. By placing a magnet opposite the articulating joint, we identified discrete steps and calculated the dynamic ROM during unprovoked ambulation in both the human knee and the porcine stifle. Importantly, the measured ROM for the porcine stifle was consistent with previously reported values for healthy swine,[6] indicating functional joint recovery by 3 months. Standard gait parameters, such as cadence and swing/stance phase ratio, may also be derived from this data. Simple and inexpensive, this magnet-based system will facilitate the longitudinal assessment of joint abnormalities and functional recovery for animal and human subjects in orthopaedic research.

Significance

This device allows inexpensive quantification of joint kinematics and activity levels in research subjects using a single integrated sensor.

Acknowledgments

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Variations in Media Formulation Impact ECM Synthesis and Retention in NP Cell-laden HA Hydrogels

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Introduction

Degeneration of the intervertebral disc (IVD) is a major cause of back pain, and tissue engineering has emerged as a promising method for the generation of replacement structures. Various growth factors and media formulations have been used to fabricate tissue engineered constructs using both progenitor and native tissue cells.[1] However, the media formulations that best produce engineered constructs during in vitro culture and maintain this state upon in vivo implantation remain to be determined. In particular, TGF-β3 has been shown to induce differentiation of MSCs, as is indicated by significant increases in chondrogenic gene expression and extracellular matrix production.[2,3] Dexamethasone, a synthetic glucocorticoid, is widely used to stimulate chondrogenesis and maintain the integrity of the cartilaginous matrix in chondrogenic cell lines.[4,5] In addition, ascorbate has been shown to play a role in collagen hydroxylation, and its absence impairs collagen secretion and assembly.[6] In the present study, starting from a chemically defined, serum free medium, we assessed the impact of three molecules (TGF-β3, ascorbate, and dexamethasone) on proteoglycan and collagen deposition by nucleus pulposus (NP) cells in a 3D hyaluronic acid (HA) hydrogel culture system, and further examined the persistence of these properties after implantation.

Methods

NP cells were isolated from adult bovine caudal discs and encapsulated in a 1% w/vol MeHA solution at a density of 20 million cells/ml.[7] Constructs (diameter: 4 mm, thickness: 2.25 mm) were cultured for 8 weeks in one of five media conditions: chemically defined media (CDM)[8,9] with/without 10 ng/ml TGF-β3 (+TGF-β3/−TGF-β3), CDM with TGF but lacking ascorbate (−Ac), CDM with TGF but lacking dexamethasone (−Dexa), or a basal, serum containing media (BM) supplemented with ascorbate (BM+Ac). For in vitro analysis, cell viability of constructs was assessed using the Live/Dead staining kit and unconfined compression tests were performed to determine construct mechanical properties as a function of different media formulations. For each construct, a constant 2g load was applied and creep displacement was monitored until equilibrium was attained (~300 s). Then, a stress relaxation test was carried out via a single compressive deformation to 10% strain at a rate of 0.05% s^{-1} followed by 20 min of relaxation to equilibrium, at which point stress and strain values were used to calculate equilibrium modulus. Sulfated glycosaminoglycan (s-GAG) and collagen content were assessed as in.[7] Sections were also stained with Alcian blue and picrosirius red to visualize proteoglycans and collagen, respectively. Based on in vitro study results, four formulations (+TGF-β3, −TGF-β3, −Ac, and −Dexa) were implanted into the rat subcutaneous space after 6 weeks of pre-culture. Biochemical and histological assessment of matrix deposition was performed 5 weeks after implantation (11 weeks).

Results

In the in vitro study, Live/Dead staining showed a similar number of live cells in all formulations except for BM+Ac, where the number of live cells was lower (Figure 1A). Mechanical properties of +TGF-β3, −Vc and −Dexa constructs were higher than all other constructs at 4 and 8 weeks, with no differences observed between the +TGF-β3 and −Dexa groups (Figure 1B). GAG content increased over time in all groups except for −TGF-β3 constructs, with the +TGF-β3 and −Dexa constructs reaching significantly higher levels than the −Ac constructs at 8 weeks (Figure 1C). Collagen content of the +TGF-β3 and -Dexa constructs were significantly higher than -Ac constructs at both 4 and 8 weeks (Figure 1D). In the in vivo study, after subQ implantation, proteoglycan staining decreased slightly for all groups (Figure 1E), while collagen staining increased markedly (Figure 1F), with similar trends observed via quantification of construct content (Figure 1C-D).

Discussion

This study explored the impact of different media formulations on the maturation of NP cell-laden HA hydrogels, both in vitro and after implantation into the rat subcutaneous space. Our findings demonstrate that inclusion of TGF-β3 is most essential for establishing disc-like mechanical properties and ECM content.

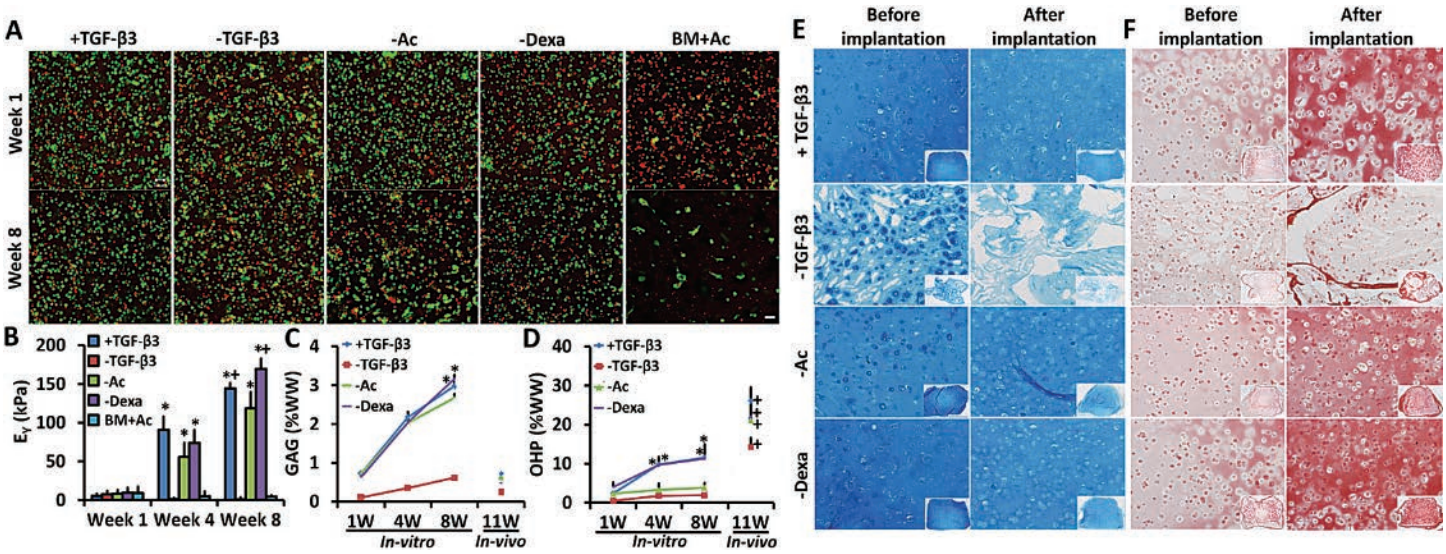

Figure 1. (A) Representative images of Live/Dead staining of NP cell-laden HA gels cultured in five different media formulations (Bar = 50 μm). (B) Equilibrium compressive modulus (n = 4~5, *:p < 0.05 vs. −TGF-β3, + :p < 0.05 vs. −Ac). (C) GAG content (n = 4 ~ 5, *:p < 0.05 vs. −Ac). (D) Collagen (OHP) content (n = 4 ~ 5, *:p < 0.05 vs. –Ac, + :p < 0.05 vs. 8 weeks). E: Alcian Blue staining of proteoglycans. F: Picrosirious Red staining of collagens.

Notably, removal of dexamethasone had no impact on construct properties, while removal of ascorbate resulted in lower mechanical properties and collagen content, consistent with the established role of this molecule in collagen crosslinking. Furthermore, we noted, for all media formulations, a decrease in proteoglycan content and marked increase in collagen deposition after subcutaneous implantation.

Significance

Our findings suggest that chemically defined media can be refined to promote NP cell-based construct maturation, but that transfer to the in vivo space alters construct composition and function, and so must be considered in any translational application.

Acknowledgements

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Audio-Visual Fellowship: Pearls and Pitfalls

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The residency program at the University of Pennsylvania is known to be hard working, particularly in the first few years. Every year, however, there are two residents who take a brief detour from the gradual slope of decreasing work as they enter their dedicated research year. These residents are referred to as the "AV fellows", although their lives and roles are poorly understood. This article will attempt better elucidate this unknown role and provide guidance to future AV fellow generations.

First, is the adjustment to a new daily schedule. Gone are the days of being in the hospital at 5am, you just need to be at conference by 6:30am, so enjoy the extra sleep. Attendance at the regular morning conferences ensures that sign-in sheets can be collected, with the side benefit of maintaining orthopaedic knowledge. Rarely does a morning conference need actual audio-visual assistance. Once you arrive at lab, usually around 7:30am, you can enjoy the silence and productivity until your remaining lab colleagues arrive by 9am. This time is most valuable to checking your email for unusual requests of your time, managing medical student rotations and preparing for Grand Rounds. At this point you are ready to work on your research projects, although if you can't find anyone you should check what time it is. In the first two years of residency time was more of a conversation piece, but in lab the 12pm-1pm timeframe is sacred. No work is to be done during this time, at all costs, because being social is apparently valued. After this lunch break, you can settle back into work mode, but be careful not to lose track of time at the risk of returning to lab at 5:01pm to find that everyone has vaporized.

All hands are on deck for Grand Rounds, the ultimate test of the electronic equipment amateur. Although thorough preparation can help limit some issues that might arise, not all events can be avoided. As a well-established meeting place, crossing many generations, the Agnew-Grice auditorium has just as many eras of technology in use. The projector represents the most critical and robust component to the audiovisual system, and will unlikely fail despite its desperate pleas for filter changes.

Delivering high quality data to the projector is the audiovisual tower located behind the podium. This tower looks sturdy-enough given the locked metal gate shielding it from prying eyes and hands; however it is the most unpredictable component of the system. Contained within this tower is a collage of seven audiovisual companies, all competing for your attention using a rainbow of lights. If one is unfortunate enough to have this system stop working during Grand Rounds, usually indicated by a built-in alarm that unhelpfully rings seconds before shutdown, the first step is to block out the glares and high pitched squealing of electronics and try a restart of the entire system. This continues to be the primary advice of all previous AV fellows, though there is no current evidence of this ever fixing any tower related issue. Following your publicly failed attempts to fix the tower with a restart, the next step is tracking down someone to professionally service it. As the tower seemingly appeared out of nowhere several years ago, finding a still existing company to take responsibility for any one of the tower components is a challenge requiring a significant number of emails and orthopedic staff. Persistence in this search is the key to success.

The last component to the Grand Rounds audiovisual system is the laptop computer that is transported to the auditorium weekly. Initially this seems to be a convenient way to have a reliable computer, but it is soon realized that it thoroughly enjoys being the most up to date piece of equipment. It is so dedicated to this quest that it will restart itself to install updates whenever it decides to. If you are lucky, it will wait 1000 minutes but too often it decides 10 minutes should be enough time for the current lecturer to wrap up their talk. This situation occurs usually at about 30min after start-up, usually during a visiting professor talk. Once this countdown has started, no amount of window closing, double clicking, or prayer can stop the computer from shutting down. In fact, the timer itself cannot even be removed from the screen as the computer proudly blocks the now-rushed speaker from displaying the entirety of his or her slides.

The design of Agnew-Grice is nostalgic for some, bringing thought of sitting in a surgical amphitheater. However, its steep ascent of rows also produces both an alpine and tropical climate, which must be managed with grace.

Anything more than a gentle breeze on the thermostat dial can cause a wild shift in temperature from the air handling units, breeding discomfort, unsightly underarm pit stains, and even anxiety regarding hurricane formation. Adjust the thermostat with precision and caution.

Although this list is by no means comprehensive, the tips contained in this article should be enough to get the new AV fellow started on his or her track to Penn AV mastery, and bring a new level of understanding to all. At times it may seem a professional might do a better job, but it is important to remember that the Titanic was built by an entire team of experts, whereas the Arc was built by one lone amateur. Be great, never give up, and don't build the Titanic.

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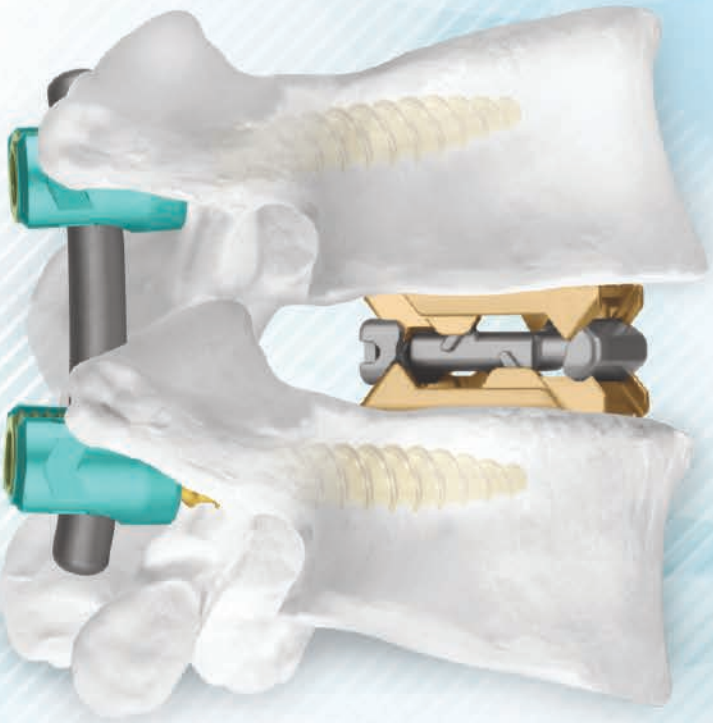

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Letter From the Chairman

L. Scott Levin, MD, FACS

I have always considered it a privilege to write a chairman's column for the UPOJ. Looking back over the last seven years, it is remarkable to reflect on our team's progress and, with pride, I will highlight our achievements this past academic year. Innovation in musculoskeletal care is part of our Penn orthopaedic culture. Our Penn Musculoskeletal Center, the MSKR service line, and the recently renewed NIH funded Penn Center for Musculoskeletal Disorders, underscores the importance we place on creating new standards of care delivery and scientific discovery that enhance our ability to treat patients.

Last July, significant media attention was given to Penn Orthopaedics from around the world when our team led the effort that performed the world's first pediatric bilateral hand transplantation. Zion Harvey, the 8 year old quadrimembral amputee, appeared on television, radio, newspapers and magazines both in the United States and abroad. This patient's clinical result has been as we all had hoped for. He now can feed and dress himself, attend to his personal hygiene, and is returning to school. Such success is a team effort, and teamwork is our watchword. Our partners in this effort included almost every aspect of medicine-surgeons, transplant physicians, neurologists, nephrologists, psychologists, social workers, to name a few. This vascularized composite allotransplantation program was built on our success in 2011 when we performed the region's first adult bilateral hand transplant. This story is really about our trajectory and momentum as a department of orthopaedic surgery. We build on our success, but carefully analyze our failures. As a leader, I applaud achievement, but always ask: "Where can we improve?" "How can we do better?" "Where are our weaknesses and can we develop action plans that help us improve in select areas?"

The answer to these questions lies in the hands of the people in our organization: our faculty, residents, fellows, physician assistants, nurse practitioners, and administrators. Over the last seven years almost all of these teams have grown, many exponentially. Almost every specialty has a fellowship program. We have over 25 PA's and NP's involved in patient care. In 2009 we had two. Growth must have purpose. Our goal to become the nations' #1 research program is within sight. Expanded NIH funding, corporate sponsored research, a growing clinical research program with dedicated research coordinators are examples of growth and diversity that make us stronger. In fact, we now have a 'council of research directors' that is meeting quarterly that includes representatives from the Penn Center for Musculoskeletal Disorders (this year the PCMD received the top score in the country on the NIH P30 grant renewal), the McKay Orthopaedic Laboratory, the CHOP MSK lab (under the direction of Mauricio Pacifici), the VA MSK Translational Research Center, the Biedermann lab, the Human Motion Lab, and the Human Tissue Lab. These seven entities reach across departments and institutes, and connect our science teams with the university in the medical school, veterinary medicine school, school of engineering, and the dental school.

Often in academic medicine, the educational mission is an afterthought. The clinical engine drives revenue, which is used to pay faculty salaries, practice overhead, and dean's tax. The historical adage: "publish or perish" resulted in a revolving door of departmental faculty-signing on to an academic department only to be told after a few years: "you have not written enough papers or secured adequate grant funding" to continue practicing in the university system. The University of Pennyslvania School of Medicine has modernized its approach to clinical faculty recruitment and retention, allowing clinical faculty to seek pathways commensurate with their passions and strengths. Clinical work, educational efforts, and research are all rewarded and acknowledged in our department and across Penn Medicine. The tenure track, clinician educator track, and academic clinician category allow our faculty to define their role in all three of our missions. Our partnership with the health system provide a line of sight compensation system that is transparent and equitable, rewarding teaching and research as well as clinical efforts. As a result, our recruitment and retention has been outstanding, adding this year Kathryn O'Connor (Mt. Sinai orthopaedic residency, Washington University fellowship) to our Foot and Ankle Division, and Benjamin Gray (Washington University orthopaedic residency and University of Cinncinati hand fellowship) to the hand division. Dr. Gray is pursuing a master's degree in clinical science at Penn, a high mark for us and a tribute to his dedication to the clinical research mission. Our growth from a faculty of less than 20 surgeons in 2009 to a complement of 43 physicians and surgeons in 2016 has been calculated and strategic. We have tremendous depth on the bench in all sections which greatly enhances our ability to educate the next generation of orthopaedic surgeons.

We have named this past year "the year of the student". Our visiting medical student rotations were revised to provide a better and broader experience for students over their 4 week stay. Our residency 'school', held for four hours every Thursday, allows every resident in the program to attend our teaching curriculum that includes grand rounds, cadaver lab, visiting professors and didactic lectures. No resident is left behind (as in stuck in the operating room, wards, or emergency room)

during critical educational time. This builds camaraderie and demonstrates the commitment of our faculty to resident education. Our enhanced education efforts have extended to our PGY-1 residents. A one month classroom experience provides an orthopaedic primer to our 'interns', teaching skills essential for success over the course of the residency. Clearly, we will strive to always improve the educational experience, but our efforts are paying off. The balance of service and education in postgraduate training must be tipped towards education if we are to attract the best and brightest medical students to our teaching program.

We believe in a robust visiting professor program with several named lectureships. Our guests this past year have included James Chang (Stanford), John Callaghan (Iowa), Chip Routt (UT Houston), and Alison Toth (Duke) to name a few. This provides our residents and faculty a chance to interface with national leaders and showcases our department's clinical and research capabilities.

A brief review of advances in our divisions will provide insight into our progress.

In 2015, CHOP Orthopaedics hired a sports medicine specialist, a non-operative orthopaedist, as well as three surgeons; moved into three new facilities; renovated the Nicholson visiting professorship; appointed a new director of orthopaedic engineering; hired a director of clinical research; awarded Chair's research grants to six winners totaling $87,000; renovated the fellowship recruitment and interviewing process; launched multiple development initiatives with a new board of visitors for orthopaedics; and partnered with CHOP office of clinical quality improvement on two major projects (sports and spine) to improve the efficiency, safety and value of orthopaedic surgery.

The division of hand surgery, under the leadership of David Bozentka, added a second full time hand and microsurgical fellow approved by the accreditation council for graduate medical education (ACGME). Dr. Bozentka was awarded the prestigious 'master clinician' award by the school of medicine this spring. The Penn Hand Surgery Section is currently leading a national trial and enrolling patients in the Axogen Company's sponsored study: a multicenter, prospective, randomized, subject and evaluator blinded comparative study of nerve cuffs and advanced nerve graft, evaluating recovery outcomes for the repair of nerve discontinuities. Dr. Stephanie Thibaudeau, along with principle investigator David Steinberg, was awarded a grant from the Bach Fund for a blinded randomized control trial to compare Tylenol 3 versus ibuprofen/acetaminophen for pain control and patient satisfaction after ambulatory hand surgery. Our hand fellows this year have been outstanding. Stephanie Thibaudeau is completing the second of a two year fellowship with the second year sponsored and supported by ASSH. We were only one of a handful of hand fellowships in the country to be awarded this support. She will be returning to McGill's Division of Plastic Surgery as an attending physician. Nickolas Kazmers will join the full time orthopaedic faculty at the University of Utah this fall.

The past year saw several changes for the Orthopaedic Trauma Service including settling into a new home at Penn-Presbyterian Medical Center and the retirement of John Esterhai, Jr - an esteemed part of not only the trauma service but also of Penn Orthopaedics. The service continues to grow in terms of volume and breadth of practice. The faculty continue to work in all three missions with recent accomplishments including grant funding from PCORI and OREF. In addition, the faculty within the division are well represented at the national and international level as president of the state society, on the board for FOT and AONA, and chairing several different courses and meetings.

The Foot and Ankle Service expanded to Radnor. Daniel Farber serves on the AAOS board and is involved in the AAOS fellowship accreditation task force. Keith Wapner represented the department globally with international presentations in Brazil, Japan, Qatar, and South Africa and nationally at AOFAS and AAOS.

The Shoulder and elbow division continues to work in close collaboration with researchers from the McKay Research Laboratory help to form one of the largest, shoulder research laboratories in the world. The Shoulder and Elbow service has 6 active research grants in 2015, including NIH, Veterans Affairs, health system and the industry grants. The Penn shoulder and elbow faculty presented 8 abstracts at national meetings, giving 15 talks at international, national, regional and local meetings in 2015.

The sports medicine division has continued to grow with the addition of Dr. Ellen Casey to the faculty. Dr. Casey is an established researcher in the area of the female and athlete and will combine efforts with Dr. Kate Temme and Dr. Brian Sennett to develop the preeminent centers for the female athlete. The educational mission has continued with multiple national and international presentations in addition to the annual Penn Cartilage Symposium, directed by Drs. James Carey and Robert Mauck, and the Penn Throwing Symposium under the direction of Dr. John Kelly. The OREF awarded a prestigious New Investigator Grant to principal investigator Miltiadis Zgonis, MD. This grant provides funding to pilot several studies investigating novel strain transfer mechanisms within the meniscus and will add to our knowledge of how the native meniscus works within the body and better inform tissue engineered scaffold designs for meniscus repair or replacement. With respect to fellowship education, the Penn Sports Medicine Division has been extremely successful in attracting the best applicants to their fellowship and hosting national and international travelling fellows in this past year. Locally, the Penn Sports Medicine Division has also served as the leader in providing care to the running athlete under the direction of Drs. Rahul Kapur and John Vasudeven. Each year, they serve as the medical directors for the Tri-rock Philly Triathlon the Philadelphia Love Run Half-Marathon.

The spine division began a spine fellowship in combination with the Shriners Hospital of Philadelphia and they subsequently matched 2 fellows. Dr. Vincent Arlet was awarded the humanitarian award from the Duncan Tree Foundation for his outreach work in the West Indies.

The Pennsylvania Hospital Orthopedic Spine Center has become the region's premier referral center for complex spine reconstruction, emphasizing adult spine deformity. Pennsylvania Hospital is the first adult hospital in the region to use the EOS advanced imaging technology in order to tailor spine reconstruction to the needs of each individual patient. Dr. Arlet served on this year's scoliosis research society teaching faculty in Minneapolis and Dr. Harvey Smith received the award from ISMRM for the T2 mapping paper (published 2001) as one of the top 20 papers in the journal. Dr. Smith was also appointed to an FDA panel on orthopaedic devices. The division was responsible for continued clinical growth at PPMC, and fostered tissue-engineered disc replacement research (in collaboration with Rob Mauck). This work was recognized as one of the top basic science submissions at IMAST in May of 2015.

The Penn Adult Reconstruction Division and Penn Human Tissue Lab held the 3rd annual revision hip and knee CME course as well as the 3rd annual Orthopaedic Resident Training Initiative in collaboration with the International Congress for Joint Replacement. The 4th annual course will be held May, 2016. Atul Kamath was awarded the prestigious Hip Society Rothman Ranawat Traveling fellowship for travel March and April, 2016. The division published dozens of peer review publications and delivered several scientific presentations in the past year. Dr. Gwo Chin Lee was awarded the James Rand Award for clinical research at the November, 2014 meeting of the American Society of Hip and Knee Surgeons.

Under the direction of Dr. Kristy Weber, the Division of Orthopaedic Oncology hired two scientists that are focused specifically on sarcoma. Dr. Weber also developed a sarcoma advocacy group that runs annual events to raise money for sarcoma research. The footprint of the division expanded to CHOP in order to provide orthopaedic oncologic care to children with bone and soft tissue tumors (January, 2015). Dr. Alex Arkader was added to the CHOP faculty in September 2015, in order to assist Dr. Weber in orthopaedic oncology and provide expertise in pediatric limb deformity.

One of the important responsibilities of a chairman is to provide support for residents and faculty. As American healthcare changes and the challenges of constrained reimbursement continues, there are less dollars for the critical missions of education and research. Our development efforts have paid off over the last several years and are continuing. The generosity of the Biedermann family, the Hans Jorg Wyss foundation, Leonard and Madlyn Abramson Family Foundation, as well as the Cali and Weldon families have all been previously acknowledged and we are forever grateful. This year, thanks to the vision and generosity of Dr. Ed Ralston and his family, we were able to secure an endowed chair for Robert Mauck PhD, who was recently promoted to the professorial rank. I hope many of you will return to campus on September 22, 2016 to celebrate this professorship with Dean Larry Jameson and other leaders from the health system and school of medicine.

The greatest tribute a department can bestow on an individual is to name a lecture or endowment in honor of that person. At the 2016 AAOS alumni reception we welcomed many of you to a very memorable evening. We announced the creation of the Barbara Weinraub educational endowment. Barbara, her husband Michael, and her children attended this event. Barb spoke at this function, and reflected on her 38 years with the department of orthopaedic surgery. She was responsible for guiding and supporting over 200 residents through their Penn orthopaedic residency journey. I hope that as each of you read this-you will reflect on what Barbara did for you and your classmates, and acknowledge her generosity of heart and spirit with a pledge to honor her legacy. Although Barb has finally 'retired' she will maintain a vibrant presence in the department through the Barbara Weinraub educational fund.

We are all at different stages of our careers, and many of us have held different practice positions in different places at different times, depending on many factors. This year marks the retirement of two giants in history of Penn Orthopaedics. Bruce Heppenstal has retired after a terminal sabbatical, and John Esterhai is retiring this April. Both of these men have given their life to Penn Orthopaedics. Both have embraced education, the research mission and clinical excellence. They have both been leaders locally and internationally, and their work in trauma ("Hepp") and infection ("St. John" as he is affectionately and genuinely referred to) have made a difference in modern orthopaedic surgery. John's role at the VA hospital cannot not be overstated, and Bruce's dedication to residents and the HUP service was monumental. We wish both icons well and look forward to their continued participation in department functions and educational programs.

In addition to the changes we continue to make in the profile of Penn Orthopaedics in Philadelphia, our health system is growing at an exponential rate. Our purchase of Lancaster General Hospital and Chester County Hospital has allowed us to broaden our reach within Pennsylvania, and we recently recruited our first faculty member to Cape Regional Hospital in New Jersey. Kevin McHale is joining our sports medicine faculty after completing Penn orthopaedic surgery esidency and a sports fellowship at the Massachusetts General Hospital. We intend to extend our Penn service line to other health care entities in our system, and will be meeting with our colleagues from LGH this April to discuss orthopaedic quality and safety initiatives across our system. This spring I traveled with Dean Larry Jameson to Monaco where we were hosted by Prince Albert. Penn Orthopaedics is "going global" to provide consultancy and guidance for international patients. A Penn Orthopaedic directed conference will be held in Abu Dhabi next February at the request of the emirates. This is exciting for our team and further expands our horizons. We continue to engage in humanitarian outreach. Dr Jaimo Ahn traveled to Africa this spring and is exploring service and educational opportunities for our residents in underserved areas of the world. In addition, Dr. Neil Sheth has been actively engaged in developing orthopaedic outreach in Tanzania, and in concert with the Leonard Davis Institute is developing plans to build a dedicated orthopaedic hospital. Penn Orthopaedics gives back locally and globally. A great source of pride for all of us.

Our future has never been brighter. This spring I was

privileged to deliver the AOA address to the medical students. The title of my lecture was: Your Next 35 Years: observations and lessons learned. I discussed the impact of Osler, Halsted, and my mentors in surgery and how this affected my career pathway. We are truly blessed for the opportunity to provide care, discover new knowledge, and to teach the next generation the skills and principles of orthopaedic surgery. We are planning a celebration of our Penn orthopaedic legacy this September 16-18, 2016. I urge all of you to return to Philadelphia to come see what we are doing and to share with us what you are doing in practice and beyond. I welcome your comments to this narrative and as always, appreciate your continued support and enthusiasm for our department.

Service Line Strategy: Procedures to Pathways

Lori Gustave & Rachel Kleinman

Introduction. The health care system is rapidly evolving in an attempt to broaden coverage to more patients while reducing costs to the federal and state agencies, commercial insurers, and providers. There are several initiatives focused on this objective, attempting to anchor reimbursement to the quality and efficiency of an episode of care, rather than the volume of procedures done and tests performed.

In the past five years, Penn Medicine has been working to get ahead of this transformation through the organization of key service lines that work across the continuum of care.These service lines include: Cancer, Heart & Vascular, Neurosciences, Musculoskeletal & Rheumatology, and Women's Health.

The Musculoskeletal and Rheumatology (MSKR) Service Line is a strategic partnership between Orthopaedic Surgery, Rheumatology, Physical Medicine and Rehabilitation, Family Sports Medicine, Pain Medicine, and Radiology. Through this collaboration, we have organized disease teams that manage patient care pathways throughout the health system. Figure 1 shows the disease teams that have been designed to manage the care pathways, listed in order of implementation.

Figure 2 highlights the priorities of the MSKR Service Line. All strategies and initiatives for the service line are designed within the context of this framework. The development of patient pathways is the core of all of the strategic goals, and it is supported by four primary efforts: improved quality and value, regional integration, profitability, and alignment.

Patient Pathways. The central theme is the management of patients through defined pathways. Pathways map the current state process of a patient's experience from initial presentation through recovery. Opportunities for improvement are highlighted and an optimal map is designed. A multidisciplinary group of physicians, nurses, therapists, social workers, and administrators meet regularly to review a sample of patients and their experience on the pathway. Through each case review, processes are refined and systems are developed to move towards an ideal state. Figure 3 shows an example of our geriatric hip fracture pathway and revisions resulting from a multidisciplinary collaborative effort.

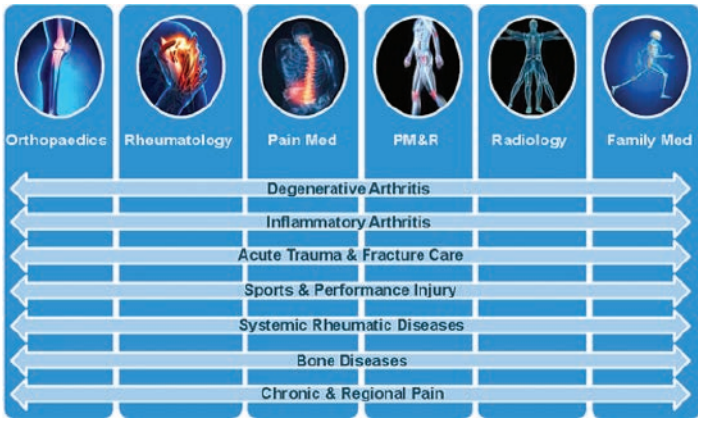


Figure 1. Moving From Departments to Diseases. Departments participating in the MSKR serviceline are listed across the top and the multi-disciplinary disease-teams" are listed vertically.

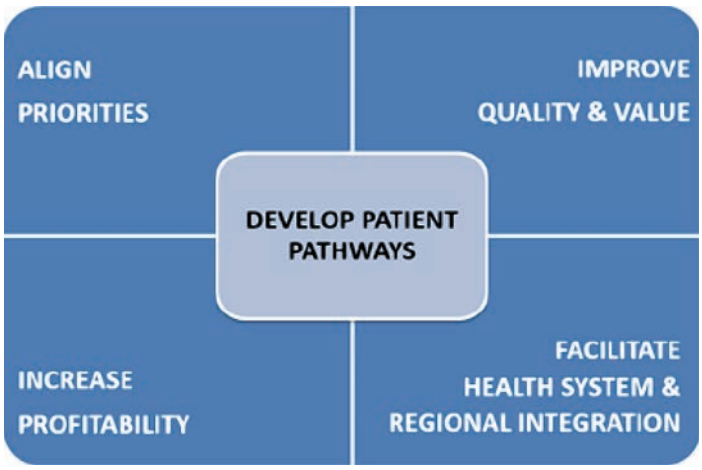


Figure 2. MSKR Service Line Goal Framework.

Improvement of Quality & Value. Some of the most significant national attention given to payment reform focuses on bundled payments. Penn Medicine has been participating in the CMS Bundled Payments for Care Improvement (BPCI) Program for just over two years. Primary hip and knee replacement, managed out of the degenerative arthritis disease team, is currently the most significant bundle for Penn Orthopaedics. Since 2014, the team has seen vast improvements on inpatient length of stay and readmissions. Now the team is focused more narrowly on the post-acute pathways for patients, partnering closely with local skilled nursing facilities (SNFs) and home care agencies.Together, this group creates pathways that delineate which patients can go home safely as well as identify length of stay guidelines for participating SNFs.

Beyond payment reform, there is an increasing environment of consumerism. More and more, patients choose their care providers based on reputation, outcomes, and cost. One driver of consumerism is the rise in higher co-pays and deductibles for patients. Higher out-of-pocket costs heighten patient awareness of what "products" are available in the market and increase their expectations of their experience. Consequently, we can no longer rely on our outstanding high quality care; we have to provide the service edge. Patient pathways give us an important patient-focused framework to think about how people interact with the health care system and what

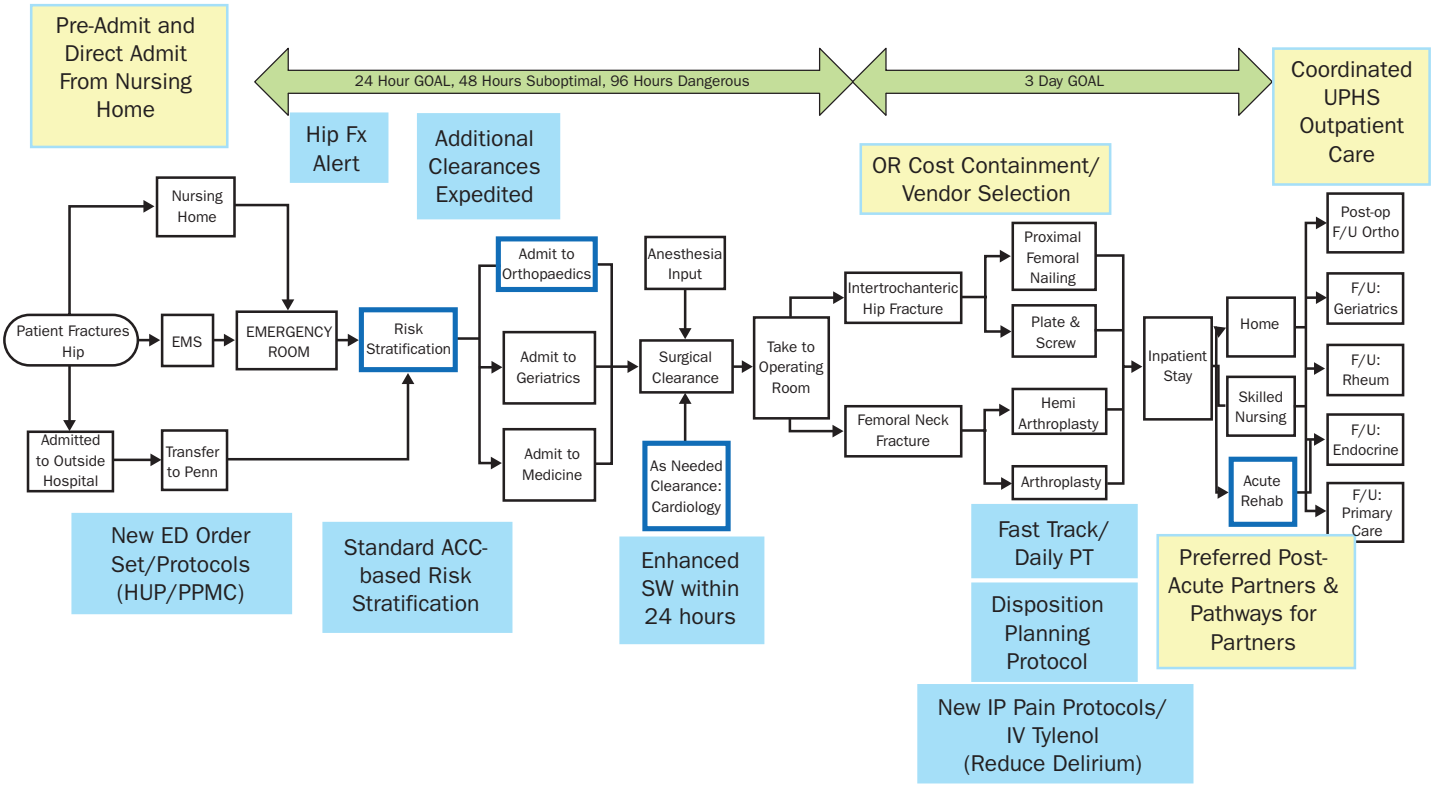


Figure 3. Geriatric Hip Fracture Pathway. This map serves as the guide for improving the geriatric hip fracture patient experience. The flow chart represents the patient's steps (highlighted boxes showing refinements). The comment boxes are improvements implemented to date and existing opportunities.

they expect of their providers. Pathways are not necessarily "evidence-based", they are more practice and experience based but normalized across providers for the ultimate quality and patient outcome experience.

Increase Profitability. In addition to new payment models, health reform is driving reductions in payments and therefore overall revenue. As such, the health system has renewed focus on decreasing unnecessary costs to improve overall margin and allow for programmatic and facility reinvestment. The service line is focused on variations in practice among both physicians and hospitals. From implants to sutures, unit costs are analyzed, identifying savings and reducing waste. Examples of cost improvements made through this initiative include: standardization of high volume surgical trays to reduce sterilization costs and labor; standardization of implants to improve pricing of high ticket items; and evaluation of the efficacy of specialty products.

Facilitate Health System and Regional Integration. Another national trend is the consolidation of hospitals and existing health systems into large regional health systems. Over the past three years, Penn Medicine has acquired one new suburban hospital and one community health system, growing the health system to five acute care hospitals in all. Pathways provide a vehicle for organizing and integrating care across disease populations within the health system and beyond, especially given the level of growth and expansion that we have experienced lately. It also facilitates the circulation of best clinical and operational practices among entities.

Align Priorities. Due to the multidisciplinary nature of pathway management, priorities of different departments must be aligned to achieve a common goal. The MSKR Service Line has a shared incentive plan that benefits all participating departments. The incentive is designed to financially benefit departments directly as well as create a central pool managed by the service line executive committee for investment in service line priorities.

Conclusion. While we have planned for a major shift in health care delivery and reimbursement, there are still quite a few unknowns. There is little certainty in how quickly CMS and private payers will move to more value-based payments or the extent to which it will impact some of the high acuity care we provide. Additionally, consumers continue to demand price transparency, on-demand access to providers, and more agile use of their own medical records. We have adopted the service line strategy and disease based pathways as one viable way, and no-regret move, for us to advance our patient centered care.

Letter from the Program Director

Craig L. Israelite, M.D.

Individually and collectively as a department, we are driven to make a demonstrable difference in the education of orthopaedic residents at the University of Pennsylvania. The education of residents and fellows has been, and continues to be, one of the greatest strengths of the Department of Orthopaedic Surgery. It is with continued pride and gratitude that I continue to serve as Program Director of one of the finest orthopaedic programs in the country. It is only with the exemplary efforts of our Chairman, Dr. L. Scott Levin, M.D., Co-Program Directors, Samir Mehta, M.D., and Jaimo Ahn, M.D., Ph.D., and the outstanding faculty that our program continues to fluorish. As a testament to our national position, we receive over 800 applicants annually for our eight, hightly coveted and competitive, positions. The committment to resident education continues to be emphasized at the highest levels from our Chairman, Vice-Chairs, Division Chiefs and faculty. It is truly a team effort and has led to the continued academic success of the department.

This last year has been a year of transition within our department with the change of Program Coordinator after Barbara Weinberg's retirement. Shanna Kurek has filled the role quite successfully and has made significant improvements in all aspects of the residency program. Her enthusiasm, knowledge and committment has helped transform the residency to a well-run and disciplined program. We can only hope that Shanna has the same longevity as Barbara, as I am sure her legacy will be as outstanding.

Currently, there are 42 residents within the department. There are eight new residents which matriculate each year, of which two residents spend an entire year doing full-time research between their post-graduate two and three years. The residents continue to rotate at the University of Pennsylvania Health System locations which include Hospital of the University of Pennsylvania, Penn-Presbyterian Medical Center, and Pennsylvania Hospital. Additionally, strong rotations at our V.A. Hospital, Children's Hospital of Philadelphia and Bay Health Community rotation continues to be very successful. In addition, several of our residents continue to participate and are encouraged to pursue global outreach health programs.

While our affiliations are large and diverse, our department continues to strive for a balanced and well-structured core curriculum. The curriculum is run on a two-year cycle and covers all areas of our specialty. Grand Rounds are required and take place every Thursday morning with four continuous hours of protected educational time. Additionally, each subspecialty delivers at least one academic didactic session each week. These morning conferences are comprised of faculty within the division, fellows, residents and students, both from the University of Pennsylvania and visiting from other medical schools throughout the country. These lectures are reviewed, critiqued and discussed with each division chief in order to maintain updated goals and objectives for each session.

In addition to our core academic mission in educational activities, this robust program is further enhanced by numerous additional opportunity. The Visiting Professor lecture series occurs each month and are sponsored and named lectureships. The lectureships are comprised of the most reknowned national and international faculty. In addition to visiting faculty, our residents are encouraged and supported to attend numerous off-site courses to enhance their learning each year. While the faculty are talented and motivated individuals and help steward our residency, it is really the residents who help continue to make daily changes and suggestions. I believe we have one of the best graduate medical education committees in an orthopaedic program. This highly functioning and motivated group consists of the Chairman, Program Directors, faculty as well as two members of each resident class who are elected by their peers. The program can, therefore, constantly review current situations and respond with ease to any needed changes. Many of our new programs have been initiated by resident suggestions during these meetings.

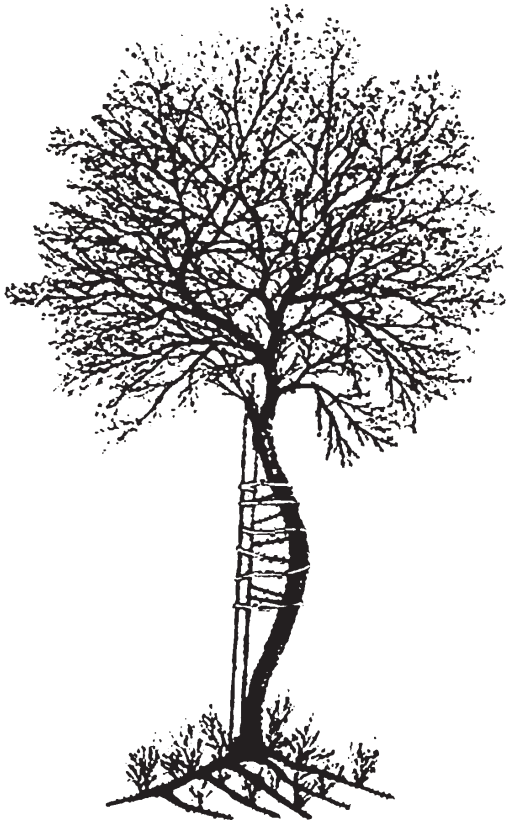
One of the suggestions which have come to fruition is the beginning of a standardized intern skills month. Under the leadership of Drs. Nicholas Pulos, as well as Nicole Zelenski, this month-long skill session endoctrinates our interns into the practice of surgery and orthopaedic surgery. Many faculty and upper level residents participate in activities ranging from knot tying to fluoroscopic guidance of instrumentation. Lectures include every subspecialty of the orthopaedic department and, as a testament to continued faculty and resident involvement, there has been no difficulty finding volunteers to give up their academic and clinical practices to spend a day with our new valuable interns.

As in all things in life, change is constant. Under the ACGME rules, strict guidelines which document mentorship must be implemented. We have created a Clinical Competency Committee at the University which semiannually reviews all orthopaedic residents. In addition to their milestone achievements, we review their academic record, research activities, and citizenship metrics. We are thus able to monitor and submit in a timely fashion to the accreditation facilities and, thus, have continued to be granted full accreditation in orthopaedics.

While our residency continues to be clinically robust, it is another testament to the depth and enthusiasm of our department that numerous grants have been awarded. These include institutional, national and industrial support grants. This allows us to support our visiting professor programs and helps in the training of our current orthopaedic residents. Our residents continue to present at local, state, national and even international meetings. Numerous publications are produced each year from all of our residents and culminate with our Research Day in June of each year. This is an outstanding program which gets better every year under the guidance of Dr. Louis Soslowsky and Dr. Jaimo Ahn.

While there are many metrics used with respect to the quality of an orthopaedic program, perhaps one of the greatest is the success of the graduating residents into fellowship programs. This year is no exception with all eight of our residents receiving their top choices for fellowships at very competitive programs. Please congratulate our graduating chief residents, Dr. Stephen Liu, who will be doing a Hand Fellowship at the University of Pittsburgh, Dr. Andrew Milby, who will be doing a Spine Fellowship at Emory University, Dr. Michael McGraw, who will be doing his Sports Fellowship at Hospital for Special Surgery, Drs. Christopher Melnic and Paul (Max) Courtney, who will both be doing Joint Fellowship at Rush University, Dr. Sarah Yannascoli, who will be doing her Hand Fellowship at Washington University, Dr. Nicholas Pulos, Hand Fellowship at the Mayo Clinic, and Dr. Jonathan Slaughter at the Mary S. Stern Hand Fellowship in Cincinnati. All of these individuals have our deep gratitude, and we expect outstanding careers as they have demonstrated their great potential where here at our program.

Finally, I can assure you that the success of our mission will continue as we have eight outstanding newly matriculated orthopaedic interns who have already demonstrated outstanding knowledge and skills during their internship year. I look forward to communicating with you next year as our future continues to grow and brighten with every year.

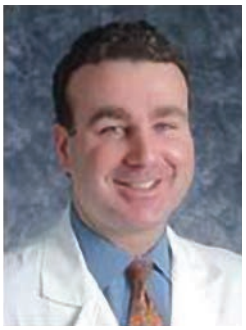


Arthroplasty

Arthroplasty Staff

Charles Nelson, MD

Craig Israelite, MD



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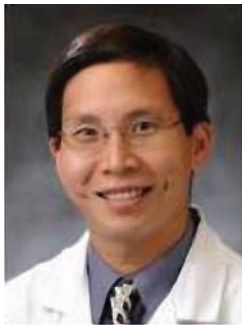


Gwo-Chin Lee, MD

Hip and Knee Arthroplasty Bundle Experience at the University of Pennsylvania, Department of Orthopaedic Surgery

Eric L. Hume, MD, Laura L. Kosseim, MD, Atul F. Kamath, MD,
Joanne J. Piscitello, MSN, RN, Finnah L. Pio, MS

Introduction

Hip and knee arthroplasty are superbly successful surgical procedures[1]. However, with increasing numbers of arthroplasties come increasing burden of failures. The cost of hip and knee arthroplasty is already a major expense for the Center for Medicare Services (CMS). In 2014, Medicare spent more than $7B on 400,000 arthroplasty patient hospitalizations alone. Kurtz *et al* predicts a large increase of number of hip and knee arthroplasty and revisions between 2005 and 2030[2]. The number of total hip arthroplasties will grow 174% to approximately 272,000 per year by 2030, and total knee arthroplasty will grow 673% to 3.4 million procedures per year by 2030. Hip revisions are predicted to double by 2026 and knee revisions to double by 2015.

Hip and knee arthroplasty cost is a major focus for CMS to evaluate payment models to improve quality and to limit costs. CMS describes the variability of arthroplasty care provided in the U.S. as measured by 1) the rate of complications such as infection or reoperation, 2) the threefold readmission rate variation and 3) expenditures ranging from $16,500 to $33,000 across regions.

Reduced variability should improve value. Value is a measure of higher quality and lower cost. Within the present bundle models, the goal is a lower rate of readmission for complications and a lower rate of post-acute care in expensive locations. Lower readmission rates will lower CMS value based purchasing penalties to UPHS. The major focus for successful bundles under CMS in the Comprehensive Care for Joint Replacement Model (CJR) is to send patients home safely. Home care will lower post-acute care costs and safely lower readmission rates.

Background

UPHS started the Bundled Payment for Care Improvement Initiative (BPCI) on January 1, 2014. Figure A shows our present involvement. Our awardee convener is Remedy Partners, who provides support, data aggregation and reconciliation for the UPHS system. The convener shares risk and benefit with the system; they offer some protection against the cost of failure and share the benefit when the bundles are successful.

In BPCI Model 2, actual expenditures from the day of surgery to 90 days postoperatively are reconciled against a target price. The target price is an adjusted average of the cost history of reimbursement for the hospital system. The CMS definition of actual pricing of any single bundle at any single hospital changes from quarter to quarter with two basic adjustments compared to the historic baseline October 2009 - September 2012. CMS looks at the national trend of non-BPCI participating hospitals in order to avoid penalizing/rewarding BPCI hospitals for broader changes in the care of patients. This cost trend should reflect technology/implant changes and other broadly applicable changes. As each bundle contains multiple diagnosis related groups (DRG) (e.g. with and without complications), the pricing is updated every quarter for the current hospital-specific trend in the changing mix of DRGs within the bundle. CMS.gov/BPCI describes the active BPCI locations. As of January 1, 2016, there are 1574 participants in Phase 2 including 409 acute care hospitals and 288 physician group practices.

- **CMS Bundled Payment Care Initiative (BPCI)**
  - Fractures of Hip, Femur or Pelvis – PPMC, PAH
  - Hip & Femur procedures except major joint - PPMC
  - Major Joint Replacement of lower extremity – PPMC, PAH
  - Revision of hip or knee – PPMC, PAH
- **Horizon Blue Cross NJ**
  - Major Joint Replacement of the Knee – PPMC, PAH
  - Knee Arthroscopy – PPMC, PAH
- **Independence Blue Cross Orthopedic Bundled Payment and Shared Savings Program**
  - Hip and Knee Replacement – PPMC, PAH

Figure A. UPHS orthopaedic surgery bundles.

To be successful, physicians, clinical colleagues, and administrative planners must be actively involved in managing patient preparation before admission, during the hospital stay, and for 90 days after discharge from the acute care hospital. This collaboration occurs within the UPHS system and with preferred provides outside the system. Our internists risk stratify and recommend care before the patient is admitted. Social workers, clinical resource management, and the home health care team support patients to be identified for safe discharge to home. Collaboration with Skilled Nursing Facilities (SNF) and Inpatient Rehab Facilitates (IRF) benefit the patient who needs inpatient rehabilitation.

Our Risk Stratification experience started January 1, 2012 for acute care hospital postoperative safety. Based on the characteristics of the intensive care unit (ICU) patient, we generated a preadmission risk tool to predict unplanned ICU need. As planned, risk stratification successfully lowered the rate of rapid responses, unplanned ICU admissions and mortality. We have observed that the risk stratification tool also predicted risk for readmission. The goal is to develop actionable guidelines that will impact readmission rate directly.

Value

Because the value equation is higher quality at a lower cost, we can impact value by improving either parameter or both. One key strategy is reducing variability. Improvement can be evaluated by process metrics. The realization of cost savings awaits cost collection and reconciliation available long after the process metrics predict the cost outcome. Therefore the process metrics are important to manage care that can be validated only when cost data becomes available.

Pre-acute:

The surgical decision between the physician and the patient is based on risk benefit perception. Some disease risk can be mitigated by preoperative guidelines. Tobacco addiction is a modifiable disease. Smokers have clear risk for poor wound healing, increased risk of venous thromboembolism, and long-term health problems. Smoking cessation should be a part of the preoperative preparation and is reinforced by our internal medicine consultants and arthroplasty class. Diabetes is also a modifiable disease. All diabetics are at higher risk of infection and perioperative complication, but the poorly controlled patients are at the highest risk. We delay surgery for a hemoglobin A1C of 10% or greater and refer to an endocrinologist. For an A1C between 8% and 10%, patients are contacted by a pharmacist for individual education. We delay surgery if fasting blood sugar is >200mg/dL on the day of surgery. Body Mass Index (BMI) above 40 increases risk of wound and other complications. While it is reasonable for a surgical procedure for a patient of a BMI above 40 to be delayed pending bariatric care, many patients are unable to significantly change their BMI. Patients should understand that BMI is a modifiable risk. Weight loss not only lowers surgery risk, but also lowers long term risk for heart disease, hypertension, and diabetes. Poor nutrition increases surgical risk. Albumin can reflect nutritional status but we have found that low albumin often predicts chronic disease such as liver disease, renal disease, and others. Hypoalbuminemia of chronic disease is not correctable but is a marker of risk perioperative infection and complication.

Chronic diseases may not be modifiable. An accurate description of risk for patients with chronic renal disease and chronic liver disease continues to be elusive. A patient with a low creatinine clearance or a high MELD score should be counseled about the risk of their underlying disease as best as possible. In patients with CKD stage 3 or greater, we avoid use of nonsteroidal anti-inflammatory medicine and hold all potentially nephrotoxic medications, including ACE inhibitors, angiotensin receptor blockers and diuretics. Low albumin and coagulopathy of liver disease are important markers of surgical risk. Patients who have a successful renal transplant appear to have an improved preoperative risk over the patients who are being chronically dialyzed. Chronic viral infection patients should have disease markers and viral loads evaluated. Patients on biological therapy for autoimmune disease are counseled to stop them under the supervision of their rheumatologist.

If a patient with a chronic disease is well-managed, the patient's decision to proceed with elective surgery has always been based on the patient's perceived risk/benefit ratio. Considering the bottom line cost for above high risk disease burden, physicians or systems will increasingly be put in the role of gate-keeper in the decision for access to elective surgery for risky patients. Is there a threshold of risk that would appropriately deny elective surgical procedure outside the patients' willingness to accept risk? This answer may come from our society's willingness to accept the excess cost of health care for the high risk patient.

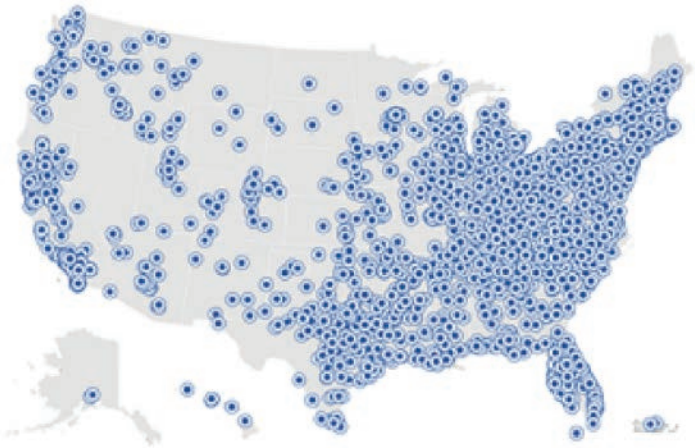
Acute Care:

Hospital programs must be in place to manage the patients based on pre-acute planning and also based on postoperative inpatient progress. The Risk Assessment and Prediction Tool (RAPT) may predict home discharge but hospital preparation must support the predicted plan. The inpatient work of the nurse and physical/occupational therapists can be amplified by a robust collaborative mobility plan. Daily processes and benchmarks are set and monitored for compliance. If processes are met, we expect lower length of stay and increased rate of discharge home safely, lower SNF discharge rates and lower readmission rates. Medical co-management discharge planning can be developed around risk predictors for higher readmission rate. Handoffs to post-acute care are an important component. Iorio *et al* published their acute care pathway with detail[3].

Post-acute care for 90 days postop:

Our post-acute location-of-care cost data suggests where to find value (Figure B). The home environment with physical therapy and occupational therapy and skilled nursing as needed provides both decreased cost and improvement in quality for appropriate patients. Our rate of discharge to home is lowering the regional and national averages. For MSKR BPCI bundles, our SNF average has a cost just under four times higher than our home health care average. Work with Penn Center for Continuing Care (PCCC) shows the benefit of active collaboration. PCCC, compared to the average MSKR BPCI for all SNFs, is half the cost, largely due to almost half the length of stay but also a lower readmission rate.

The IRF offers value for the patient with acute care needs. For our MSKR BPCI average, the inpatient rehab facility cost is about 50% higher than the average SNF stay with a readmission rate equal to SNF. Inpatient rehab facilities offer value for the patient need higher level of care (Figure C).



Source: Centers for Medicare & Medicaid Services

Figure B. CJR sites, as of January 1st, 201.

Post Acute Provider	Number of Episodes	HHA Cost Per Episode	Avg # of Visits	Episodes with a Readmission
HHA average	199	$3,298	15.1	10.05%
Penn Care At Home	111	$3,400	15.4	9.91%

Post Acute Provider	Number of Episodes	SNF Cost Per Episode	Average Length of SNF Stay	Episodes with a Readmission
SNF average	370	$13,247	21.6	20.00%
PPCCC	135	$6,130	12.7	17.78%

Post Acute Provider	Number of Episodes	IRF Cost Per Episode	Average Length of IRF Stay	Episodes with a Readmission
IRF average	69	$18,376	12.6	21.74%
Penn Rehab Unit	28	$16,530	13.5	17.86%

Figure C. Remedy Partners reported CMS PPMC MSK Q4 2013—Q3 2015 Post-acute location of care.

To predict safe home discharge, RAPT score predicts SNF need. Prehab PT visits are also potentially important so patients are better prepared for surgery physically and better prepared about the home. Literature for the prehab demonstrates its potential usefulness[4].

Readmissions are a marker of low value: both low quality and high cost. Readmission rate from home is lower than the readmission rate from an inpatient post-acute facility. Higher readmission rate from SNF's and inpatient rehab facilities is partially related to higher risk patients. Our experience is that increased rate of discharge to home has maintained unchanged readmission rate from home.

Clinical guidelines for evaluation before readmission, such as the hot joint protocol for septic arthritis, or SIRS and VTE guidelines have lowered "unnecessary" readmission. Workups can be done as an outpatient or in the emergency room.

Process metrics can show variability that may or may not be of benefit. The quality improvement/process improvement is iterative. Metrics should be **SMART**: Specific—target a specific area for improvement, **M**easurable—quantify or at least suggest an indicator of progress, **A**ssignable—specify who will do it, **R**ealistic—state what results can realistically be achieved, given available resources, **T**ime-related—specify when the result can be achieved. Quality improvement with process metrics is well-documented in the industry and has become accepted within medicine and should be useful in medicine to lead to improved outcomes.

The antidote to the "NEVER" event are "ALWAYS" events. The goal to never have postoperative infections is appropriate, but unreachable. Focus on the "always" events supports active reduction of variability. Always do best-practice skin preparation before surgery, always give the right antibiotics at the right time and always provide appropriate wound care after surgery. "ALWAYS" processes should be SMART.

Conclusion

The home safely idea has recently become a central concept in total joint arthroplasty. Prepare the patient before admission for surgery, prepare for discharge to the best value post-acute care location while in the hospital, and collaborate with the post-acute care based on the value metrics of both quality and cost. The safety component has been the evaluation of unnecessary readmissions. Programs such as our Hot Joint Program were developed when we recognized that some patients admitted for infection were unnecessarily admitted for an infection workup. Careful review of readmissions for opportunity should a central activity.

Our bundle work has been driven by value. Quality and cost improvements often overlap. Reduce variability and always apply best practices to improve patient quality and safety. Savings will reward successful implementation. Participation in bundles, initially driven by cost, has led to important improvement in quality and safety.

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Foot & Ankle

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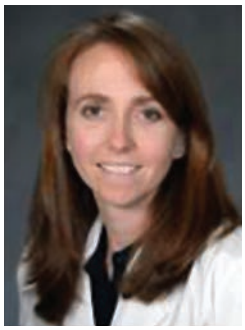

Kathryn O'Connor, MD, MSPT

Department of Foot & Ankle Update

Daniel Farber, MD

The Foot and Ankle service continues to grow with the addition of our newest faculty member, Kathryn O'Connor MD, who completed her fellowship at Washington University at St. Louis. Our growth has allowed us to expand our advanced foot and ankle services from Center City to University City and to the Main Line. We now have four physician assistants to better serve our patients needs and expand our abilities as the city's premier foot and ankle care team. Over the past year, we have seen 16,602 patients and performed over 1,029 surgical procedures.

From a research perspective, our division has been exploring the capabilities of the region's only weight-bearing CT scanner as well as continuing many other projects. We are forging relationships with the Human Motion Laboratory and the Biedermann laboratory to take full advantage of Penn's impressive resources. Finally, we continue to be involved in the basic science pursuits of the McKay Lab, attempting to answer many of the difficult questions surrounding Achilles tendon ruptures and their optimal treatment.

Our faculty also serve the Orthopaedic community as whole. Dr. Daniel Farber continues in his role as a board member of the AAOS and Chair of the AOFAS fellowship curriculum task force. Dr. Keith Wapner has presented nationally and internationally, including Brazil, Japan, Qatar, and South Africa and was recently elected to the AOFAS nominating committee. Dr. Wen Chao is an Orthopaedic Consultant to the Pennsylvania Ballet.

Hand

[Staff

David Bozentka, MD

David Steinberg, MD

L. Scott Levin, MD, FACS

Benjamin L. Gray, MD

Robert B. Carrigan, MD

Department of Hand and Upper Extremity Update

David Bozentka MD

It has been a historic year for the Hand and Upper Extremity Section of the Department of Orthopaedic Surgery in the Perelman School of Medicine at the University of Pennsylvania. Dr. L Scott Levin MD, FACS, led a team of pediatric and adult hand and microvascular surgeons to perform the world's first pediatric bilateral hand vascularized composite allotransplantation. The eight year old child Zion Harvey was the recipient. Zion, a quadramembral amputee due to sepsis, is on life-long immunosuppression therapy for a kidney transplant. After 18 months of planning the hand transplant team developed the surgical protocol through multiple training sessions and cadaveric trials. The collaboration of 40 surgeons, anesthesiologists, nurses and support personnel were required to coordinate the 11 hour procedure that took place at Children's Hospital of Philadelphia. With the extensive preparation the procedure proceeded smoothly as planned. Post-operatively Zion is progressing well while undergoing an extensive hand therapy program. It is expected that Zion's hands will grow normally over time as he is followed closely for evidence of rejection.

The hand surgery section has expanded as Dr. Benjamin Gray MD has joined the group. After his orthopaedic surgery residency at Washington University in St. Louis, Dr. Gray trained under Dr. Peter Stern MD at the University of Cincinnati for his hand surgical fellowship. Dr. Gray has quickly become an integral component of the department with a rapidly expanding clinical practice based at Pennsylvania Hospital, a keen interest in clinical research and strong dedication to teaching. Dr. Gray is currently pursuing a Masters of Science in Clinical Epidemiology. The experience will allow Dr. Gray to better develop his focus on out-comes research.

Under the direction of Dr. David Steinberg, the hand surgical fellowship also has expanded. The Accreditation Council for Graduate Medical Education (ACGME) has approved a second full time hand and microsurgical fellow. Our current fellows have been a remarkable asset to the section and will continue their academic pursuits when they finish their training. Dr. Stephanie Thibaudeau is completing her second year of fellowship and will return to McGill University to take a position in the Department of Plastic Surgery where she performed her residency training. Dr. Nikolas Kazmers, who completed his orthopedic surgery residency at Washington University at St. Louis, has accepted a position on the hand surgery service at the University Of Utah School Of Medicine in the Department of Orthopaedic Surgery.

The second Penn Flap course for hand and microsurgery fellows and residents will take place July 9th and 10th at the Penn Orthopaedics Human Tissue lab. Local, regional and free tissue transfers will be reviewed during the two day course. In addition to soft tissue reconstruction, faculty from across the country will review nerve transfers and brachial plexus procedures with didactic lectures combined with cadaveric dissection.

The hand research program is expanding with the support of Annamarie Horan PhD, Colleen Pellegrini and Kara Napolitano. The service is currently involved in the NIH funded study "A clinical trial for the surgical treatment of distal radius fractures in the elderly." In addition, Dr. Levin is the principle investigator for the multicenter Axogen-sponsored study: A Multicenter, Prospective, Randomized, Subject and Evaluator Blinded Comparative Study of Nerve Cuffs and Advance Nerve Graft Evaluating Recovery Outcomes for the Repair of Nerve Discontinuities. Dr. Levin will oversee the fifteen participating sites throughout the country. Dr. Stephanie Thibaudeau MD with principle investigator David Steinberg MD was awarded the Bach Fund for the blinded randomized control trial to compare Tylenol 3 versus Ibuprofen/Acetaminophen for pain control and patient satisfaction after ambulatory hand surgery. The group is actively enrolling patients at this time.

The hand service hosted two visiting professors. Dr. Andrew Weiland MD, Professor of Orthopedic Surgery and Professor of Surgery (Plastic) at the Weill Cornel Medical College, discussed the evolution of the treatment of distal radius fractures and led a cadaveric dissection of thumb cmc arthroplasty and distal biceps repair. Dr. James Chang MD, the Johnson & Johnson Distinguished Professor and Chief of the Division of Plastic and Reconstructive Surgery at Stanford University, was the Leo Leung lecturer. During the combined plastic surgery and hand surgery grand rounds, Dr. Chang provided a captivating discussion of hand surgery and the art of Rodin. He later led cadaveric demonstration of radial forearm facial and post Interosseous flaps.

As this remarkable year comes to a close, it is exciting to consider the bright future in store for the hand surgical section.

The First Bilateral Pediatric Hand Transplant

Colen D, Thibaudeau S, McAndrew C, Levin S

Since the first successful hand transplantation performed in France in 1998, the field of vascularized composite allotransplantation (VCA) has gained widespread acceptance as a means of reconstruction in upper extremity amputees. Until recently, hand transplants had only been performed on adult patients with 72 patients having received a total of 107 transplanted hand or arms over the past 17 years.[1] Under the guidance of Drs. L. Scott Levin and Benjamin Chang, the CHOP Hand Transplant Team was formed in the fall of 2013 in conjunction with the Shriners Hospital for Children to pursue the first ever hand transplant in a pediatric patient. After one and a half years of planning and just under 11 hours in the operating room, this goal became a reality. On July 7, 2015, Zion Harvey became the first child to receive a double hand transplant.

At the age of two, Zion was hospitalized with staph auerus bacteremia and sepsis resulting in fulminant renal failure and bilateral below knee amputations, left upper extremity transcarpal amputation and right transradial amputation. He underwent a successful renal transplant from his mother two years later. Zion, now 8, adapted well to his lower extremity prostheses, but unfortunately failed a trial of passive hand prostheses. After initial consultation with Dr. Kozin and Dr. Zlotolow at the Shriner's Hospital, Zion was referred to CHOP to determine his eligibility for a vascularized composite allotransplant.

Hand transplantation in a child carries significant ethical considerations. In addition to opportunistic infections, lifelong immunosuppression puts patients at significant risk of *de novo* malignancy compared to the general public (SIR = 1.4-3.6), with younger recipients even higher risk than their adult counterparts (SIR = 2.2-2.4) .[2,3] This risk has thus tempered much enthusiasm about VCA in pediatric patients. By virtue of his history of renal transplant, however, Zion was a uniquely qualified candidate, as the procedure and postoperative immunosuppressive regimen would not put him at increased risk for malignancy.

A multidisciplinary team was formed, including physicians from orthopedic surgery, plastic surgery, transplant surgery, pediatrics, cardiology, infectious disease, nephrology, anesthesia and psychology, as well as members from social work and physical and occupational therapy. Zion was evaluated by each member of the team prior to being listed for the transplant. Several rehearsals of the surgical procedure were held in the fresh tissue laboratory to achieve an efficient surgical protocol. CTs of his forearms were used to create 3D printed models and cutting guides which would be used intraoperatively to improve efficiency through precise osteotomies of the radius and ulna and accurate plate placement. 3D models of an upper extremity for a child of Zion's weight and stature were made to allow for appropriate size match at the time of procurement. After detailed informed consent, Zion was listed and to the surprise of many, a donor was found in a matter of 10 short weeks.

The procedure was divided into four parts with three surgeons on each team: one team to prepare each donor hand (2) and one team to prepare each recipient forearm (2). The patient was brought to the operating room and dissection of the recipient forearms was begun while the donor hands were simultaneously dissected with all of the neurovascular structures and tendons meticulously labeled. The 3D printed cutting guides were then used to make precise osteotomies and drill holes prior to plating. The donor hands were plated first and then brought to the patient where they were fixated to the recipient radius and ulna bilaterally. Ulnar arteries were anastomosed and the donor hands were reperfused to end ischemia time and to allow for venous egress to flush preservative solution prior to continuing with the reconstruction. Next, radial arteries were anastomosed along with the venae comitantes of both arteries, followed by repair of the median and ulnar nerves and the volar tendons. The hands were then pronated and the dorsal tendons and radial nerve branches were repaired. After closing skin, Drs. Levin and Chang notified Zion's family of a successful operation as the patient was brought to the intensive care unit to recover.

Revision of the right arterial and venous anastomoses was required on the first postoperative day due to an acute arterial thrombus. Zion's recovery was closely monitored by a multidisciplinary team, with weekly skin biopsies to monitor for signs of rejection during the first 12 weeks after his surgery. Despite low grade rejections (grade 1-2), Zion responded well with modifications to his immunosuppression regiment. Six weeks after his transplant, there was evidence of bone formation at his osteotomy sites. A protocol of intensive physical and occupational therapy was started early in his post-operative course to allow for tendon and nerve gliding Zion continues to engage in intensive physical and occupational therapy with continually improving function and sensation of his new hands. He can now perform activities of daily living with his hands including eating and writing.

This marks an exciting milestone in the field of reconstructive surgery. This goal could never have been realized without important contributions from many people, including the tireless work of the entire CHOP team and the diligent leadership of Dr. Chang and Dr. Levin. We must also thank the family of the donor who, despite their tragedy, found a way to help others. But most importantly, we owe the greatest gratitude to the unrelenting courage and exquisite character

of Zion and his family for trusting us with his care. We look forward to watching Zion grow into an amazing young man and learning from his experience as more children stand to benefit from vascularized composite allotransplantation.

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Pediatrics

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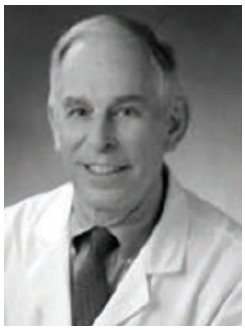
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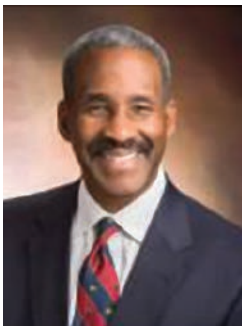
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Introduction

The Division of Orthopaedic Surgery at the Children's Hospital of Philadelphia (CHOP) enjoyed another year of significant growth, accomplishment, and innovation. Upholding our mission to provide the most comprehensive care to patients, we have continued to expand our clinical, research, and teaching programs. In 2015, *US News and World Report* ranked the Division of Orthopaedic Surgery 1st in the nation in pediatric orthopaedic surgery.

In 2015, CHOP Orthopaedics hired three surgeons, a sports medicine specialist and a non-operative orthopaedist, moved into three new facilities, renovated the Nicholson Visiting Professorship, appointed a new Director of Orthopaedic Engineering, hired a Director of Clinical Research, awarded Chair's research grants to six winners totaling $87,000, renovated the Fellowship recruitment and interviewing process, launched multiple Development initiatives with a new Board of Visitors for Orthopaedics and partnered with CHOP Office of Clinical Quality Improvement on two major projects (Sports and Spine) to improve the efficiency, safety and value of orthopaedic surgery.

This year marked the opening of a number of state-of-the-art facilities. In May 2015, the Division began seeing patients at the new Specialty Center at King of Prussia (Figure 1). The new center is comprised of 68 exam rooms, two operating rooms, an urgent care center, and the latest imaging equipment. The facility also houses a sports medicine gym and separate physical-therapy and occupational-therapy gym. With these resources, it serves as a "one-stop shop" for children and families in need of outpatient care. In addition to King of Prussia, CHOP orthopaedic physicians see patients at the new Brandywine Valley Specialty Care and Ambulatory Surgery Center that opened in October 2015 (Figure 2). The 44,000 square-foot facility, with an ambulatory surgery center and physical therapy gym, allows CHOP clinicians to see patients

Figure 1.

Figure 2.

Figure 3.

in Pennsylvania and northern Delaware closer to home. At our main campus, CHOP, orthopaedic faculty began seeing patients at the new Buerger Center for Advanced Pediatric Care in November 2015 (Figure 3). The building features a "children in motion" theme that uses images to promote a culture of wellness and activity. These new facilities across the greater Philadelphia area demonstrate CHOP's commitment to being a world leader in patient care.

This past year CHOP orthopaedic surgeons joined in a multi-surgeon team effort to perform bilateral hand transplant surgery for Zion Harvey's historic bilateral. Drs. Benjamin Chang, Ines Lin, and Robert Carrigan all held significant roles in throughout the surgery. They join other surgeons, CHOP physical therapists, social workers, and psychologists to support Zion in his care.

Clinical Program

Our orthopaedic faculty continues to expand and is currently comprised of thirty total providers, including nineteen specially trained pediatric orthopaedic surgeons

(fifteen operative and four non-operative), five pediatricians with sports medicine training, and three transition-to-adult care faculty.

CHOP Orthopaedics is pleased to announce the addition of four new providers: Dr. Patrick Cahill, Dr. Alexandre Arkader, Dr. Apurva Shah, and Dr. Danielle Magrini.

Figure 4.

Dr. Patrick Cahill (Figure 4) obtained his medical degree from the University of Illinois College of Medicine in Chicago, IL. He completed his residency in orthopedic surgery at Loyola University Medical Center and a fellowship in molecular genetics at the National Institutes of Health in Bethesda, MD. Dr. Cahill is currently a member of a number of spine-related multi-center study groups including the Chest Wall and Spine Deformity Study Group and the Harms Study Group.

Figure 5.

Dr. Alexandre Arkader (Figure 5) began his medical education in Brazil at F.T.E Souza Marques in Rio de Janeiro (medical school), Hospital of Santa Casa de Misericordia (internship in international medicine), and Hospital de Traumatologia-Ortopedia (residency in orthopaedic surgery). In 2003, Dr. Arkader moved to the United States to continue his training and completed a fellowship in pediatric orthopaedics at The Children's Hospital of Philadelphia and a second fellowship in musculoskeletal oncology at Memorial Sloan-Kettering Cancer Center.

Figure 6.

Dr. Apurva Shah (Figure 6) joins the Division of Orthopaedics after working as an orthopaedic surgeon at Boston Children's Hospital and the University of Iowa. Dr. Shah earned his medical degree from Columbia University College of Physicians and Surgeons and his MBA at Columbia Business School. He completed a residency in orthopaedic surgery at the University of Michigan and two Hand and Upper Extremity fellowships at Brigham and Women's Hospital and Boston Children's Hospital. Dr. Shah has been recognized as a "Young Leader" by the American Society for Surgery of the Hand.

Figure 7.

Dr. Danielle Magrini (Figure 7) obtained her medical degree from New York College of Osteopathic Medicine in Old Westbury, New York. She served as chief resident in her final year of residency in pediatrics at Robert Wood Johnson University Hospital in New Brunswick, NJ. She subsequently completed a fellowship in pediatric sports medicine at the Children's Hospital in Colorado in Aurora, CO. Dr. Magrini joined the Division of Orthopaedics after completing a second fellowship in non-operative pediatric orthopaedics here at CHOP.

In 2015, the department also saw significant growth in the mid-level provider staff. There are currently twenty-one nurse practitioners, seven physician assistants, and six athletic trainers who evaluate, diagnose, and treat a full range of musculoskeletal disorders.

Education Program

CHOP Orthopaedics currently funds four one-year clinical fellowships and one one-year research fellowship. The 2015-2016 clinical fellows are Evan Curatolo, MD (Figure 8); Lloydine Jacobs, MD (Figure 9); Sarah Nossov, MD (Figure 10); and Sheena Ranade, MD (Figure 11). Following the completion of their clinical fellowships, Dr. Curatolo will be returning

Figure 8.

Figure 9.

Figure 10.

Figure 11.

to his hospital of residency, Monmouth Medical Center in New Jersey, and joining a private group, Atlantic Pediatric Orthopaedics. Dr. Jacobs plans to serve the needs of a medically underserved community in the South by practicing the full scope of orthopedics - pediatric and adult. Dr. Nossov will pursue a career in general pediatric orthopaedics. Dr. Ranade will be focusing on sports medicine, complex hip, and lower extremity deformity in an academic medical setting. This year's research fellow is Mazen Ibrahim, MD from Egypt (Figure 12). While at CHOP Dr. Ibrahim has focused his research efforts in lower limb deformity and basic science research.

Figure 12.

The 2014-2015 clinical fellows have continued to practice and train across the country (Figure 13). Aristides Cruz, MD joined the faculty at Brown University Orthopedics, focusing on pediatric sports surgery. Peter Fabricant, MD MPH is completing a sports medicine fellowship at Boston Children's Hospital and plans to return to HSS. Andrew Georgiadis, MD joined Gillette Children's in St. Paul, Minnesota as a pediatric orthopaedic surgeon. Mark Seeley, MD is an attending surgeon at Geisinger Health System in Wilkes-Barre, PA. To celebrate the graduation of the 2014-2015 clinical fellows, the Division hosted the Nicholson Visiting Professor Program and Fellows Graduation & Reunion

Figure 13.

in June 2015. This year's Visiting Professor was Dr. Vince Mosca from Seattle, an internationally renowned pediatric foot and ankle expert (Figure 14, center). The program consisted of a mix of short lectures and discussion, a cocktail reception, and research and end of the year remarks from the four fellows.

Figure 14.

The Division also continues to host visiting scholars and provide the opportunity to observe clinical care of pediatric patients in a high volume, academic setting. Over the past year, the Division has hosted Dr. Kunbo Park, Assistant Professor of Pediatric Orthopaedic Surgery at Inje University Haeundae Paik Hospital in Busan South Kora, and Dr. Giuseppe Orlando, School of Medicine University of Messina, Messina, Italy.

Research Program

Basic Science and Translational Research

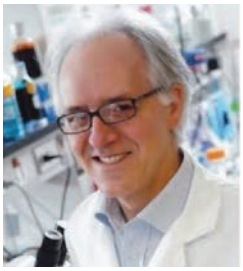


Figure 15.

This past year, our Orthopaedic Basic Research Program, led by Maurizio Pacifici, Ph.D. (Figure 15) has continued to make impressive progress and has generated new, exciting and far-reaching data and insights on basic and translational medicine aspects of skeletal biology and pediatric musculoskeletal pathologies. Our faculty members and their associates—including postdoctoral fellows, visiting scientists and research technicians - continued to tackle and fulfill the goals of several current NIH R01 grants, one Department of Defense (DOD) grant, one Muscular Dystrophy Association (MDA) grant and one Veterans Administration (VA) grant. These biomedical research projects which focus on basic cellular, biochemical and genetic mechanisms of skeletal formation and growth, also addressed the very complex tissue-tissue interplays that orchestrate the morphogenesis and three-dimensional organization of limb and craniofacial skeletal elements. The resulting data and insights continue to be used to uncover pathogenic mechanisms that subtend pediatric and adult conditions including Hereditary Multiple Exostoses (HME), Fibrodysplasia Ossificans Progressiva (FOP), Duchenne Muscular Dystrophy and other musculoskeletal pathologies. Work on HME was carried out under the supervision of Drs. Pacifici and Eiki Koyama and is supported by one of the RO1 NIH grants. This rare and orphan disorder is characterized by benign cartilaginous tumors that form at multiple locations in the growing skeleton of children. The tumors can often exceed a total number of 100 and thus, can cause a number of problems including skeletal deformities, growth retardation, chronic pain, interference with blood vessel and nerve function, and other problems. In 2 to 5% of the children, the benign tumors can become malignant and thus life threatening. Our clinical Division remains a major national and international center of diagnosis, care and surgical treatment for children affected by HME. Under the auspices of the NIH, we are actively engaged in understanding the cellular and molecular pathogenesis of HME, using animal models and cells in vitro. We have made much progress and have published a number of papers over the last two years that have revealed novel aspects of the pathophysiology of HME and have uncovered new and previously unsuspected possible targets of therapeutic intervention. To extend these basic research efforts and accelerate the pace of research toward translational medicine outcomes, our senior investigator—Dr. Paul Billings—has further developed new cell-based bioassays to screen chemical libraries and identify drugs able to correct the specific polysaccharide deficiency that causes HME. Such pharmacological treatment could be used in combination with surgical interventions to provide a more effective and comprehensive therapy for HME patients in the future. In a felicitous development, Drs. Billings and Pacifici received funding from the U Penn Center on Rare and Orphan Diseases that has permitted an acceleration and expansion of the research and a search for possible drugs using additional and more diverse chemical repositories. Together, these considerable investments of time, effort and funds are paying off in a considerable manner and have provided not only new insights into the mechanisms of HME pathogenesis, but also on how a basic polysaccharide deficiency could be corrected in this and related pathologies.

Studies carried out under the auspices of the Muscular Dystrophy Association (MDA) and led by another one of our faculty members—Dr. Masahiro Iwamoto - have made progress

toward testing an effective therapy to stimulate muscle tissue repair and/or decrease muscle cell death after trauma or in congenital conditions such as muscular dystrophies, using pharmacological treatments. Our faculty member Dr. Eiki Koyama joined forces with a faculty member in the CHOP Division of Plastic and Reconstructive Surgery—Dr. Hyun-Duck Nah—to understand the development and growth of the temporomandibular (TMJ) joint and to identify possible therapeutic means to treat TMJ osteoarthritis, a condition particularly common in women and quite debilitating. The data and insights stemming from their work led to the publication of several important studies, and all their work and dedication were rewarded by a new 5 year NIH RO1 grant they received last year. An equally important area of research led by another faculty member—Dr. Motomi Enomoto-Iwamoto - was supported by a R21 grant from the NIH and focused on tendon and ligament biology. These research efforts aim to find ways to stimulate structural and functional repair in those essential structures when damaged by trauma or overuse. Some of this work is carried out in close collaborations with members of the Department of Orthopaedic Surgery at Penn and in particular Dr. L. Soslowsky. Work by Dr. Enomoto-Iwamoto was also supported by a grant from the Arthritis Foundation to study a cell membrane component that affects the behavior and function of surface cells in articular cartilage, cells that are essential for the frictionless movement of the joints and are responsible for the production of key lubricating macromolecules including hyaluronate and phospholipids. These ongoing studies should shed important light on the biology of cartilage surface cells, should suggest ways to maintain their function during normal function and even aging. These studies will also pave the way to identify more effective and aggressive means by which function of joint surface cells could be restored in severe and chronic conditions including osteoarthritis and after acute joint injury in pediatric and adult patients. In a related development, Dr. Pacifici joined forces with Dr. Robert Mauck in the Department of Orthopaedic Surgery at Penn to study whether progenitor cells isolated from developing embryonic synovial joints can repair articular cartilage more effectively than generic stem or progenitor cells - including bone marrow—or fat tissue-derived mesenchymal stem cells—currently used by most groups. These innovative studies are supported by a grant from the VA that Drs. Pacifici and Mauck received recently.

As indicated in our report last year, our basic research work on FOP has led to an ongoing phase 2 clinical trial sponsored by the Canadian-based pharmaceutical company Clementia. FOP is an extremely severe pediatric disorder in which extraskeletal bone (collectively called heterotopic ossification or HO) forms and accumulates throughout the body over time, progressively limiting the ability of patients to carry out daily functions and often leading to premature death. Studies we first published in 2010 and 2011 showed for the first time that synthetic agonist ligands for nuclear retinoic acid receptors are very potent inhibitors of HO in experimental animal models of FOP. The clinical trial was launched in July 2014 in close collaboration with our colleagues at the U Penn FOP Research Center Drs. Fred Kaplan, Bob Pignolo and Eileen Shore and is expected to be completed later this year. This is a major milestone achievement for our basic Research Division and shows that years of basic research can and do translate into possible new treatments for severe pediatric skeletal disorders. We continue to use our two-pronged approach that combines basic and translational medicine to tackle, and hopefully find treatments for, other orphan musculoskeletal disorders affecting and afflicting children and their families worldwide.

Genetic Research

CHOP Orthopaedics continues to work in collaboration with the Center for Applied Genomics (CAG), led by Dr. Hakon Hakonarson and Dr. Struan Grant, to compile a registry of DNA and RNA samples. These samples are obtained from patients and families with a variety of orthopaedic conditions including adolescent idiopathic scoliosis (AIS), osteocondritis dissecans (OCD) of the knee, and multiple hereditary extoses (MHE). This past year, in conjunction with colleagues in genetics and basic science at CHOP and St. Luke's Orthopedics in Boise, ID preliminary results from a study of genetic predispositions for OCD were published in the *Journal of Pediatric Orthopaedics.* The study discussed the relevance and applicability of genome-wide association study (GWAS) in studying a genetic basis for OCD. The study also identified top signals that may suggest loci involved in coordinated expression as well as a transcription factor involved in development that may be highly relevant to this trait. Additional advances in 2015 include identifying a novel copy number variation (CNV) deletion that was common between a mother and daughter who both suffered from the absence of the anterior and posterior cruciate ligaments of the knees. Future efforts in genetics research include a push to perform whole exome sequencing and GWAS of OCD samples collected from the Research in Osteochondritis Dissecans of the Knee (ROCK) group.

Orthopaedic Engineering

In 2015 Dr. Saba Pasha, PhD was appointed as the new Director of Orthopedic Engineering. Dr. Pasha's research focuses on application of 3D imaging and computer simulation in surgical planning, use of predictive models in surgical decision making, and exploring gait and motion analysis for a more personalized treatment.

With new emerging technology, such as the EOS x-ray imaging system, comprehensive information about a patient's condition is now readily available. Dr. Pasha's work utilizes advanced imaging and motion analysis to collect data on a range of conditions and patient populations. These tools will help us to visualize and determine the best treatment options for patients. Currently, research is focused in two areas—skeletal deformities and sports medicine.

Clinical Research

The Division of Orthopaedic Surgery is currently conducting 117 IRB approved clinical research projects. This includes 50 prospective randomized clinical trials and

observational studies. CHOP Ortho faculty are also members of a number of multicenter study groups including the Harms Study Group (HSG), Research in Osteocondritis Dissecans of the Knee (ROCK), and International Hip Dysplasia Institute (IHDI). Investigators within the division have been awarded funding from both internal and external sources to conduct these studies. In 2015, the Division published over 107 articles in major orthopaedic journals, including JBJS, Spine, JPO, and CORR.

Our pediatric orthopaedic faculty continues to present research studies at orthopaedic conferences around the world, including the American Academy of Orthopaedic Surgeons (AAOS), the Pediatric Orthopaedic Society of North America (POSNA), the European Pediatric Orthopaedic Society (EPOS), the Scoliosis Research Society (SRS), the American Orthopaedic Society for Sports Medicine (AOSSM), the International Meeting on Advanced Spine Techniques (IMAST), the Societe Internationale de Chirurgie Orthopedique et de Traumatologie (International Society of Orthopaedic Surgery and Traumatology, SICOT) and many more.

Figure 16.

The Division continues to award the annual Benjamin Fox Scholarship Award for medical students who are interested in conducting a year of clinical research within orthopaedics. In June, Christopher Brusalis (Perelman School of Medicine at the University of Pennsylvania) and Christian Refakis (Stony Brook University School of Medicine), were awarded with the scholarship. While at CHOP, Chris (Figure 16) has concentrated his research on novel educational interventions for orthopaedic surgery residents, the impact of surgeon experience on clinical outcomes, and supracondylar humerus fractures. Christian (Figure 17) has focused his research on the complications of posterior spinal fusions for scoliosis, the management of neuromuscular scoliosis and hip dislocation in the setting of cerebral palsy, the incidence and management of concussion in sports, the management of pediatric shoulder instability, and the patient-reported outcomes of All-Epiphyseal ACL reconstruction.

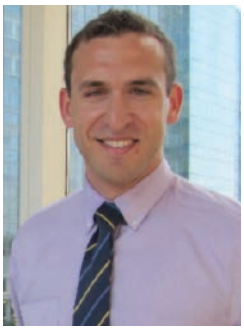


Figure 17.

Recognition and Achievements

Our Attendings have assumed several leadership roles within the pediatric orthopaedic community over the past year.

Alexandre Arkader, MD was the International Guest and Keynote Speaker at the 9th TROIA (Brazilian Pediatric Orthopedic Trauma Meeting) in Curitiba, Brazil. He also served as a guest speaker and International Faculty at the 4th Combined SLAOTI/POSNA/EPOS meeting in Bogota, Columbia.

Keith Baldwin, MD, MSPT, MPH is the current Director of Clinical Research and Associate Director of Orthopaedic Trauma in the Division of Orthopedic Surgery. This past year he earned the Jacqueline Perry Award Paper from the Orthopaedic Rehabilitation Organization. Dr. Baldwin currently serves as a reviewer for a number of journals including the *Journal of Orthopedic Trauma*, *Journal of Bone and Joint Surgery - American*, and the American Academy of Pediatrics and an editorial board member of the *American Journal of Orthopedics* and *World Journal of Orthopedics*.

Patrick Cahill, MD was awarded the Scoliosis Research Society Traveling Fellowship. Dr. Cahill joined other North American fellows to visit major spinal deformity centers in Asia under the guidance of a senior SRS fellow. He continues to serve as an Associate Editor for *Spine Deformity Journal* and reviewer for the *Journal of Bone and Joint Surgery – American* and the Thrasher Research Fund. Dr. Cahill served as faculty for the 2015 Early Onset Scoliosis Seminar in Nagoya, Japan.

Robert Campbell, MD continues to expand and develop the Center for Thoracic Insufficiency at CHOP. He was also awarded the Pennsylvania Bio's Patient Impact Award. The award recognizes a company or organization that has made a significant contribution to the quality of healthcare or length of life of patients." Previously, the University of Pennsylvania and CHOP were jointly given the award for their groundbreaking immune therapy research. In 2015, Dr. Campbell and Dr. Udupa (Perelman School of Medicine) were awarded a NIH R21 Grant for their project "Dynamic MRI Image Analysis for Studying Thoracic Insufficiency Syndrome". Drs. Campbell and Udupa plan to study quantitatively pre- and post-operative dynamic MRIs of TIS patients and compare these quantitative measures to pulmonary function tests and patient outcome to understand the effectiveness of surgery.

Robert Carrigan, MD is a team member of the Hand Transplantation Program and took part in the first pediatric double hand transplant. Dr. Carrigan continues to serve on the POSNA Resident Newsletter Committee. Together with his wife, Dr. Carrigan and his family welcomed their third child, Sam, in 2014.

B. David Horn, MD is the current chair of the AAOS Pediatric Evaluation Committee and is in the process of editing the 2016 Pediatric Self Assessment Examination. He also served as the organizer and moderator of the instructional course Office Pediatric Orthopedics at the AAOS annual meeting in Las Vegas, NV. In August, Dr. Horn traveled with Dr. Davidson to serve as facilitators the 25th Annual Baltimore Limb Deformity Course.

Jack Flynn, MD, Chief of the Division of Orthopaedic Surgery, continues serve his 10-year term as a Director of the American Board of Orthopaedic Surgery and a 4-year term as AAOS Chair of Continuing Medical Education. He also Co-Chairs the International Pediatric Orthopaedic Symposium and the sold-out Spine Surgery Safety Summit. Dr. Flynn is co-editors of two textbooks: *Rockwood and Wilkins': Fractures in Children* and *Operative Techniques in Orthopaedic Surgery—Pediatrics*. He continues his service on the Board of Directors of the

Children's Spine Study Group, and is active in the Harms Study Group, a multi-center collaboration of researchers studying care improvements for pediatric spine deformity surgery.

Theodore Ganley, MD is the Sports Medicine Director at CHOP, supporting clinical, research, and outreach initiatives which continue to grow. He was selected as moderator or instructor at instructional course lectures for the following annual meetings: AAOSM, AAOS, AAP, IPOS, and POSNA. In 2015, he was invited to speak the Penn Cartilage Repair Symposium. Dr. Ganley continues to serve on the Advisory Board of the International Pediatric Orthopaedic Symposium, on the Executive Committee of the Research in Osteochondritis Dissecans of the Knee (ROCK) group, and as Co-founder and Treasurer of the Pediatric Research in Sports Medicine (PRISM) group.

John Todd Lawrence, MD, PhD, through an OMeGa grant, recently completed the development of a distal radius fracture model (patent pending) which will improve resident performance in fracture reduction and casting techniques. The model was recently validated in conjunction with a multicenter pediatric simulation group and is currently being market. He continues to serve as a reviewer for the *American Journal of Sports Medicine (AJSM)* and *Journal of Shoulder and Elbow Surgery (JSES)*.

Wudbhav Sankar, MD is the Director of the Young Adult Hip Preservation Program at CHOP. In 2015, he was promoted to Associate Professor of Orthopaedics Surgery. Dr. Sankar completed his term on the POSNA Board of Directors and is currently Chair of the POSNA Fellowship committee. He remains active in several study groups including Academic Network of Conservational Hip Outcomes Research (ANCHOR) and International Perthes Study Group. Dr. Sankar is currently a reviewer for the scientific program of the POSNA annual meeting and an Editorial Board Reviewer of the journal *Techniques in Orthopaedics*. In addition to his professional accomplishments, Dr. Sankar and his wife welcomed their third child, Kiran Oliver on November 20, 2015.

Apurva Shah, MD, MBA currently serves as co-PI on the POSNA Directed Research Grant "Improving value delivery in pediatric distal radius fracture care". The primary aims of the grant include accessing practice pattern variation and comparing treatment costs across institutions and geographic regions and between low and high volume centers. This past year, he has continued to serve as senior investigator on two OREF grants studying time-driven activity based costing and trigger finger injections. At the 2015 Mid-America Orthopaedic Association Annual Meeting, he received the award for Best Poster. In October 2015, Dr. Shah served as team leader and traveled to Sigua Tepeque, Honduras for a pediatric hand surgery medical mission.

David Spiegel, MD was awarded the Children's Hospital of Philadelphia Global Health Pilot Grant. Together with Dr. Bibek Banskota the funds will be used in Nepal to conduct the longest follow-up in the world's literature of patients treated by the Ponseti method in a low-middle income country. Dr. Spiegel continues his work with the World Health Organization, traveling to Geneva, Switzerland and Nepal. Following the Nepal earthquake in April 2015, Dr. Spiegel traveled to the country to support his friends with their work to treat patients. In December he took part in four sessions at the 12th Annual International Pediatric Orthopaedic Symposium in Orlando, FL.

Lawrence Wells, MD is the Associate Director of the Sports Medicine Performance Center at CHOP and Director of Quality, Safety, Value, and Patient Experience in the Division of Orthopaedic Surgery. In 2015, he joined the POSNA EPIC Steering Committee and POSNA Quality, Value, Safety committee, and the Pediatric Research in Sports Medicine Society (PRISM). Dr. Wells was also appointed as an external reviewer on the Committee of Appointments and Promotions at the University of California San Diego School of Medicine.

Shoulder & Elbow

Staff

David Glaser, MD

G. Russell Huffman, MD, MPH

Andrew Kuntz, MD

Department of Shoulder & Elbow Update

David Glaser, M. D.

It has been another outstanding year for the Shoulder and Elbow division of the Department of Orthopaedic Surgery in the Perelman School of Medicine at the University of Pennsylvania. With continued commitment to manage the most complex cases, the section's tertiary referral network has dramatically increased along with the complexity of cases. In 2015, the group performed over 10,000 visits and performed 1054 surgical cases.

As Director of research, Andy Kuntz is leading our research effort, with close collaboration with Louis Soslowsky and others in the McKay Research Laboratory. Together, we form one of the largest, shoulder research laboratories in the world. The Penn shoulder and elbow faculty presented 8 abstracts at national meetings, and delivered 15 talks at international, national, regional and local meetings in 2015. The Shoulder and Elbow service has 6 refereed research grants in 2015, including NIH, Veterans Affairs, health system and the industry grants. Highlights from this work include investigations into the "Effects of Autologous Juvenile, Adult, and Aged Tenocyte-Seeded Nanofibrous Scaffolds in Rotator Cuff Repair", which was funded by the Penn Institute on Aging, was presented at the 2016 ORS Annual Meeting (presented by Julianne Huegel), and received the New Investigator Research Award honors. Andy was the PI, with Lou as a Co-Investigator on this study. Additional studies include "Effect of Scaffold-Delivered Growth Factors in Rotator Cuff Repair" with Andy as the PI. Ongoing clinical studies measure outcomes using multimodal pain control, outpatient shoulder arthroplasty, long term outcomes of prosthetic implants and cost efficiency in delivering health care. Penn was also selected as a study site to investigate 10-year outcomes of Integra Titan Reverse Shoulder Prosthesis (contract negotiations / legal finalization continue, new budget approved), with Dr. Glaser selected as site PI.

Spine

Staff

Vincent Arlet, MD

Harvey E. Smith, MD

Department of Spine Update

Vincent Arlet MD, James Friedman MD

The Spine Section of the Department of Orthopedic Surgery at the University of Pennsylvania had another excellent year. In 2015 the spine section boasted 2,133 patient visits with 429 surgical cases.

Vincent Arlet, MD, chief of the Spine service, presented at a number of events and courses in 2015 including the French Spine Society; the European Spine Journal/AO Spine Meet the expert forum on Spine Osteotomies in Barcelona, Spain; the Depuy Synthes Spine Course; and the Inaugural Philadelphia Spine Summit where both he and fellow Penn Orthopaedics spine surgeon Harvey E. Smith, MD served as course directors. Dr. Smith also presented at the AAOS instructional course lecture in Las Vegas, NV and was named chair of the Biologics Committee for the Association for Collaborative Spine Research Subspecialty Group Meeting in Miami, FL.

The Spine service was also the recipient of numerous grants in 2015. These include grants to study the Impact of Pre-Culture and In Vivo Remobilization on Engineered Disc Replacement; and the Regeneration of the Intervertebral Disc using Notochord- Derived Cells and Mesenchymal Stem Cells. 2015's grants also included a career development award for Biomedical Laboratory Research & Development Service of the VA Office of Research and Development.

Sports

Staff

Brian Sennett, MD

James Carey, MD, MPH

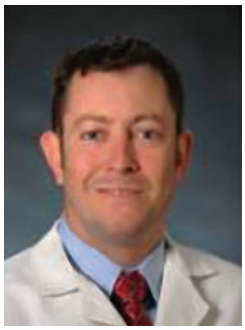
John Kelly, MD

Miltiadis Zgonis, MD

Trauma

Staff

Samir Mehta, MD



Derek Donegan, MD

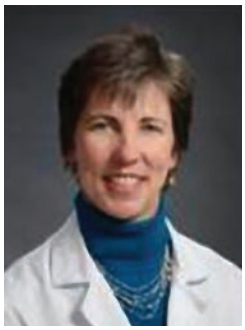
Jaimo Ahn, MD, PhD

John Esterhai, MD

L. Scott Levin, MD, FACS

Tumor

Staff



Kristy Weber, MD

Advancing Clinical Care and the Science of Sarcoma Orthopaedic Oncology at Penn

Kristy Weber, MD

Abramson Family Professor in Sarcoma Care Excellence, Chief, Orthopaedic Oncology Director, Penn Sarcoma Program at the Abramson Cancer Center

The Orthopaedic Oncology service at Penn is part of a multidisciplinary team of caregivers that is focused on our patients with bone and soft tissue tumors. This includes the care of patients with benign and malignant primary tumors as well as patients with metastatic bone disease. With the departure of Dr. Richard Lackman and the arrival of Dr. Kristy Weber in 2013, the program shifted to the Hospital of the University of Pennsylvania (HUP) and the Perelman Center for Advanced Medicine (PCAM). It is now in close proximity to the Children's Hospital of Philadelphia (CHOP), the Abramson Cancer Center administration, the U. Penn School of Veterinary Medicine, and the laboratories of investigators focused on sarcoma research. The Orthopaedic Oncology core team involves Dr. Weber, Bethany Sterling, NP, and Angeline Mombrun, Administrative Coordinator along with a PGY4 orthopaedic resident. A PGY2 orthopaedic resident also participates on the service in addition to managing their primary role of evaluating and treating the HUP inpatient and ER consults. Patients are seen 2 days each week in PCAM clinic, and surgeries are performed 1-2 days per week at HUP. Starting in April, 2016, Bethany Sterling, NP will run a weekly Bone Metastasis Clinic at our Valley Forge location.

There is a comprehensive clinical multidisciplinary team that treats patients with bone or soft tissue sarcoma.This group meets weekly at PCAM for a clinical care conference to discuss the presentation and differential diagnosis of new patients as well as the ongoing multimodal therapy for existing patients. Our musculoskeletal radiology team leads the conference under the direction of Dr. Ronnie Sebro who joined Penn in July, 2015. Our musculoskeletal pathology team provides expertise about the tumor biopsies and teaches all of us about the histologic appearance of these rare tumors. The medical oncology team is led by Drs. Chip Staddon and Lee Hartner who practice at Pennsylvania Hospital and have provided high level care for sarcoma patients for many years in the Penn community. Dr. William Levin is the lead radiation oncologist at PCAM/HUP who utilizes proton radiation and IMRT for patients with sarcoma,and he works with a network of radiation oncology colleagues throughout the Penn hospital system. Dr. Giorgos Karakousis is a surgical oncologist who treats patients with melanoma as well as retroperitoneal, abdominal and extremity sarcoma. In addition, we have a large surgical team including neurosurgery, plastic surgery, colorectal surgery, urology, and gynecologic oncology surgery that collaborate to surgically resect complex tumors about the spine and pelvis and reconstruct the defects to allow maximal function. Finally, there is a large supportive care team at Pennsylvania Hospital and HUP/PCAM to who assist patients with bone and soft tissue cancers including nurse practitioners, nurses, social workers, nutrition specialists, physical/occupational therapists, prosthetic/orthotic specialists and others focused on alternative therapies. Patients are generally seen in the PCAM orthopaedic oncology clinic within 1-2 days (and often same day) with new bone and soft tissue lesions. For those with concerning lesions, an image-guided biopsy is arranged within a few days by our MSK Radiology team. The diagnosis is made within 2 days in the vast majority of patients by our MSK Pathology team. All of the members of our clinical Sarcoma team prioritize seeing patients expediently and efficiently to make an accurate diagnosis and provide expert treatment.

Dr. Weber took over the orthopaedic oncology practice at CHOP immediately upon departure of Dr. John Dormans in January, 2016. She sees patients in CHOP clinic during 2 days each week and operates there 1 day each week. Dr. Alex Arkader joined the CHOP orthopaedic team in August, 2015 as an expert in orthopaedic oncology and pediatric deformity. He was recruited from LA Children's Hospital where he had a robust pediatric orthopaedic practice. He is originally from Brazil and completed prior fellowship training at CHOP with Dr. Dormans. There is capacity to see children with bone or soft tissue tumors every day of the week. Patients are now diagnosed via image-guided needle biopsy in the vast majority of cases. The current team of Drs.Arkader and Weber have the ability to perform innovative and complex limb salvage procedures in children with these diseases. All types of reconstructions of the shoulder, pelvis, hip, knee and elsewhere in the skeleton are performed utilizing cadaveric allografts, vascularized bone and periosteal grafts, and standard or expandable joint megaprostheses. Dr. Arkader's expertise in pediatric skeletal deformity brings added ability to recreate equal leg lengths in skeletally immature patients with limb length discrepancies due to tumor resection or systemic chemotherapy. An active marketing effort is underway to build the Bone and Soft Tissue Tumor Program along with our pediatric and radiation oncology colleagues.

One of the features that allows the Penn Sarcoma program to stand above other centers in the region and nation is the presence of a collaborative scientific team focused on new discoveries in sarcoma. Within the past year, Karin Eisinger, PhD and Malay Haldar, MD, PhD were recruited to help build this team and have joined Celeste Simon, PhD (senior investigator at Penn focused on hypoxia and metabolism in sarcoma and other diseases), Margaret Chou, PhD at CHOP, and Nicola Mason, PhD, BVetMed. These investigators utilize sophisticated murine and canine spontaneous sarcoma models to study these diseases. They are focused on immunology,

hypoxia, and epigenetics among other areas of study in both bone and soft tissue sarcomas.

In summary, the goals of the Penn Sarcoma program are to prioritize scientific discovery, translational research and outstanding clinical care to result in better outcomes for our patients with bone and soft tissue tumors. The Penn Orthopaedic Oncology service plays a key role in achieving these goals.

Philadelphia Veterans Affairs Medical Center Update

John Esterhai, MD

PVAMC Chief of Orthopaedics

"The willingness with which our young people are likely to serve in any war, no matter how justified, shall be directly proportional to how they perceive the veterans of earlier wars were treated and appreciated by our nation."

—George Washington, President

"There are those who speak about you and say, he lost an arm, he lost a leg, she lost her sight. I object. You gave your arm, you gave your leg, you gave your sight as gifts to your nation that we might live in freedom. Thank you. And to your families, families of the fallen, and families of the wounded you've sacrificed in ways that those of us who have not walked in your shoes, can only imagine."

—Peter M. Pace, General

Since 1776 Americans have been blessed by the opening words of our Declaration of Independence: "We hold these truths to be self-evident, that all men are created equal, that they are endowed by their Creator with certain unalienable Rights, that among these are Life, Liberty, and the pursuit of Happiness." For more than two centuries our men and women have been prepared to fight to protect these principles. Although less than one percent of our population bears the military responsibility for our national defense, and although just over seven percent of all Americans living today have worn our Nation's uniforms, that still amounts to 26.5 million veterans (of whom 1.7 million are women) deserving of special care.

The Philadelphia VA Medical Center (PVAMC) provides health care to 90,000 veterans living in America's fifth largest metropolitan area. Our four-fold mission is to honor America's veterans with world-class health care, advance medical knowledge through research, train health care professionals, and be prepared to serve in the event of a crisis.

The PVAMC is a tertiary referral center with more than 135 acute care beds, 95 of which are medicine-surgery beds, and a total yearly operating budget of more than $380 million dollars. It is a fifteen minute walk from the Penn Presbyterian Medical Center and the Orthopaedic offices at 3737 Market Street. Our Orthopaedic residents and faculty are honored to help care for those who have served their country. Abraham Lincoln articulated the primary mission of the Veterans Administration Penn Orthopaedic Service more than a century ago: "To care for him who shall have borne the battle."

The VA is the largest health care system (122 medical facilities) supporting graduate medical education in the United States and the second largest funding source for resident training (31,000 resident physicians) after the Centers for Medicare and Medicaid Services. It is affiliated with 107 of the nation's 129 medical schools.

The University of Pennsylvania Orthopedic rotation at the Philadelphia VAMC allows our PGY-2 and PGY-5 residents to care for veterans in an intensive, general orthopedic practice under the direct supervision of Drs. Ahn, Bernstein, Ecker, Esterhai, Farber, Gentchos, Hume, Kelly, Kuntz, Sheth, Steinberg, and Zgonis. Dr. Harvey Smith, our spine surgeon, teaches and works with a PGY-3 resident. Dr. Levin volunteers his time without compensation. The veterans who require care at a level of sophistication that we cannot provide are referred to sub-specialists in Universities throughout the region.

In addition to their dedication to direct patient care and resident education, Drs. Bernstein, Esterhai, Kuntz, Steinberg, and Zgonis have each applied for or been awarded research funding through the Veterans Administration competitive grant system. Under the direction of Drs. Mauck and Dodge our department's Translational Musculoskeletal Research Center has five Merit Grants. Dr. Smith is actively engaged in his basic science spine studies funded by a prestigious five year Career Development Award. Our PVAMC clinical faculty members collaborate actively with intra and extra mural physicians and basic scientists including Drs. Jonathan Black,

Jason Burdick, George Dodge, Dawn Elliott, Kurt Hankenson, Annamarie Horan, Russ Huffman, Robert Mauck, Samir Mehta, Lachlan Smith, and Lou Soslowsky.

Drs. Mauck and Dodge direct our Translational Orthopedic Research Facility, in 4,500 square feet of superbly equipped research space. They have energized collaboration with scientists in Rheumatology and Physical Medicine and Rehabilitation.

We have been able to improve our preoperative patient evaluation process to expedite surgery scheduling with the addition of preadmission testing offices embedded in our clinic, improved peri-operative pain management, and post-operative floor care. We look forward to operating room rennovations this year and adding OR and PACU personnel to extend the operating room duty day.

Mitchell (Chip) Staska and John Wheeler, our superb Physician Assistants, are our boots on the ground every day. They provide seamless, exemplary, tender care from initial patient referral through appropriate triage, outpatient evaluation, scheduling of appropriate testing and consultations, surgery, and post hospitalization care. Chip and John provide immediate, timely interaction with referring physicians and outside consultants, coordination of pre-bed evaluations, surgery scheduling, interaction with the primary care providers, liaison with VA referral health centers, acute and chronic pain management, and assist in the operating room! John coordinates Dr. Smith's Orthopaedic spine care for our veterans.

We have the best electronic medical record system in the country. All veteran records including consent forms and imaging studies are electronic. In-patient and out-patient progress notes, laboratory results, and imaging studies are available at the workstations on the in-patient units, offices, out-patient care areas and individual examination rooms from local and satellite VA care facilities across the country.

Ours is a surgical service. We do not attempt to provide primary musculoskeletal care. We have patient office hours on Mondays, Wednesdays, Thursdays, and Fridays. In FY 2015 Orthopaedics and Orthopaedic Spine saw 3471 consultations and 7068 office visits. We receive more than 15 new consultations five days a week. Every effort is made to insure that new patients are scheduled within thirty days of their primary physician's request for consultation. The Emergency Room is very busy. We perform scheduled surgery four days each week. In FY2015 Orthopaedics and Orthopaedic Spine performed 636 surgeries. Orthopedics performs more major surgeries than any other service. None of this would be possible without the professional expertise, personal sacrifice, and wisdom of the Chief of Surgery, Lew Kaplan, and the anesthesiologist, nurses, administrative support personnel, and physician staff of the PVAMC.

Vince Lombardi said, "The achievements of an organization are the results of the combined effort of each individual." By God's providence and the hard work and daily diligence of everyone in anesthesia, instrument processing, nursing, and orthopaedics the infection rate for our total joints replacement patients has remained excellent. Several factors specifically contributed, including: improved pre-operative patient screening and preparation, rigorous instrument processing and packaging, heightened awareness of potentials for intraoperative contamination, optimal perioperative antibiotic dosing, and patient retention for on-site rehabilitation before discharge to the patient's home. In this time of increasing financial constraint and federal budget review we will likely be called upon to deliver more direct care and perform more research with fewer resources.

As I write this I anticipate that Dr. Marlene DeMaio, Captain, Medical Corps, U.S. Navy (retired) will be coming on board in the near future. For the first time the PVAMC will have a full time Chief of Orthopaedic Surgery. Dr. DeMaio's credentials are impeccable: twenty years of active duty Navy military service, President of the Association of Bone and Joint Surgeons, Member of the Board of Trustees of Clinical Orthopaedics and Related Research, and Oral Examiner for Recertification and Part II for the American Board of Orthopaedic Surgery.

Many of the veterans for whom we care commute a long distance from central and northeastern Pennsylvania, southern New Jersey, Maryland, and Delaware. Many have significant co-morbidities such as HCV and difficult psychosocial environments. Many have had multiple operations making reconstructive surgical approaches and wound healing more difficult. Not infrequently they have had a difficult time reintegrating into society after their military service. It has been said that "a veteran is someone who wrote a blank check, payable to the United States of America, for an amount up to and including his own life." Providing Philadelphia-level, state of the art, complication free, compassionate care requires extra, special diligence. It is a worthy goal to which we are fully committed.

McKay Orthopaedic Research Laboratory

Robert L. Mauck, PhD and Louis J. Soslowsky, PhD

The McKay Orthopaedic Research Laboratory of the Department of Orthopaedic Surgery in the Perelman School of Medicine continues to explore important problems in musculoskeletal research. The research facility, including labs and offices, occupies just over 16,000 sq. ft. of space on the 3rd, 4th and 5th Floors of Stemmler Hall. There are over 100 full- and part-time staff and trainees now in the labs. It is an active, thriving research and educational environment. Furthermore, our building is in the midst of a >$100 million dollar renovation, which will culminate in 2018 in a fully modernized and aesthetically pleasing facility in which to grow our laboratory space, faculty, and research and training endeavors.

Currently, the lab has an annual research budget from extramural grants, gifts, and endowments > $12,000,000 and continues to rank within the top 5 orthopaedic programs in the country in terms of funding from the National Institutes of Health (NIH) with a 2015 ranking of #4. This past year has seen a very impressive and continued rise in new grant activity amongst the faculty.

We have had several new grants (>$25,000) awarded this year. These are:

- Andrew Kuntz, M.D., is PI of a VA SPiRE grant entitled "Effect of Scaffold-delivery Growth Factors in Rotator Cuff Repair."
- Xiawei Liu, Ph.D., is PI of a grant from the NIH entitled "The effect of parathyroid hormone on modeling-based bone formation."
- Robert Mauck, Ph.D. and Maurizio Pacific, Ph.D. are PIs of a VA SPiRE grant entitled "Cartilage Repair with Synovial Joint Precursors."
- Robert Pignolo, M.D., is PI of a grant entitled "Natural History Study Protocol A Natural History, Non-Interventional, Two-Part Study in Subjects with Fibrodysplasia Ossificans Progressiva (FOP)."
- Robert Pignolo, M.D., is PI of a grant entitled "Identification of plasma soluble biomarkers for Fibrodysplasia Ossificans Progressiva disease progression and treatment response."
- Ling Qin, Ph.D., is PI of a grant from the NIH entitled "Mechanism of radiotherapy-induced osteoporosis and its treatment."
- Lachlan Smith, Ph.D., is PI of a grant from the NIH entitled "Mechanisms of Vertebral Bone Disease in Mucopolysaccharidosis VII."
- Louis Soslowsky, Ph.D., is PI of an NIH supplement grant entitled "Challenging Treatment Paradigms for Achilles Tendon Ruptures in an Animal Model Supplement."
- Louis Soslowsky, Ph.D., is PI of an NIH grant entitled "Mouse Models for SLRP Roles in Tendon Aging and Impaired Healing in Aging."
- Miltiadis Zgonis, M.D., is PI of a Young Investigator Grant from the OREF entitled "Strain Transfer in the Knee Meniscus: Novel Mechanisms to Guide Treatment and Inform Tissue Engineering Strategies."

In addition to the above-mentioned new grants this year, each of the McKay Laboratory faculty remains well-funded through existing research grants not identified in this new grants list. Further, there were several new industry grants and clinical trials for our research and surgeon faculty this year. These are:

- Dana Farber, M.D., is PI of a grant entitled "A Phase 3 Randomized, Placebo-Controlled, Blinded Study to Investigate the Safety and Efficacy of a Topical Gentamicin Collagen Sponge in Combination with Systemic Antibiotic Therapy in Diabetic Patients with an Infected Foot Ulcer."
- Samir Mehta, M.D., is PI of a SRA entitled "A Phase 2a Randomized, Single-Blind, Placebo-Controlled, 24-week Escalating Dose Study to Assess the Safety,Tolerability and Clinical Activity of 3 Concentrations of Locally Applied MBN-101 to Infected Osteosynthesis Sites."
- Robert Pignolo, M.D., is PI of a SRA entitled "A Phase 2, Open-Label Extension, Efficacy and Safety Study of a RAR(gamma)-Specific Agonist (Palovarotene) in the Treatment of Preosseous Flare-ups in Subjects with Fibrodysplasia Ossificans Progressiva (FOP)."
- Robert Pignolo, M.D., is PI of a clinical trial entitled "A Phase 2, In-Home, Safety and Efficacy Evaluation of Episodic Administration of Open-Label Palovarotene in Subjects with Fibrodysplasia Ossificans Progressiva (FOP)."
- Louis Soslowsky, Ph.D., is PI of a SRA entitled "sNAG in a Rotator Cuff Tendon Healing Rat Model."
- Louis Soslowsky, Ph.D., is PI of a SRA entitled "Unilateral supraspinatus injury + repair."
- Kristy Weber, M.D., is PI of a clinical trial entitled "Open-label, multicenter, dose escalation Phase 1a/1b study with expansion phase to evaluate safety, pharmacokinetics and activity of RO5509554, administered as an intravenous infusion as monotherapy and in combination with Paclitaxel in patients with advanced solid tumors."

Growing musculoskeletal research in the Department of Orthopaedic Surgery and across the Penn campus has been a primary objective for our program, and this effort has been particularly fruitful thus far. We look forward to another exciting year of continued growth and success.

The Center for Research in FOP & Related Disorders Update

Frederick S. Kaplan, MD, Robert J. Pignolo, MD, PhD, Eileen M. Shore, PhD

This article was truncated and modified from a pre-existing report with permission from the authors.

Since it was established in 1991, the Fibrodysplasia Ossificans Progressiva (FOP) Collaborative Research Project at The Center for Research in FOP& Related Disorders has had a singular mission—to determine the cause of FOP and to use that knowledge to advance the treatment and a cure for FOP. During the past twenty-five years, we moved from the wastelands of a rare disease to the watershed of clinical trials. We identified the genetic cause of FOP and used that knowledge to spearhead worldwide research efforts to develop therapies that will transform the care of individuals with FOP.

In partnership with our benefactors, we have expanded the frontiers of discovery and drug development in this rare and catastrophically disabling condition, dismantled the physical and perceptual barriers that have impeded progress, and inspired global research in small molecules, antibodies, and gene therapy for FOP. The Center for Research in FOP & Related Disorders has provided the infrastructure of flexibility and intellectual space needed for serendipity and continuity.

Here, at The Center for Research in FOP & Related Disorders, our work is broad and comprehensive while focused on seven spheres of FOP activity: Clinical Care and Consultation Worldwide, Clinical Research and Infrastructure Development, Basic Research (Identification of Therapeutic Targets), Translational Research (Preclinical Drug Testing & Biomarker Discovery Program), Developmental Grants Program, Clinical Trial Development and Proof-of-Principle Investigation in Patients, Education.

The Center for Research in FOP & Related Disorders is unique. It is the world's first and only comprehensive program in FOP. Here at The Center, we have had a very busy year, and have achieved many milestones. Clinically, The Center directs the world's largest FOP clinic and referral center here in Philadelphia, while conducting international FOP clinics in Italy, Serbia, England, Germany, and Russia.

In clinical research The Center has completed a global surgery survey of FOP flare-ups and designed and validated the FOP Cumulative Analogue Joint Involvement Scale (CAJIS). Clinical trials have also commenced with Palovarotene, an inhibitor of endochondral bone formation, in adult FOP patients. This represents the first randomized double-blind placebo-controlled trial in the history of FOP and was developed with Clementia Pharmaceuticals and the FDA

At the benchtop The Center has made great strides in elucidating the molecular mechanisms by which FOP flare-ups occur and have identified potential therapeutic targets for the treatment of FOP. Additionally, The Center has completed the second-year of a comprehensive pre-clinical drug-testing and biomarker discovery program in FOP mouse models. In this program, a promising compound was discovered that partially inhibits heterotopic compound that partially inhibits heterotopic ossification in a mouse model of FOP. Ongoing studies, both *In vivo,* and *In vitro* are also looking at other potential therapeutic compounds. For this promising work The Center was awarded a new 2-year developmental grant on induced pluripotent stem cell modeling for FOP.

Our work at **The Center** is at the forefront of the FOP world. The work is constantly evolving as we cross the bridge daily between the clinic and the laboratory and back again in a process that builds knowledge and deep understanding to help us accomplish our mission.

Penn Center for Musculoskeletal Disorders

Louis J. Soslowsky, PhD

(Founding Director of the Penn Center for Musculoskeletal Disorders)

The Penn Center for Musculoskeletal Disorders (PCMD) was initiated in 2004 with a goal to bring musculoskeletal researchers across campus together at the University of Pennsylvania. In 2006, the National Institute of Arthritis and Musculoskeletal Skin Diseases of the NIH funded our proposal (P30 AR050950) at which time we became one of five such NIH-recognized Centers in the country (www.med.upenn.edu/pcmd). In 2011, this Center grant was renewed for another five years and was the only one of the three up for renewal that was re-funded that year. Through the review by the NIH, Penn scored a perfect "ten" and was hailed as "exceptional" by the review panel! In 2016, we received another "exceptional" score by the NIH review panel and we are awaiting official notification of another five years for our NIH-supported Center.

The overall goal of this Center is to promote cooperative interactions among investigators, accelerate and enrich the effectiveness and efficiency of ongoing research, foster new collaborations and new research, and ultimately, translate our research efforts into better and new therapies for musculoskeletal disorders. The central theme of the Center continues to be "Musculoskeletal Tissue Injury and Repair". This theme is broad (as it includes all musculoskeletal tissue types, such as bone, cartilage, disc, ligament, meniscus, muscle, and tendon), focused (as takes advantage of commonalities in approaches across tissue types), and clinically significant (as it fosters development of assays, procedures and knowledge in pre-clinical animal and human models of translational relevance). It is important to note that our PCMD is not a "bone center" nor is it a "muscle center". Rather, it is truly a "musculoskeletal center" and has emerged as the recognized home for musculoskeletal research across the Penn campus and as a technical and intellectual resource for the broader Philadelphia musculoskeletal research community. Thus, the primary overall aims of this Center are to enhance and advance the research productivity of investigators in musculoskeletal tissue injury and repair by: 1) Providing innovation within critical resource core facilities in areas that cross disciplines, length scales, and hierarchies. These core facilities are CT Imaging, Biomechanics, and Histology, 2) Developing a pilot and feasibility grant program for investigators, with direct mentorship, whereby new approaches, ideas, and collaborations can be developed prior to seeking extramural funding, and 3) Developing educational and research enrichment programs spanning tissue types, research approaches, and paradigms, through which members can learn from national leaders and from each other. High quality musculoskeletal research is currently being conducted by many groups at Penn. While many bring sophisticated approaches to bear on musculoskeletal problems, few groups have the required expertise and facilities to perform high quality and specialized assays in their own labs. Furthermore, most investigators are not aware of approaches utilized, and results obtained, in other tissues that may have direct relevance on their research questions. Ultimately, close cooperation, communication, and collaboration among researchers across musculoskeletal tissue types and from a wide variety of disciplines will significantly enhance the research of our members. The Center will provide opportunities to integrate multi-disciplinary techniques to determine mechanisms for tissue function, injury, degeneration, repair, and regeneration, with the ultimate goal of advancing the diagnosis, treatment, and prevention of diseases and injuries of the musculoskeletal system.

The Center currently has a membership of more than 125 faculty across five schools at Penn (Perelman School of Medicine, School of Engineering and Applied Science, School of Veterinary Medicine, School of Dental Medicine, and School of Arts and Sciences). We also now have faculty members for 10 Philadelphia-area institutions as we expand the reach and impact of our Center. For more information on the PCMD, please visit our website at www.med.upenn.edu/pcmd.

Update on the Biedermann Lab for Orthopaedic Research

Michael W. Hast, Ph.D.

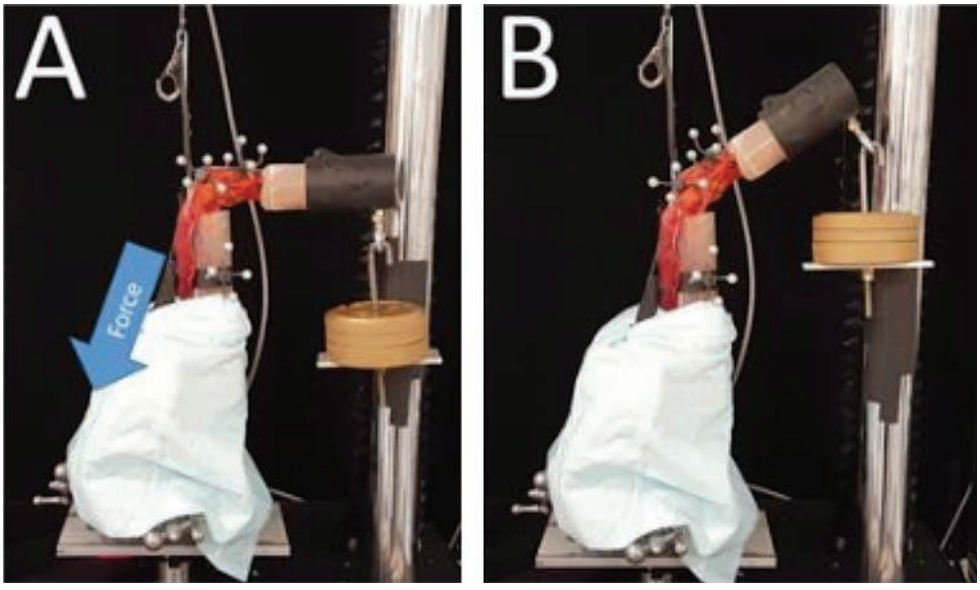
Director, Biedermann Lab for Orthopaedic Research

The Biedermann Lab for Orthopaedic Research had its grand opening in June of 2015. Since that time, the Lab has worked to develop partnerships with surgeons and researchers from the Philadelphia area and beyond. Thus far, research collaborations have been established with clinicians and scientists from the University of Pennsylvania, Thomas Jefferson University, Children's Hospital of Philadelphia, Emory University, and several orthopaedic implant companies.

Currently, the Biedermann Lab is working on over 15 biomechanical research projects that are in various stages of development, execution, or publication. The ongoing projects have a wide scope of interests and employ a variety of techniques including computational modeling, 3-D printing of bones, cyclic testing of implants, 3-D motion capture, and cadaveric simulations of activities of daily living.

Recently, the Lab completed two experiments focused on implant designs and surgical techniques used to address upper extremity fracture fixation. These projects have been submitted as abstracts to the annual meeting of the Orthopaedic Trauma Association and will also be developed into full-length manuscripts. The full abstracts to these projects can be found within this journal and represent two examples of the opportunities the Biedermann lab brings to Penn. The first study utilized the labs' ability to precisely cyclically load implants to test the strength of different screws implanted at varying angles into plates. The second study utilized the lab's ability to reproduce physiologic forces on cadeaver tissue to test screw placement strength for fracture fixation (Figure 1).

These two studies exemplify the continuous goal of the Biedermann Lab– to perform research that is relevant and translatable so that the standard of care and quality of life for patients is improved. In order to continually achieve this objective, a steady flow of research ideas and scientific collaborations is essential. Going forward, the Biedermann Lab will work hard to continually develop and foster academic relationships at Penn and throughout the world. If you have a research interest that may be suitably addressed with the research competencies of the Biedermann Lab, you are encouraged to contact Michael Hast directly. For more information about the Biedermann Lab, please see its website: www.med.upenn.edu/biedermann/



International Orthopaedic Surgery Update

James Friedman, MD

I will never forget watching the fear in my patient's face as he received a below knee amputation with only lidocaine for anesthesia, the third one of the day. Since his house collapsed in an earthquake, he had spent the last four months trying to provide for his family walking on an infected tibial non-union. Although this fracture would have received earlier definitive treatment in a country like the US, this was not an option for a poor Haitian farmer. With the increasing pain and weakness associated with infection, amputation became his only option.

Of course, this is not an isolated story. Estimates report that 11-33% of global morbidity is secondary to surgical disease. Furthermore, the treatment of traumatic injury in developing countries positively influences not only the patient acutely, but also the livelihood of his family and even country. The economic impact of surgical disease is staggering, representing a loss of up to 2.5% of GDP in some countries. Although this may not sound like much, this is equivalent to at least 12.3 trillion dollars between today and 2030. Trauma has been reported to make up over 1/3 of these costs and, in some developing countries, it is the primary cause for GDP loss. Regaining these losses has been suggested by economist Jeffrey Sachs to be enough to raise some countries out of extreme poverty.

Over the past few years the Penn Orthopaedic Surgery Department has benefitted from its' increasing involvement in global orthopedics through improved learning opportunities, increased interaction with other fields, enhanced interaction with medical students, and by opening further opportunities for growth. Attending and resident education has benefitted from increased options to travel to low resource areas from recent trips to Trinidad, Nicaragua, and India. It is on these trips that residents and attendings have described the benefit of relying on basic principles of orthopedic management to operate, as opposed to relying on the instruction manual of the most recent hardware. At this year's first annual Global Surgery Symposium, Dr. Spiegel and Dr. Sheth represented orthopedics and helped lead discussion with other fields including plastic surgery, general surgery, oncology, and urology to discuss funding, delivery methods, and ethical considerations in future trips. Involvement abroad has also allowed new opportunities for Penn Orthopaedics to interact with the medical students. This year, multiple well attended medical student lunch talks and interactive sessions have been given by Dr. John Kelly, Dr. Neil Sheth, Dr. David Spiegel, and Dr. Derek Donegan. Furthermore a large number of students have begun research projects and have published papers with our attendings and residents directly related to global surgery. Finally, as Penn Orthopaedics is becoming increasingly recognized as a potential source of global outreach, we have received multiple offers to participate in sites as far away as Botswana and Vietnam, allowing Penn a unique opportunity to participate at these sites.

As our role in global orthopaedics evolves, it is becoming increasingly realized that in order to make an impact on reducing the global burden of surgical disease, better long term strategies need be developed. This includes cross-departmental cooperation (including nursing and anesthesia), longer term surgical infrastructure investment, and reliable patient follow-up systems. A few of our faculty have already taken the lead in developing these systems, including Dr. David Spiegel establishing clubfoot clinics in Nepal, Dr. Neil Sheth developing a self-sustaining arthroplasty/trauma hospital in Tanzania, and Dr. Jaimo Ahn with Dr. John Esterhai working to identify a permanent orthopedic site in Botswana alongside our medicine colleagues. In addition to all this, the University of Pennsylvania Center for Global Health has offered both advice and financial support for our endeavors.

The importance of delivering orthopaedic care to low-resource countries is only just beginning to be recognized. Delivering orthopaedics in developing countries helps individual patients, reduces family economic burden, and may even help raise entire economies out of extreme poverty. For our own department it allows us to provide unique learning, research, and leadership opportunities to our residents and attendings. It is therefore important as we move forward to continue to support our attendings interested in international program development, to find ways to allow residents time abroad without affecting patient care at Penn, and to continue to seek new opportunities in cross-departmental collaboration and research. With these goals in mind, Penn orthopaedics, with its history of interest, involvement, and resources, has a great opportunity to become one of the leaders in the field of global orthopaedics over the next few years.

Acknowledgements:

Thank you to Dr. John Esterhai, Dr. Derek Donegan, Dr. Neil Sheth, and Dr. Spiegel for their advice regarding this article.

Visiting Professor Lecture Series

Guest Lecturer: Dr. Bruce Reider July 2, 2015

James M Friedman MD

The University of Pennsylvania Department of Orthopedic surgery was honored to welcome Dr. Bruce Reider, Professor of Orthopaedic Surgery, Emeritus, of the University of Chicago as a visiting professor this past July. A New York native, he completed his undergraduate degree at Yale University, and received his medical degree from Harvard Medical School. He subsequently completed a general surgery internship at Columbia Presbytyrian Hospital and an orthopedic residency at the Hospital for special Surgery (HSS), followed by multiple fellowships at HSS, the University of Wisconsin, and Kantonspital Bruderholz. Thereafter, Dr .Reider arrived at the University of Chicago (UC) in 1981.

With over 20 book chapters, and over 40 peer-reviewed articles Dr. Reider has contributed greatly to sports medicine. In addition, he has held a number of leadership positions and has received many prestigious awards for his work. At UC he continues to serve as the head team physician for the athletic programs and was the Director of Sports Medicine for over thirty years. He is also credited with founding the sports medicine fellowship program and in 2013 received the Starkey Duncan award for his care for varsity athletes. As an active member of the AOSSM, he held the position of program chair from 1999-2000, and was president of the Herodicus Sosciety from 2004-2005. He has also participated in multiple committees including research, education, traveling fellowship, nominating and program. In 2014 he was nominated to the AOSSM hall of fame for his invaluable contribution to sports medicine. He continues to work as the editor-in-chief of the American Journal of Sports Medicine and as the Executive Editor of the Medical Publishing Group for the AOSSM.

Dr. Reider delivered two lectures during his visit. His first lecture focused on current philosophies of ACL repair from a historical perspective. He described the initial controversy regarding the importance of the ACL compared to other knee structures and detailed the evolution of the diagnosis of an ACL tear including physical exam and usage of MRI. He then focused on the recent increased interest in the ACL and the ongoing development of fixation strategies. He finished his talk with the conclusion that in the future better outcomes will be found in a compromise between ACL fixation and fixation of surrounding soft tissue, lending to the title of his talk: *Swings of the Pendulum*.

Dr. Reider's second talk, pulled from his experience as an editor-in-chief to discuss strategies for getting research published. He described how to organize a paper and stressed the importance of on-topic, organized paragraphs that lead to a logical conclusion. He also discussed the importance of format. Finally he discussed reasons for rejection of papers and gave great insight into both how the rejection process works and on how authors can best respond to rejection.

The University of Pennsylvania Department of Orthopedics was honored to have Dr. Bruce Reider as a visiting professor on July 2nd, 2015.

Guest Lecturer: Dr. Stephen Kates July 22, 2015

James M Friedman MD

The University of Pennsylvania Department of Orthopedic surgery was honored to welcome Dr. Stephen Kates, Professor or Orthopaedics and Rehabilitation, Director of the Geriatric Fracture Center, Chief of Oncology, Metabolic Bone and Geriatric Division of the University of Rochester Medical Center. A native of the Philadelphia area, he earned his undergraduate and medical degrees at Northwestern University, where he also completed his internship before completing an orthopaedic surgery residency at the University of Rochester Medical Center.

Dr. Kates has practiced at the University of Rochester since 1989, making significant contributions to the care of geriatric hip fracture patients. As the director of the Geriatric Fracture Center, he has developed an internationally recognized hip fracture program, and published over 40 peer-reviewed publications, with focus on the management of geriatric fractures. Dr. Kates is the Editor of Geriatric Orthopaedic Surgery and Rehabilitation and an International Program Editor for AOTrauma. He has lectured extensively across North America, Europe and Asia promoting system improvement and management of geriatric fractures, infections and osteoporosis. He is past president of the International Geriatric Fracture Society, dedicated to collaboration on the delivery of evidenced-based, patient-centered care for the treatment of geriatric or fragility fractures.

Dr. Kates delivered a lecture titled "Geriatric Fracture Center—A Holistic Model Care" during his visit. To begin his talk, he presented the future impact of fragility fracture treatment to the cost of medical care and how a collaborative effort can maximize efficiency of health care spending. He described the model of a geriatric fracture center, which focuses on decreasing morbidity and mortality, reducing lengths of stay and time to surgery while improving quality of care. Dr. Kates established a protocol driven system for co-management of geriatric fractures between orthopaedists and geriatricians. The center has developed processes to support achievement of quality and cost goals, such standardized order sets for ED providers to fast track fragility fracture patients and evidence-based pre-operative consultation checklists to quickly facilitate optimizing and risk stratifying patients for surgery. Dr. Kates also described implementing treatment algorithms, which reduced peri-operative errors and facilitates cost effective implant decisions. Furthermore, the goals of care extend beyond hospital care to post-discharge recover. In this continuum model of treatment, the Gertiatric Fracture Center has developed partnerships with rehabilitation facilities to promote efficient post-operative recovery and reduce unnecessary extended stays in skilled nursing facilities. Dr. Kates delivered strong evidence that standardized care and co-management not only provided better quality care and outcomes for patients, but is effective at controlling expense for this important component of an aging health care population. The University of Pennsylvania Department of Orthopedics was honored to have Dr. Stephen Kates as a visiting professor on July 23rd, 2015

Guest Lecturer: Dr. Jo A. Hannafin October 15, 2015

James M Friedman MD

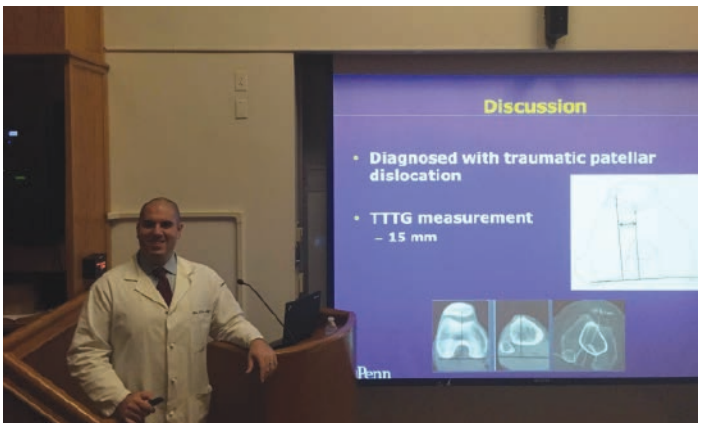
The University of Pennsylvania Department of Orthopedic Surgery had the privilege of welcoming Dr. Jo A. Hannafin as a guest lecturer this past October. Dr. Hannafin is an attending orthopedic surgeon at the Hospital for Special Surgery in Manhattan and recipient of the 2014 HSS Lifetime Achievement Award. She also serves as director of the HSS Women's Sports Medicine Center and as a professor in the Department of Orthopedic Surgery at Weill Cornell Medical College. She has been listed as one of the "Best Doctors in America" multiple times over the course of her career, and has been voted president of the Herodicus Society and AOSSM, as well as serving on the board of the OREF. Herself a former silver medalist in the 1984 World Rowing Championships and three-time gold medalist at the US National Rowing Championships, she has served as a team physician at multiple Olympics, as well as the team physician of the US Rowing team since 1994. Dr. Hannafin has published nearly 100 peer-reviewed journal articles and over 20 book chapters, and has been the recipient of numerous research grants, including NIH RO1 funding. She has been recognized as a national and regional leader in patient care, resident education, and high-caliber research, and it was a true honor to host her as a guest lecturer and visiting professor.

Dr. Hannafin began her visit with a lecture detailing her experience with Adhesive Capsulitis and current treatment regimens, *"Adhesive Capsulitis from Bench to Bedside and Back."* With a deft mixture of clinical anecdotes and evidence based medicine, she proved her expertise and familiarity with the care of patients suffering from the disease. With an emphasis on her own practice, which specializes in disorders of the shoulder as well as the treatment of the athletic population, she was able to showcase how innovative her practice model truly is.

In her second hour of lectures, Dr. Hannafin delineated her thoughts on ACL reconstruction in a talk entitled, *"The 'Anatomic' ACL Reconstruction: Myth or Misnomer?"* In her talk, she spoke about the history and evolution of the ACL reconstruction, from both a personal perspective as well as from the field itself. Emphasizing clinical outcomes and evidence based measurements, she gave a detailed description of the challenges facing ACL-deficient patients and the surgeon attempting to help them. In particular, she emphasized a thorough, in-depth understanding of the biomechanics of the knee and how anatomy guides function.

Dr. Hannafin and our own Dr. Miltiadis Zgonis then held court over the residency during several "interesting case presentations" by a number of the residents and fellows. During the session, cases were presented highlighted unusual and informative cases seen by members of the sports services. Dr. Hannafin lent her vast experience in highlighting the approaches she takes in patient diagnosis and care, as well as common pitfalls that can affect outcomes following surgical intervention. She was able to highlight important considerations in delineating operative and non-operative courses for several common injury patterns, and even learned a few things about the TT-TG interval from Orthopaedic PGY-1 intern Mark Hasenauer!

The morning Dr. Hannafin spent with the residency program was invaluable in its rich exchange of ideas and approaches to patient care. The lectures and case presentations were able to highlight common problems and areas of interest seen in the world of orthopaedic sport medicine, and hopefully represent a rich collaboration between our departments for many years to come.



Guest Lecturer: Dr. Robert D. Fitch November 15, 2015

James M Friedman MD

The University of Pennsylvania, Department of Orthopedics was pleased to host Dr. Robert Fitch, Professor of Orthopaedics at Duke University and Director of the Section of Pediatric Orthopaedic Surgery at Duke Children's Hospital and Health Center, as a visiting lecturer. Dr. Fitch completed his medical school and residency training at Duke University in 1976, followed by a pediatric orthopedic fellowship at the Texas Scottish Rite Hospital for Children in Dallas, Texas, before returning to Duke. Dr. Fitch maintains both a highly successful clinical practice and proliferative research program with particular focus on congenital and developmental deformities of the spine, hip, and extremities.

Dr. Fitch delivered two talks. His first talk focused on pediatric tibia pseudoarthroses, a difficult problem to treat with scant literature to help guide practicing orthopedists. In his talk he described 12 cases of his own with short, medium, and long term followups. He described a relatively high success rate with careful debridement, intramedullary nailing, and bone grafting. However, he also stressed that pseudoarthrosis is not a local problem confined to the fracture site and that complications, especially refracture, should be carefully monitored for. The second talk described cases of severe developmental dysplasia of the hip. Multiple cases were presented and a discussion was held regarding surgical approaches, and various osteotomies used to locate and stabilize the hip. Mid-term outcomes were also presented.

The remainder of Dr. Fitch's time was spent with the residents in the human tissue lab. Sawbones were used to demonstrate and practice the application of Ilizarov frames to reduce and correct a fractured tibia. Concepts included how to assemble the frame, how to fix the frame to bone, and how to use computer models to determine the correct sequence of pin modification to correctly align the fracture.

The University of Pennsylvania of Orthopedics was honored to have Dr. Fitch as a visiting professor. His honesty regarding outcomes, and his willingness to share his experience with the residents was invaluable.

June C. Wapner Memorial Lectureship December 3, 2015 Guest Lecturer: Sigvard T. Hansen, MD

Cody D. Hillin, MD MS

This past December, the University of Pennsylvania Department of Orthopedic surgery was honored to welcome Dr. Sigvard T. Hansen, Professor Emeritus of Orthopaedics and Sports Medicine at the University of Washington School of Medicine and Director of the Sigvard T. Hansen Foot and Ankle Institute at the University of Washington Medical Center. Dr. Hansen attended medical school at the University of Washington School of Medicine, and completed residency at University of Washington Medical Center. He began his career as an Assistant Professor at the University of Washington in 1971.

Known as the "father of modern traumatology", his innovations of aggressive and expeditious orthopaedic care of polytrauma patients has revolutionized the field. Moreover, he is recognized as a leading expert on complex foot and ankle reconstruction after trauma as well as the treatment of congenital conditions and deformities of disease. He is also continues to be involved with research, as an editorial board member of Clinical Orthopedics and Related Research, and is on the AO International Board of Directors and Maurice E. Müller Foundation of North America Board of Directors. Additionally, he is on the Hansen Chair Committee, and a member of the Board of Directors for Prosthetics Research Study in Seattle, and Director of Special Teams for Amputations, Mobility, Prosthetics/Orthotics (STAMP) in Seattle.

Dr. Hansen delivered two thought provoking lectures during his visit. The first lecture focused on current principles of muscle balancing and tendon transfer. Focusing on the foot, he discussed the importance of understanding the normal biomechanics before developing an operative plan to treat a disorder. The eventual division of treatment normally involves fusion of stability joints while performing soft tissue procedures for mobility joints.

Following a short break, Dr. Keith L. Wapner introduced the lectureship, which is dedicated to his late wife. Delivering a moving speech about his wife, her tenacious spirit and devotion to their two sons, he discussed the establishment of the June C. Wapner Memorial lecture.

After Dr. Wapner's remarks, Dr. Hansen discussed the role of shear strain in fixation with examples from the foot and ankle. He noted that compression is not the only important factor for producing a bony union but rather a construct that adequate limits movement in a stable fashion. These treatment principles can be difficult with small short bones in the foot, but he gave examples to demonstrate techniques to overcome these challenges.

The remaining two hours of Grand Rounds was spent discussing case presentations presented by residents and fellows. The University of Pennsylvania Department of Orthopedics was honored to have Dr. Sigvard T. Hansen as the June C. Wapner Memorial Lectureship Visiting Professor on December 3nd, 2015.

Guest Lecturer: Dr. Andrew J. Weiland January 28, 2016

Cody D. Hillin, MD MS

The University of Pennsylvania Department of Orthopedic surgery was honored to welcome Dr. Andrew J. Weiland, Professor of Orthopaedic Surgery and Plastic Surgery at the Weill Cornell Medical College. Dr. Weiland attended medical school at the Bowman Gray School of Medicine of Wake Forest University, and completed residency in orthopaedic surgery at the Johns Hopkins Hospital. He subsequently completed ASIF fellowship training in fracture internal fixation in Switzerland, and in upper extremity surgery at the Christine Kleinert Institute.

Clinically, he focuses on disorders of the hand, wrist, and elbow, including reconstructive microsurgery. His leadership ability is shown by his numerous positions including past President of the American Society for Reconstructive Microsurgery (1991), the American Society for Surgery of the Hand (1995), the American Orthopaedic Association (1998-1999), and the American Board of Orthopaedic Surgery (1998-1999). He has also served as the Treasurer of AAOS (2000-2003) and has authored over 250 papers dealing with the hand, wrist, and elbow. Currently he is on the Board of Trustees of the Journal of Bone and Joint Surgery.

Dr. Weiland delivered two thought provoking lectures, followed by a cadaver dissection in the Human Tissue Lab during his visit. The first lecture focused on the approach to scaphoid fracture management. He discussed the treatment algorithm and commonly encountered problems related to scaphoid fractures, which were demonstrated by case examples. For the second talk, he discussed the current trends in distal radius fracture treatment, covering common and new techniques.

Following the first two lectures, attendees moved to the Human Tissue Lab. In the laboratory, Dr. Weiland gave a short talk on basal joint arthritis of the hand. He discussed the importance of identifying the location of disability and pain in order to determine the best treatment. Moving to the cadaver, he demonstrated his preferred techniques for stabilizing the CMC joint, as well as performing prosections of the forearm.

The University of Pennsylvania Department of Orthopedics was honored to have Dr. Andrew J. Weiland as a visiting professor on January 28th, 2016.

Guest Lecturer: Robert Turcotte, MD, FRCS(C) February 11th 2016

Russell Stitzlein, MD

The University of Pennsylvania Department of Orthopaedics was privileged to host Dr. Robert Turcotte as a guest lecturer on February 11, 2016. Dr. Turcotte came to us from McGill University, where he practices orthopaedic oncology and serves as the Maurice E. & Marthe Muller Chair for the Division of Orthopaedic Surgery and as the Medical Director for the Supraregional Sarcoma Program. Additionally, he holds the position of International Professor of the Orthopaedic Research Chair in the Department of Orthopaedics at King Khalid University Hospital in Riyadh, Saudi Arabia.

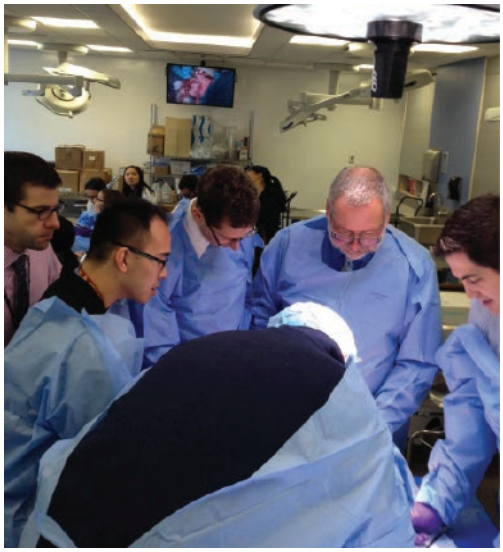
Dr. Turcotte attended Université de Montréal for medical school and then furthered his training in the Édouard-Samson Orthopaedic Program at Université de Montréal. He then had the opportunity to train in orthopaedic oncology and adult reconstructive surgery of the lower limb at the prestigious Hôpital Cochin in Paris, France. He followed that with a fellowship in orthopaedic oncology at Mayo Clinic, under the direction of master clinician, Dr. Franklin Sim. While at McGill, Dr. Turcotte has become a leader in the field of musculoskeletal oncology and limb salvage. His dedication to training the next generation of surgeons led to the establishment of a fellowship program in 2000, training fellows in orthopaedic and general surgical oncology. Dr. Turcotte is also a dedicated clinical researcher, holding numerous active externally-funded research grants and authoring several basic science and clinical research peer-reviewed publications.

Dr. Kristy Weber provided a welcoming opening introduction befitting of a friend and colleague, having also trained under Dr. Frank Sim for her orthopaedic oncology fellowship. Dr. Turcotte then lectured to a mixed audience of Penn medical students, residents and faculty on his more than 25 years experience with endoprosthetic stem fixation. Dr. Turcotte explained that tumor surgeons face much different scenarios than in standard joint replacement surgery. For hip and knee replacements, minimal bone is resected, leaving the metaphysis intact and the traditional teachings of cementless and cemented fixation likely hold true. In tumor surgery, quite often the metaphyseal bone is also resected along with a varying portion of diaphyseal bone, leaving a "tube" of bone within which to obtain fixation. He argued that the traditional teaching of leaving a 2mm cement mantle means that a smaller implant must be used or more strong cortical bone must be removed to allow for a larger implant. Both of these weaken the construct and lead to failures. He showed excellent long-term results for a "line-to-line" reaming with cemented fixation, noting that often the cement mantle is discontinuous in this technique. Dr. Turcotte's talk was very well received and led to engaging discussion among the audience.

After a brief interlude, Dr. Turcotte's second talk was preceded by a nostalgic introduction by Dr. Scott Levin, reflecting on their time together travelling as a part of the 1995 ABC Travelling Fellowship. Dr. Turcotte then gave an excellent lecture geared towards resident education on the management of Giant Cell Tumors (GCT) of bone. Dr. Turcotte described how the classification and location of GCTs determines whether curettage and void filling techniques are adequate or whether more extensive resection +/- reconstruction is required. He also discussed newer targeted therapies for GCT, including denosumab, can be utilized as an adjunct or as definitive treatment. He did, however, warn against indiscriminant use of these agents as they can complicate the surgical management in some instances.

Upon completion of the second lecture, Dr. Turcotte led a cadaveric session in the Penn Human Tissue Lab on lower extremity amputations. Dr. Turcotte progressively took the residents through the Chopart, Symes, transtibial (below knee), knee disarticulation and transfemoral (above knee) amputations, providing "tips and tricks" for each of the amputation levels. The session was well received by the residents, many of whom have not yet had the opportunity to perform many of these levels of amputation.

The University of Pennsylvania Department of Orthopaedic Surgery was honored to host Dr. Robert Turcotte as a visiting professor on February 11, 2016. Dr. Turcotte's passion for his work and dedication to resident/fellow education were evident in his time spent with our department. We hope his visit inspires our residents and medical students to be lifelong learners and to show such commitment to their chosen fields of specialty.



Guest Lecturer: Dr. Fred Sweet, MD February 18, 2016

Chase Woodward MD MPH

The University of Pennsylvania Department of Orthopaedic Surgery was honored to welcome spine surgeon Dr. Fred Sweet, a co-founder of the Rockford Spine Institute and clinical assistant professor at the University of Illinois College of Medicine, as a visiting professor this past February. The educational visit was generously supported by AOSpine North America. Dr. Sweet attended Rush University Medical School in Chicago and completed an internship at the Naval Medical Center in San Diego. He subsequently completed his residency in orthopaedic surgery at Washington University in St. Louis and then stayed to complete their prestigious spinal deformity fellowship. He is a member of the American Academy of Orthopaedic Surgeons, North American Spine Society, and the Scoliosis Research Society. Notably Dr. Sweet served in the United States Navy as a medical officer aboard the U.S.S. Coronado during the first Gulf War.

After arriving in Philadelphia Dr. Sweet was welcomed at the home of Dr. Vincent Arlet, faculty host of his visit and chief of orthopaedic spine surgery at the University of Pennsylvania. Thereafter he was escorted to a nearby restaurant where he met many of the spine surgery staff members, clinical and research residents, and their significant others. Dr. Sweet shared many stories about his career including the rigorous nature of his spine deformity fellowship, founding and managing a successful private practice, and maintaining his interest in basic science research. He specifically mentored residents interested in spine surgery about what they should seek in a fellowship program and how it will benefit their future practice. There was also a rich discussion about new concepts in spinal surgery and the changing landscape of orthopaedics in general, and the residents enjoyed hearing Dr. Sweet's perspective as a private practice surgeon. We were pleased to learn about his aviation hobby and that he had actually flown himself from Illinois to a Philadelphia regional airport earlier that day.

On the second day of his visit Dr. Sweet lectured at the department's Grand Rounds meeting where he was introduced by Dr. Arlet to the large gathering of faculty, residents, and medical students. Dr. Sweet's first lecture was on the transforaminal anterior release (TFAR) technique that he developed and published. The technique involves a circumferential soft tissue release of the annulus of an intervertebral disc at a strategically chosen level allowing for significant coronal and sagittal deformity correction in a setting typically necessitating an osteotomy. Dr. Sweet has series of more than 50 adult deformity patients who have undergone this technique with good results, specifically with low blood loss and low rate of neurologic complication compared to traditional multiple column osteotomies. The second lecture was an overview of his experience applying vancomycin powder to surgical wounds. This lecture was particularly interactive, for example he asked residents to list common techniques employed to lower the risk of surgical site infections, and often times Dr. Sweet challenged the effectiveness of certain techniques we historically believe lower infection rates. He presented very provocative animal research he has personally conducted showing the efficacy of intra-wound local antibiotic delivery compared to intravenous administration.The residents left the auditorium with a critical new outlook regarding perioperative antibiotic use.

After the morning lecture Dr. Sweet was escorted to the department's state of the art Human Tissue Lab to demonstrate the transforaminal anterior release he presented earlier in the morning. Residents took turns assisting Dr. Sweet as he exposed the posterior spine of a cadaver, placed thoracic and lumbar pedicle screws, performed the transforaminal soft tissue release, and finally realigned the spinal column and fixed with an instrumentation system. The residents asked to see Dr. Sweet's technique for placing sacral screws and he gladly demonstrated his preferred method and carefully explained his rationale. Several residents commented on the high quality of the dissection and how it was one of the most instructive cadaver sessions of the academic year.

To conclude his visit Dr. Sweet had a chance to visit and tour the department's prized McKay Orthopaedic Research Laboratory under the direction of Drs. Louis Soslowsky and Robert Mauck. Thereafter the residents transported him across town to Pennsylvania Hospital for the remainder of the day so he could observe the surgical practice of Dr. Arlet. The University of Pennsylvania Department of Orthopaedic Surgery was truly fortunate to have Dr. Sweet as a visiting professor, and pleased to start what we hope to be is a successful and mutually beneficial relationship.

Guest Lecturer: Dr. Andrea Ferretti, MD March 10, 2016

James M Friedman MD

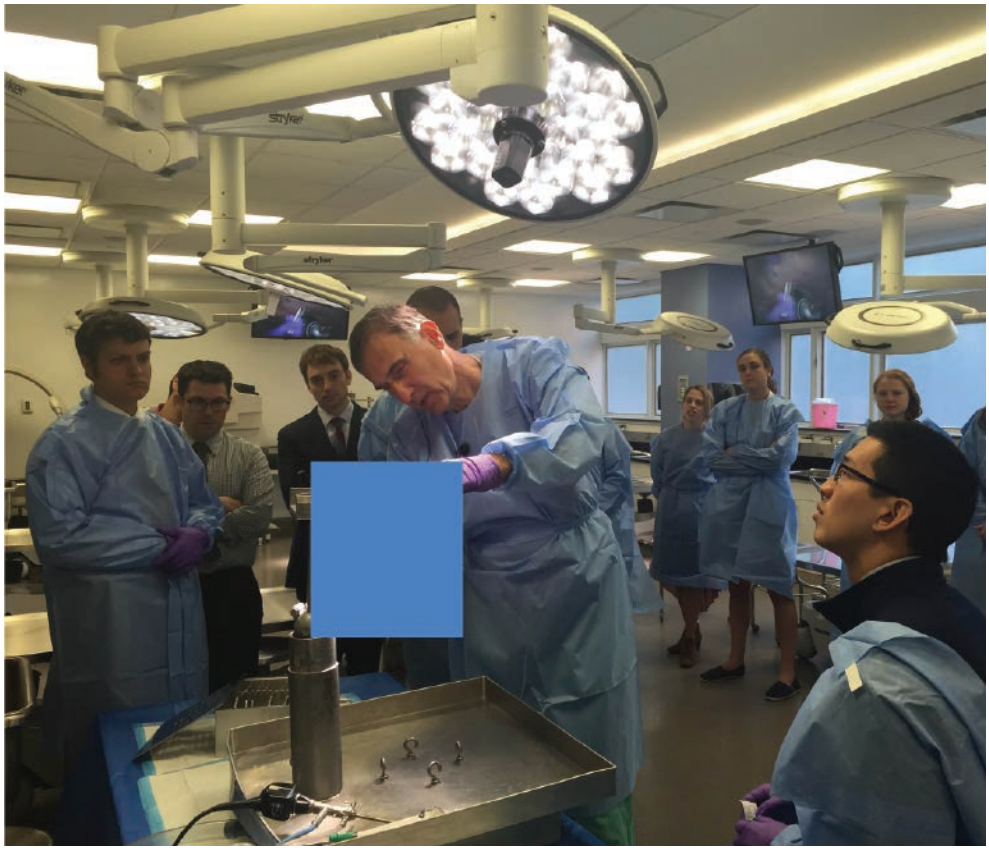
On March 3, 2016 The University of Pennsylvania, Department of Orthopedics was pleased to welcome Dr. Andrea Ferretti, MD from La Sapienza University, Rome, Italy. Dr. Ferretti serves as the chairman of orthopaedics and traumatology at La Sapienza University as well as the director of the orthopaedic postgraduate school. He also serves as the orthopaedic department chief of the Sant'Andrea Hospital. Dr. Ferretti specializes in sports related trauma and has authored or co-authored over 200 articles related to athletic injuries.

Dr. Ferretti delivered two lectures, both focused on his work regarding the anterolateral ligament (ALL) of the knee. In his first lecture he described the ALL as a potential secondary stabilizer of the ACL. He first described the anatomy of the ALL, and showed that anatomically it should provide protection from tibial internal rotation. Using cadaver models as well as human case studies, Dr. Ferretti showed that high grade pivot shifts following known ACL tears correlate with damaged ALL structures. Alternatively an intact ALL following ACL injury is associated with low grade pivot shift. His conclusion is that the ALL acts a secondary rotary stabilizer to the ACL.

In his second lecture Dr. Ferretti expounded on his findings to surgical management of the unstable knee following ACL injury. He offered that a pivot shift should be performed under anesthesia for every ACL deficient knee. In knees with a high grade pivot shift the ALL should be examined and repaired in addition to the ACL reconstruction. To back up his philosophy Dr. Ferretti included pictures of recent surgeries where significant ALL damage was discovered following high grade pivot shifts.

Following his lectures Dr. Ferretti led a human cadaver session. The ALL was dissected out and the anatomy explained. Techniques for ALL reconstruction was then discussed.

Dr. Ferretti delivered a series of outstanding lectures and led a comprehensive cadaver course. His ability to explain his findings, supported by both data and personal experience, proved an invaluable learning experience surrounding a controversial topic.



Leo Leung Memorial Lectureship March 17, 2016 Guest Lecturer: James Chang, MD

Cody D. Hillin, MD MS

This March, the University of Pennsylvania Department of Orthopedic surgery was honored to welcome Dr. James Chang as the 10th annual Leo Leung endowed lectureship. Dr. Chang is a Johnson & Johnson Distinguished Professor and Chief of the Division of Plastic and Reconstructive Surgery at Stanford University. Dr. Chang was an undergraduate at Stanford University, then attended Yale Medical School, and was subsequently a Sarnoff Laboratory Research Fellow at UCSF. He then completed a residency in plastic and reconstructive surgery at Stanford University Medical Center, and a fellowship in hand & microsurgery at UCLA. He is currently professor of both plastic and orthopaedic surgery with primary clinical interests in microsurgical extremity reconstruction, pediatric hand, and post-oncologic head and neck reconstruction.

Dr. Chang's basic science research interests include modulation of TGF-β in scarless flexor tendon wound healing and tissue engineered flexor tendon grafts for hand reconstruction. He is the recipient of numerous grants and has been federally-funded for his research since 1998. Dr. Chang continues his passion for research as an Associate Editor for Journal of Hand Surgery, Annals of Plastic Surgery, Hand, and Microsurgery. His leadership abilities are clear as he is in the presidential line for the ASSH, currently serving as vice-president and previously as treasurer and research director. Dr. Chang is dedicated to education and is vice-chair of the ACGME Plastic Surgery Residency Review Committee and the secretary/treasurer of the American Board of Plastic Surgery.

Dr. Chang started by giving an inspiring lecture on the use of art to teach anatomy. He discussed his inspiration from Rodin's collection of hand artwork, which always seemed to mesmerize him. Seeking more knowledge about them he developed relationships with the museum staff, eventually leading to the development of an installation at the museum. The exhibit he helped teach the underlying anatomy of the hand and demonstrate disease processes well illustrated by Rodin. This lecture emphasized the importance of observation, and dedication to dissemination of knowledge to others.

After a brief intermission, Dr. Bozentka introduced the lectureship, which was established in memory of Dr. Leo Leung, an orthopaedic surgery resident at University of Pennsylvania from 1998-2002. Dr. Leung passed away suddenly during his chief year of residency in 2002. His mentors and

colleagues founded the lectureship to honor his commitment and dedication to hand surgery, education, patient care, and research. He was affectionately known as "Leo the Lion" and "The Iron Leung" for his extraordinary work ethic and unrelenting commitment to the residency program.

Following Dr. Bozentka's remarks, Dr. Chang discussed the various ways to address the absence of a thumb. Physical exam and knowledge of anatomy are vital to understand what components are available to restore both structure and function. Case examples were utilized and discussion with the audience provided additional perspective to this important problem.

Following these lectures, the attendees moved to the Human Tissue Lab. After a brief lecture by Dr. Chang, he led a cadaveric dissection of commonly used forearm flaps. He was assisted by several residents, and provided valuable pearls and pitfalls to performing these varied procedures. The University of Pennsylvania Department of Orthopedics was honored to have Dr. Chang as the Leo Leung Memorial Lectureship Visiting Professor on March 17th, 2016.

Chief Residents

P. Max Courtney, MD

Hometown: Norristown, PA
Undergraduate: Washington & Lee University
Medical School: Georgetown School of Medicine
Residency Highlights: Marrying my beautiful wife Courtney, mentorship from our dedicated faculty, and becoming lifelong colleagues and friends with our phenomenal class
Future Directions: Adult Reconstruction Fellowship at Rush University

Stephen Y. Liu, MD

Hometown: Andover, MA
Undergraduate: Tufts University
Medical School: Tufts University School of Medicine
Residency Highlights: Trauma moving to PPMC and the opening of PMUC and ASC
Future Directions: Pittsburgh for Hand Fellowship and then wherever the wind takes me

Michael H. McGraw, MD

Hometown: Lansdale, PA
Undergraduate: Howard University
Medical School: Howard University College of Medicine
Residency Highlights: Mentorship of Dr. John Kelly in Sports Medicine and arthroscopy
Future Directions: Sports Medicine and Shoulder Fellow at Hospital for Special Surgery

Christopher M. Melnic, MD

Hometown: Allentown, PA
Undergraduate: Boston College
Medical School: Tufts University School of Medicine
Residency Highlights: Forming incredible friendships within my class, and all the teaching from them and the attendings.
Future Directions: Adult Reconstruction Fellowship at Rush University

Andrew H. Milby, MD*

Hometown: Abington, PA
Undergraduate: Washington University
Medical School: University of Pennsylvania
Residency Highlights: Working in the trenches with fellow residents and students, spending a year in the McKay lab, interacting with numerous visiting professors and seeing the incredible growth of the department over the past 10 years.
Future Directions: Spine Fellowship at Emory University, followed by an academic career.

Nicholas Pulos, MD

Hometown: Los Angeles, CA
Undergraduate: University of Pennsylvania
Medical School: University of Pennsylvania
Residency Highlights: At some point residency stopped feeling like work, but nothing compares to time spent at home with Bridget, Ryan and Andrew
Future Directions: I hope to one day train the next generation of orthopedic surgeons.

Jonathan Slaughter, MD

Hometown: Tipp City, OH
Undergraduate: University of Pennsylvania
Medical School: Boonshoft School of Medicine at Wright State University
Residency Highlights: The birth of my second child, my son Luke, working with my classmates, enjoying time outside the hospital with the best orthopaedic residents in the country, training under master surgeons, becoming part of the Penn Orthopaedic family, and exploring the east coast with my family.
Future Directions: Mary S. Stern Fellowship in Hand Surgery in Cincinnati, Ohio.

Sarah M. Yannascoli, MD*

Hometown: Syracuse, NY
Undergraduate: Cornell University
Medical School: Albert Einstein College of Medicine
Residency Highlights: The trenches of intern and second year, a peaceful and productive lab year when I married my amazing husband John, the incredible faculty mentorship throughout residency, the lifelong friends, and eventually being allowed to graduate!
Future Directions: Career in academic Hand and Upper Extremity Surgery

Current Residents

Clinical Year 4

Jason B. Anari, MD

Undergraduate:
The College of New Jersey

Medical School:
Robert Wood Johnson Medical School at Rutgers University (UMDNJ)

Joshua Gordon, MD*

Undergraduate:
Pitzer College

Medical School:
David Geffen School of Medicine at UCLA

Philip A. Saville, MD

Undergraduate:
University of Leicester

Medical School:
University of Leicester (England, UK)

Vishal Saxena, MD*

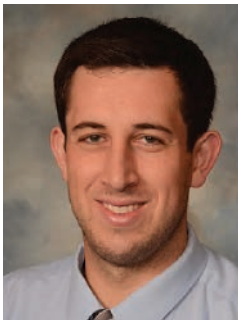
Undergraduate:
Northwestern University

Medical School:
Pritzker School of Medicine at the University of Chicago

Russell N. Stitzlein, MD

Undergraduate:
Miami University

Medical School:
Cleveland Clinic Lerner College of Medicine



Michael T. Talerico, MD

Undergraduate:
University of Notre Dame

Medical School:
Saint Louis University School of Medicine

Nathan A. Wigner, MD, PhD

Undergraduate:
North Carolina State University

Medical School:
Boston University School of Medicine

Chase Woodward, MD, MPH

Undergraduate:
Northwestern University

Medical School:
Feinberg School of Medicine at Northwestern University

Clinical Year 3

Keith P. Connolly, MD

Undergraduate:
Michigan State University

Medical School:
University of Central Florida College of Medicine

Daniel P. Lim, MD

Undergraduate:
University of Southern California

Medical School:
Keck School of Medicine at USC

Tyler R. Morris, MD*

Undergraduate:
The University of Pennsylvania

Medical School:
Drexel University College of Medicine

Alexander L. Neuwirth, MD*

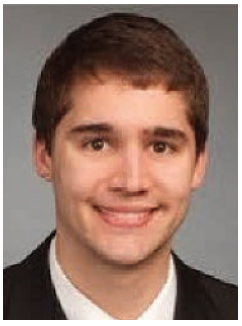
Undergraduate:
Rutgers University

Medical School:
Robert Wood Johnson Medical School at Rutgers University (UMDNJ)

Joshua C. Rozell, MD

Undergraduate:
Emory University

Medical School:
Drexel University College of Medicine



Joshua T. Steere, MD

Undergraduate:
Creighton University

Medical School:
Stritch School of Medicine at Loyola University Chicago

Chia H. Wu, MD, MBA

Undergraduate:
University of Pennsylvania

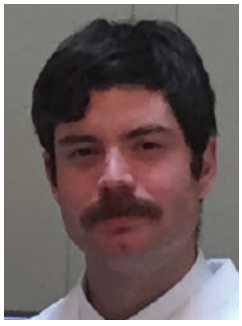
Medical School:
Perelman School of Medicine at the University of Pennsylvania

Zachary R. Zimmer, MD

Undergraduate:
Colgate University

Medical School:
Stony Brook University School of Medicine

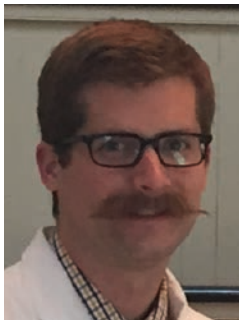
Research Year



James M. Friedman, MD*

Undergraduate:
Duke University

Medical School:
Duke University School of Medicine

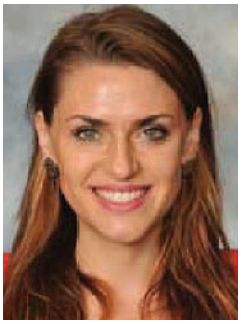


Cody D. Hillin, MD, MS*

Undergraduate:
University of Rochester

Medical School:
Baylor College of Medicine

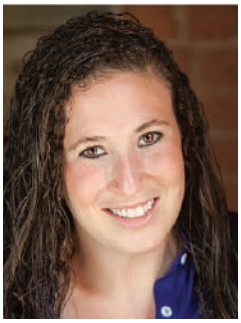
Clinical Year 2



Blair S. Ashley, MD*

Undergraduate:
The College of
William and Mary

Medical School:
University of Pittsburgh
School of Medicine



Jenna A. Bernstein, MD

Undergraduate:
Cornell University

Medical School:
University of Connecticut
School of Medicine

Kristin Buterbaugh, MD

Undergraduate:
Northwestern University

Medical School:
Icahn School of Medicine
at Mount Sinai

Jose A. Canseco, MD, PhD

Undergraduate:
Massachusetts Institute
of Technology

Medical School:
Harvard Medical School

Jonathan R. Dattilo, MD

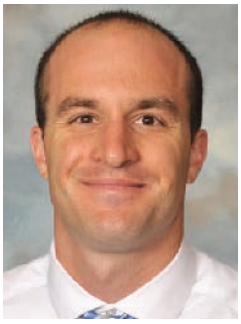
Undergraduate:
Northwestern University

Medical School:
Johns Hopkins University
School of Medicine

Daniel Gittings, MD*

Undergraduate:
Providence College

Medical School:
Boston University
School of Medicine



Luke A. Lopas, MD

Undergraduate:
University of
Wisconsin-Madison

Medical School:
University of Wisconsin
School of Medicine &
Public Health

Nicole A. Zelenski, MD

Undergraduate:
Bryn Mawr College

Medical School:
Duke University
School of Medicine

Clinical Year 1

Alexei Adan, MD

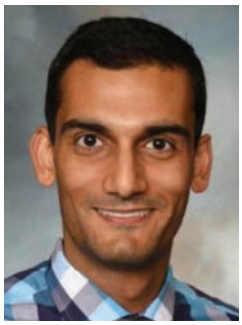
Undergraduate:
Cornell University

Medical School:
University of Pennsylvania

Ryan Charette, MD

Undergraduate:
University of Connecticut

Medical School:
University of Connecticut School of Medicine



Adnan Cheema, MD*

Undergraduate:
University of Missouri-Kansas

Medical School:
University of Missouri-Kansas School of Medicine

Michael Eby, MD, MS*

Undergraduate:
University of Pennsylvania

Medical School:
Georgetown University School of Medicine

Rikesh Gandhi, MD

Undergraduate:
Boston College

Medical School:
Duke University School of Medicine

Mark Hasenauer, MD

Undergraduate:
Boston College

Medical School:
New York Medical College

Matthew Sloan, MD, MS

Undergraduate:
University of Massachusetts

Medical School:
University of Massachusetts Medical School

Andrew Tyler, MD, PhD

Undergraduate:
Harvard University

Medical School:
University of Texas at Dallas Southwestern Medical School

University of Pennsylvania Orthopaedic Surgery Alumni Class of 2001

Juan C. Alvarez, MD
Adult Reconstruction
Florida Bone and Joint Centers
Winter Haven, FL

Keith R. Brookenthal, MD
Pediatrics
Children's Hospital Los Angeles
Calabasas, CA

Max W. Cohen, MD
Spine
Greensboro Orthopedic Center
Greensboro, NC

John N. Diana, MD
Adult Reconstruction
Napa Valley Orthopaedics
Napa, CA

Paul J. King, MD
Adult Reconstruction
Anne Arundel Medical Group
Arnold, MD

Stephen C. Umansky, MD
Sports Medicine
Lexington Clinic
Versailles, KY

Steven Y. Wei, MD
Sports Medicine
Seacoast Orthopedic Surgery & Sports Medicine
Groton, CT

Kirk L. Wong, MD
Hand & Upper Extremity
Northwest Surgical Specialist
Vancouver, WA

George L. Yeh, MD
Hand & Upper Extremity
Potomac Valley Orthopaedics Associates
Rockville, MD

The University of Pennsylvania Orthopaedic Journal

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