The PSRS, founded in 2004, was set up to bring together members of the Greater Philadelphia community of basic scientists, bioengineers and spine surgeons concerned with studies of the intervertebral disc and the associated spinal tissues.

Sriram Balasubramanian & Michele Marcolongo | Drexel University
Meeting Co-Chairs

ORGANIZING COMMITTEE
Dawn Elliott | University of Delaware
James Iatridis | Mount Sinai, NY
Neil Malhotra | University of Pennsylvania
Makarand Risbud | Thomas Jefferson University
Tom Schaer | University of Pennsylvania
Irving Shapiro | Thomas Jefferson University
Lachlan Smith | University of Pennsylvania
Ed Vresilovic | Pennsylvania State University

JEFFERSON MEDICAL SCHOOL • UNIVERSITY OF PENNSYLVANIA • DREXEL UNIVERSITY
UNIVERSITY OF DELAWARE • MOUNT SINAI • PENNSYLVANIA STATE UNIVERSITY
Program

7:30 – 9:00 AM – Poster Setup
8:00 – 9:00 AM – Registration and Breakfast
9:00 – 9:05 AM – Welcome

Session One – Research In Industry
9:05 – 10:15 AM – Moderators: Michele Marcolongo, Irving Shapiro

Brian S. Snyder
Keynote Talk: Growth Modulation of The Spine for Treating Scoliosis: Establishing the Safety and Efficacy of a New Treatment Paradigm

Kyle Malone
Research and Innovation in Spine: A Perspective from Industry (NuVasive, Inc.)

Brandon Bucklen
Closing the Gap of Translational Medicine in Spine: A Practical Framework for R&D (Globus Medical, Inc.)

Lara Ionescu Silverman
Preclinical Development of an Allogeneic Cell Therapy (DiscGenics, Inc.)

Question and Answer Session

10:15 – 10:30 AM – Coffee Break and Poster Session
Poster presenters available to answer questions

Session Two – Development and Homeostasis
10:30 AM – 12:00 PM – Moderators: Makarand Risbud, James Iatridis

John R. Bethea
Keynote Talk: Sex/Gender and Chronic Pain: The Role of TNF

Timothy Jacobsen
Rho Pathway Activation Protects Against Inflammatory Induced Alterations in Biomechanics of Cells from the Nucleus Pulposus in a Myosin-II Contractility Dependent Mechanism

Zariel I. Johnson
TonEBP Maintains Inflammatory Gene Expression in the Hyperosmotic Nucleus Pulposus

Rose G. Long
GF-β1 Induces a Contractile CD146+ Phenotype of Human Annulus Fibrosus Cells with Affinity to Collagen Scaffold for Annulus Fibrosus Repair

Question and Answer Session 1

Sun H. Peck
Effects of Hypoxia and TGF-β Exposure during Monolayer Expansion on the Survival and Matrix Producing Capacity of Mesenchymal Stem Cells

Elizabeth S. Silagi
HIF-1α-Carbonic Anhydrase Axis Controls Acid-Base Metabolism in Hypoxic Nucleus Pulposus Cells

John P. Dougherty
Region-Specific Orthotropic Growth of the Pediatric Thoracic Spine through Finite Element Methods

Question and Answer Session 2

12:00 – 1:15 PM – Boxed Lunch and Poster Session
Poster presenters available to answer questions

Session Three – Pathophysiology
1:15 – 2:50 PM – Moderators: Neil Malhotra, Dawn Elliott

Amer Samdani
Keynote Talk: Growth Modulation Strategies to Treat Skeletally Immature Scoliosis

James R. Peters
Normalcy of Vertebral Morphology for Lenke-types 1, 2, and 5 Two-Years Following Spinal Fusion Surgery

Thomas Evashwick-Rogler
Disc Height Loss, But Not Pro-Inflammatory Cytokine or Substance P Expression, Predicts Intervertebral Disc Degeneration-Related Pain in a Rat Model

Paul Millhouse
Does Baseline Sagittal Balance Influence the Surgical Outcomes of Patients with Cervical Myelopathy?

Question and Answer Session 1
Bhranti S. Shah ........................ High Mobility Group Box-1 Promotes Pro-inflammatory Signaling in Human Nucleus Pulposus Cells via Toll-Like Receptor 4

Sara Ernst .......................... Neuropeptide Y Promoter Variant Correlates with Decreased Pain at Baseline in Older Adults with Mild to Moderate Lumbar Spinal Stenosis but a Blunted Functional Response to Exercise-based Treatment Protocols

Jason Wong .......................... Identification of the Compositional Traits and Permeabilities of the Cartilage Endplate that are Required for Nutrient Transport and Disc Cell Survival

Shania Shaji .......................... Changes in Muscle Mass and Bone Morphology of the Spine and Lower Limb after Spinal Chord Injury and Treadmill Training

**Question and Answer Session 2**

2:50 – 3:30 PM – Coffee Break and Poster Session
Poster presenters available to answer questions

**Session Four – Diagnostics and Therapeutics**
3:30 – 5:05 PM – Moderators: Lachlan Smith, Thomas Schaer

Daniel H. Cortes ........................ Keynote Talk: Imaging Back Pain

Christian I. Weber ........................ Load-sharing of the Intervertebral Discs During Standing Determined by Open-Upright Magnetic Resonance Imaging

Forbes E. Howington ..................... Predicting Intradiscal Pressure in the Cervical Spine Based on Disc Height

Olivia M. Torre .......................... Development of an In Vivo Model of Neonatal Intervertebral Disc Injury and Regeneration

**Question and Answer Session 1**

Sarah E. Gullbrand ..................... Engineered Endplates Enhance the In Vivo Performance of a Replacement Disc-Like Angle Ply Structure (DAPS)

Kyle Meadows .......................... FLASH MRI for Potential Clinical Diagnostics of Cartilaginous Endplate Discontinuities

Thomas Christiani ...................... Injectable Scaffolds with Bioadhesive Properties for the Regeneration of the Annulus Fibrosus and Nucleus Pulposus of the Intervertebral Disc

Huizi Anna Lin .......................... Injectable Cellulosic Hydrogels as Nucleus Pulposus Replacements: Assessment of Herniation Risk, Fatigue Behavior, and In Vivo Biocompatibility

**Question and Answer Session 2**

5:15 – 5:30 PM – Concluding Remarks and Presentation of Awards

5:30 – 7:00 PM – Networking Reception and Posters

Gift Card Prizes for Top Three Podium Presentations and Poster Presentations
1st – $300, Certificate, Souvenirs
2nd – $200, Certificate, Souvenirs
3rd – $100, Certificate, Souvenirs
Posters

Justin R. Bendigo
Novel Techniques for the Evaluation of Physical Activity in a Large Animal Intervertebral Disc Degeneration Model

Hyowon Choi
TonEBP-COX-2 Axis Promotes Nucleus Pulposus Cell Survival Under Hyperosmotic Conditions

Michelle A. Cruz
Cell-Seeded Adhesive Biomaterial for Repair of Annulus Fibrosus Defects in Intervertebral Discs

John DeLucca
Effect of Multiaxial Mechanical Loading on Creep Recovery in Human Intervertebral Discs

Philip F. Giampietro
Approaches to Understanding the Genetics of Vertebral Malformations

Weiyoung Gu
Effect of Dynamic Loading on the Transport of Charged Antibiotics in Human Intervertebral Discs

Sarah E. Gullbrand
Scale Up of Disc-Like Angle Ply Structures (DAPS) for Total Disc Replacement in Preclinical Animal Models

Michelle A. Cruz
Cell-Seeded Adhesive Biomaterial for Repair of Annulus Fibrosus Defects in Intervertebral Discs

John DeLucca
Effect of Multiaxial Mechanical Loading on Creep Recovery in Human Intervertebral Discs

Philip F. Giampietro
Approaches to Understanding the Genetics of Vertebral Malformations

Weiyoung Gu
Effect of Dynamic Loading on the Transport of Charged Antibiotics in Human Intervertebral Discs

Sarah E. Gullbrand
Scale Up of Disc-Like Angle Ply Structures (DAPS) for Total Disc Replacement in Preclinical Animal Models

Paul Millhouse
Which Domains of the NDI Improve Most After Surgery for Cervical Myelopathy?

Paul Millhouse
Does Baseline Sagittal Balance Influence the Surgical Outcomes of Patients with L4/5 Degenerative Spondylolisthesis?

Paul Millhouse
Does Sagittal Balance Influence Baseline Pain in Patients with L4/5 Degenerative Spondylolisthesis?

Quynhhoa T. Nguyen
Effects of Inflammation on Cellular Deformation of Nucleus Pulposus Cells: A Biphasic Finite Element Model

Sun H. Peck
Rescuing Chondrocyte Hypertrophic Differentiation Potential and Exploring Therapeutic Approaches for Enhancing Bone Formation in Mucopolysaccharidosis VII Dogs

Sun H. Peck
Growth Factor and Extracellular Matrix Expression and Localization during Nucleus Pulposus Formation

James R. Peters
Normative Morphology and Growth of the Pediatric Lumbar Vertebrae

Evan Phillips
Arthritic Human Cartilage Molecular Engineering Using Biomimetic Proteoglycans Shows Infiltration throughout the Cartilage Extracelluar Matrix Ex Vivo

Matthew Piazza
Comparison of Quantitative Imaging Techniques for Detecting Degenerative Changes in a Mouse Caudal Intervertebral Disc Injury Model

Gregory D. Schroeder
Biologic Response of Human Nucleus Pulposus Cells to Treatment with Soluble Collagen VI

Gregory D. Schroeder
Correlation Between Interleukin-6 Serum Levels and Pre-Operative, Health-Related Quality of Life Outcome Metrics in Patients Undergoing an Anterior Cervical Discectomy and Fusion for Cervical Radiculopathy

Mingkun Wang
Grow and Differentiate Stem Cells in Macroporous Scaffolds and Potential Application to Intervertebral Disc Repair

Wenhai Wang
How Does Rod Diameter and Material Affect Motion and Stress Concentrations in Lumbopelvic Reconstructions? - A Finite Element Analysis

Timothy Walden
Serum MMP-9 levels Correlate with the Severity of Intervertebral Disc Herniation in Patients

Tian Zhang
Overexpression of Human Interleukin (IL)-8 in Mouse Intervertebral Disc Tissue to Model Patients with Back Pain
Keynote Talks
John R. Bethea, PhD
Professor and Head of Biology
Drexel University

Keynote Talk: The role of TNF signaling in neuropathic pain and gender differences

Abstract
Tumor Necrosis Factor (TNF) is a proinflammatory cytokine which is involved in physiological and pathological processes, systemically and within the central nervous system (CNS), and has been found to be crucial in pain development. There are two biologically active forms of TNF, soluble TNF (solTNF) and transmembrane TNF (tmTNF) that preferentially bind to TNFR1 and TNFR2 respectively. In the current study, we are interested in the effects of specifically blocking TNFR1 signaling in neuropathic pain following peripheral nerve injury in a mouse model with the use of both transgenic mice and drug therapy. After undergoing chronic constriction injury (CCI) in the sciatic nerve, knockout mice lacking TNFR1 (TNFR1 -/-) fail to develop a standard neuropathic pain response and depressive symptoms which typically occur after the pain. Furthermore, following CCI we observe a decrease in hippocampal neurogenesis following pain onset, but this decrease is not observed in TNFR1-/- mice. To investigate the therapeutic effects of inhibiting TNFR1 signaling after injury, we delivered XPro1595, a novel drug which creates a biologically inactive form of solTNF, to mice following CCI both systemically and within the CNS. Inhibition of solTNF signaling both via peripheral and central delivery of XPro1595 resulted in an accelerated recovery from neuropathic pain which began after 2 weeks of drug delivery. To begin exploring a mechanism, we investigated changes in glutamatergic receptors and synaptic markers since neuropathic pain has been shown to invoke an increase in glutamatergic signaling and changes in synaptic plasticity. In male mice, inhibiting solTNF systemically following injury reduces this glutamatergic signaling in the hippocampus and cortex while in female mice we actually observe a decrease in NMDA receptor in the cortex following injury without treatment. Following injury there is a decrease in postsynaptic density which is greater in females than males and which is restored by solTNF inhibition in both genders. Synaptophysin changes in the cortex following injury are female specific. Together, these results suggest that solTNF signaling plays a role in pain induction following CCI in males but not females. This could partly be due to the sexually dimorphic effects of solTNF inhibition on glutamate signaling components and synaptic markers following CCI.

Biosketch
John R. Bethea, PhD, is a professor and head of the Department of Biology at Drexel University. Dr. Bethea received his BS in Biology from Florida International University, and thereafter his PhD in Neuroscience from the University of Alabama at Birmingham. He went on to be a post doctoral fellow of Neuroscience at Case Western Reserve University and of Immunology at The Cleveland Clinic Foundation. His research interests include cell biology, disease biology, genetics and molecular biology, microbiology and immunology, and neuroscience. Dr. Bethea has published his research in several peer-reviewed journals, including the Journal of Neuroscience Research, Journal of Immunology, Journal of Neuroinflammation, Stem Cells, Brain Research, as well as several others.
**Amer Samdani, MD**  
Chief of Surgery  
Shriners Hospitals for Children, Philadelphia, PA

**Keynote Talk: Growth Modulation Strategies to Treat Skeletally Immature Scoliosis**

*Abstract*

Current standard for treatment for skeletally immature scoliosis is either brace or surgery. These strategies offer good results, but several limitations. Brace wear can lead to psychosocial issues decreasing compliance, and surgical fusion limits mobility and some patients may have long term pain. Newer strategies that modulate a child’s growth to control scoliosis may result in a straighter and more mobile spine. These products are in early clinical use, and are currently not FDA approved.

---

**Daniel H. Cortes, PhD**  
Assistant Professor  
Mechanical Engineering Department  
Biomedical Engineering Department

**Keynote Talk: Imaging Back Pain**

*Abstract*

Low-back-pain (LBP) remains a major burden to society with devastating consequences to patients. Current diagnosis and assessment tools heavily depend on the patient’s own perception of symptoms, which can be challenging for populations with cultural, language and cognitive barriers. As a result, evaluating the effectiveness of treatment strategies is difficult due to the subjective nature of these assessment tools. The vast majority of low back pain is mechanical in nature. The intervertebral disc degeneration, herniation and rupture are several common mechanical causes of LBP. In addition to the intervertebral disc, LBP can also come from muscles and ligaments around the spine. Strains and sprains are the most common cause of acute LBP. Although the majority of LBP is acute, 20% of these patients later develop chronic symptoms [11]. Unfortunately, the transition from acute to chronic LBP is not entirely understood. Therefore, quantitative biomarkers for diagnosis, evaluating recovery from LBP, and identifying pathways for chronification of LBP. The purpose of this talk is to present recent advances in imaging techniques to evaluate the function of the intervertebral disc and spinal muscles with special emphasis on imaging of mechanical properties.

The function of the intervertebral disc is mechanical. It transmits forces through the spine while allowing relative motion between the vertebral bodies. Therefore, tissue mechanical properties would be a useful biomarker for diagnosing disc degeneration. Magnetic Resonance Elastography (MRE) has been used to quantify the shear modulus of the disc non-invasively. Ex-vivo MRE experiments showed that it is possible to measure mechanical properties and that these properties decrease with disc degeneration. In-vivo MRE measurements showed also a similar decrease of mechanical properties. However, a fundamental difference between ex-vivo and in-vivo MRE measurements is the frequency of the applied mechanical vibration. In-vivo measurements used a frequency lower than 100 Hz, however, ex-vivo measurements suggested an optimum frequency of one order of magnitude higher. Current efforts to develop a high frequency MRE method for the spine will be presented.

LBP has also been often associated with abnormal muscle properties. The multifidus is the most studied muscles in relation to LBP. Numerous studies have reported associations between LBP, decreased multifidus area, electromyography (EMG) signals, and increased fat infiltration [13]–[20]. It is unknown whether abnormal multifidus properties are a cause or an effect of LBP [17]. Ultrasound shear wave elastography (SWE) is emerging as a superior tool to quantify muscle function. A protocol to evaluate the function of the multifidus muscle using SWE is proposed. The protocol consists of shear-modulus measurements taken at several muscle locations for static and dynamics tasks. For the multifidus muscle, one value of shear modulus is collected on the right and left sides of the L5, L3, L1, T7, and T1 vertebrae. Although the focus of the protocol is to evaluate the lumbar spine, measurements in the thoracic region helps building a robust average for the within-patient analysis (this is also typically done in EMG evaluations). For the static assessment, SWE measurements are taken at two static postures: prone and standing. Dynamic assessment consists symmetric or asymmetric arm lift (symmetric: both arms, asymmetric: one arm at the time). Average values of multifidus muscle shear modulus are presented.

Medical imaging can play a crucial role on the diagnosis and evaluation of treatments for back. More research is needed to develop novel techniques to measure functional parameters of spinal tissues in relation to LBP. Without this techniques, translation of novel treatment strategies could be very difficult. Elastography offers an interesting alternative to this goal.
Industry Talks
Kyle Malone, MS
Director, Clinical Resources and Biostatistics
NuVasive, Inc. San Diego, CA.

Industry Talk: Research and Innovation in Spine: A perspective from industry

Abstract
Preclinical and clinical research on available technology in spine surgery is regularly performed independent of industry sponsorship. This independent venue for the collection of objective evidence plays an important role in supporting evidence-based decision making. While there is a perception of conflict within industry sponsored research, it is more accurate to consider sponsored research as occupying a separate role in either supporting research that otherwise wouldn’t be able to be performed due to financial or material limitations or in pursuing analyses of emerging innovative technology. The purpose of this presentation is to review historical research output on NuVasive’s surgical solutions and to describe future directions for innovation and research.

Lara Ionescu Silverman, PhD
Senior Manager, R&D
DiscGenics, Inc. Salt Lake City, UT.

Industry Talk: Preclinical Development of an Allogeneic Cell Therapy

Abstract
Researchers at DiscGenics discovered a way to generate a therapeutic cell population for use in treating disease of the intervertebral disc. How was this concept translated into a clinically-feasible product? This talk will outline the steps taken to develop, test and optimize a novel cell therapy for the treatment of degenerative disc disease that is appropriate for use in a clinical setting. The in vitro and in vivo supportive studies will be outlined, along with the business, regulatory, manufacturing and quality aspects that contribute to successful translation from the bench to the bedside.

Brandon Bucklen, PhD
Group Manager, BioMechanics Research
Globus Medical, Inc. Audubon, PA.

Industry Talk: Closing the Gap of Translational Medicine in Spine: A Practical Framework for R&D

Abstract
Technical advances or emerging technologies in spine occur in a different manner through industry than what occurs in traditional translational medicine. Specifically, industry is concerned with the practical end of that spectrum, and such advances transpire incrementally, as either part of an R&D framework, through un-planned ‘accidental’ clinical discoveries which are later tested and confirmed through research, and with foreknowledge about the medical scope of regulatory likelihood for individual product approval. In all cases, this corresponds to an adjusted translational medicine approach which involves quantitatively smaller advances in knowledge occurring over a much quicker rate than is typically seen in academics. Examples which will be discussed include developing biomechanical models for pedicle rod fracture, the use of finite element computational methods and scanning electron microscopy as means of developing safety checks on technology, industry research for the sake of producing clinically relevant guidelines, mechanisms for un-planned research questions, and pre-clinical animal studies on safety and efficacy of surface coatings for osseointegration.
Podium Presentations
Rho Pathway Activation Protects Against Inflammatory Induced Alterations in Biomechanics of Cells from the Nucleus Pulposus in a Myosin-II Contractility Dependent Mechanism

Timothy Jacobsen1,2, Paula Hernandez3, Nadeen Chahine1,2
1 Feinstein Institute for Medical Research, Manhasset, NY, 2 Hofstra Northwell School of Medicine, Hempstead, NY; nchahine@northwell.edu

Disclosures: The authors have no conflicts of interest to disclose.

Introduction: Disc Degeneration (DD) is associated with elevated levels of pro-inflammatory cytokines, such as TNFα [1]; pro-inflammatory stimulation of nucleus pulposus (NP) cells within the disc promotes catabolic breakdown of disc matrix and a positive feedback of further loss of tissue integrity and inflammation [2]. Previously we have shown that pro-inflammatory stimulation of NP cells when cultured in 2D leads to significant changes in cellular biomechanical properties specifically increased hydraulic permeability and altered actin organization [3]. To determine which pathways connect TNFα stimulation with altered actin cytoskeleton in rounded NP cells, a chemical screen was conducted on NP cells in 3D culture (alginate beads), which revealed that inhibition of ROCK signaling (Y27632) or myosin II (blebbistatin) phenocopied alterations in biophysical properties (hydraulic permeability) seen with inflammatory stimulation. These findings indicate that Rho pathway plays a role in mediating the biomechanical responses of NP cells to pro-inflammatory stimulation. To further test this hypothesis, here we have investigated the effects of modulating Rho signaling on protection of NP cells from TNFα induced biomechanical disruption. We evaluated the effects of a Rho activator (CN03) against TNFα induced mechanobiological alterations and compared the efficacy of Rho activation as a therapeutic relative to an anti-inflammatory agent (Dexamethasone, DEX). We hypothesize that increased Rho activity will increase myosin II contractility (indicated by pMLC levels), mitigate TNFα induced changes in hydraulic permeability and elastic modulus, and exhibit efficacy comparable to anti-inflammatory drugs.

Methods: NP Cell Isolation: NP tissue was isolated from young bovine lumbar discs. Cells were cultured in 1.2% alginate beads to promote round morphology. Treatments: Cells were left untreated or exposed to the following treatments for 24h: (1) 1ug/ml Rho activator CN03 with or without 10ng/ml TNFα, (2) 1ug/ml DEX with or without 10ng/ml TNFα, (3) 10ng/ml TNFα alone; for co-treatment groups cells were pretreated with DEX or CN03 for 90 or 60 minutes respectively before addition of TNFα. Immunofluorescence: Beads were fixed, permeabilized, and immunostained with α-pMLC primary antibodies and counterstained with Draq5 nuclear stain. Cells were released from alginic beads and run through an Amnis Imagestream X Mark II Imaging Flow Cytometer that images individual cells in a high throughput manner (N≈40,000). pMLC staining intensity was determined for individual cells, averaged for each group, and normalized to untreated. Observational properties: Cells were seeded into a custom microfluidic chamber [3, 4] and placed under an inverted microscope for observation. Step hyper-(333 to 466 mOsm/L NaCl) and hypo-(466 to 333mOsm/L NaCl) osmotic loads were applied. Volume response was analyzed using multiphase mixture theory [5, 6] to compute hydraulic permeability (Lp). AFM: Cells were placed in a heated biochamber and mounted in an Asylum MFP-3D-BIO atomic force microscope. Cells were probed with a 3um radius spherical indenter and force displacement curves were fit to a Hertz contact model to determine elastic modulus. Data was analyzed with 1-way ANOVA and Fisher LSD post-hoc test, with p<0.05 significant.

Results: Stimulation with TNFα significantly reduced pMLC staining in NP cells confirming that myosin II contractility decreases with inflammatory stimulation (Fig 1). Treatment with CN03 (Fig 1A) or DEX (Fig 1B) both significantly increased myosin II contractility as indicated by increased pMLC staining, and were both able to mitigate reduced myosin II contractility induced by TNFα stimulation. TNFα stimulation caused an increase in hydraulic permeability (Lp) under hyper-osmotic loading (Fig 2), in agreement with our previous findings [3]. Furthermore treatment with CN03 (Fig 2A) or DEX (Fig 2B) both mitigated TNFα induced increase in Lp. TNFα stimulation also significantly reduced NP cell elastic modulus (Fig 3), and these changes were also mitigated by treatment with either CN03 (Fig 3A) or DEX (Fig 3B).

Discussion: The goal of this study was to examine the effects of Rho activity in regulating single cell biophysical properties. Our findings confirm that inflammatory stimulation with TNFα generalized reduced hydraulic permeability, decreased cell elastic modulus and decreased myosin II contractility. Increasing myosin-II contractility through activation of Rho pathways with CN03 mitigated changes in hydraulic permeability and elastic modulus due to TNFα. We postulate that inflammatory stimulation disrupts the biomechanical properties of NP cells in a mechanism that is dependent on myosin II contractility and have furthermore shown increasing myosin II contractility is sufficient to prevent inflammatory induced changes in NP cells. This is the first report that directly links cortical contractility with the regulation of cellular osmotic properties in NP or other chondrocyte-like cells, whose cell-ECM interaction are a key regulator of biological and biomechanical function. Modulating the Rho signaling by using a Rho activator fully restores cell stiffness and cell contractility, suggesting a potential intervention strategy to maintain the cellular biomechanical homeostasis in the threat of inflammatory stimulation.

Significance: These findings indicate non-muscle myosin-II contractility plays a key role in how inflammation alters NP cell mechanobiology and consequently in disc degeneration and identifies myosin II contractility as a potential point for therapeutic intervention. These findings advance the understanding of how inflammation alters the biomechanical properties of disc cells as well as their ability to detect and respond to biomechanical stimuli.

Acknowledgements: Funded in part by NSF CAREER Award 1151605, NIH R41AG050021, R01AR069668.


Figure 1: TNFα decreases pMLC staining. CN03 and DEX increase pMLC staining and mitigate TNFα induced changes (*p<0.05 vs. untreated).

Figure 2: TNFα significantly increases Lp for the hyperosmotic step. Both CN03 and DEX mitigate TNFα induced changes (*p<0.05 vs. untreated).

Figure 3: TNFα significantly decreases NP cell elastic modulus. Both CN03 and DEX mitigate TNFα induced changes (*p<0.05 vs. untreated).
TonEBP Maintains Inflammatory Gene Expression in the Hyperosmotic Nucleus Pulposus

Zariel I. Johnson¹, Irving M. Shapiro¹, Makarand V. Risbud¹,²
¹Department of Orthopaedic Surgery and ²Graduate Program in Cell and Developmental Biology,
Thomas Jefferson University, Philadelphia, PA

**Disclosures:** None

**INTRODUCTION:** The transcription factor TonEBP (NFAT5) is critical for the osmotic response and matrix homeostasis activities of nucleus pulposus (NP) cells in their native hypertonic environment (1-3). Interestingly, recently published studies have shown that TonEBP can also play a role in propagation of inflammation in response to hypertonicity (4). In this study, we aimed to elucidate whether TonEBP performs alternative functions in NP cells.

**METHODS:** RNA-sequencing was performed on control and TonEBP-silenced NP cells cultured under physiologically hypertonic conditions. Ingenuity Pathway Analysis was used to identify pathways, networks, and diseases relevant to the list of TonEBP-dependent genes. Selected targets and their gene promoters were further studied using gain- and loss-of-function techniques along with site-directed mutagenesis. The organ-level response to hypertonicity was evaluated using an ex vivo organ culture model, employing discs harvested from TonEBP haploinsufficient mice, according to a protocol approved by Thomas Jefferson’s IACUC. Crosstalk between TonEBP and NF-κB signaling pathways was studied using activity reporter and inhibitor experiments.

**RESULTS:** We identified 1140 TonEBP-dependent genes in NP cells, many of which were categorized into cytokine/chemokine and matrix homeostasis-related pathways. Knockdown studies confirmed that TonEBP was required to maintain levels of CCL2, IL6, TNF, and NOS2, all of which were also induced by hypertonicity. Investigation of gene promoters revealed that the CCL2 promoter was osmo-sensitive in a TonEBP-dependent manner. However, IL6 and NOS2 promoters were not inducible by TonEBP, despite harboring several predicted TonEBP binding sites. Transcript levels of CCL2, IL6, TNF, and NOS2 were induced by hypertonicity in discs from wild-type animals. Interestingly, this response to hypertonicity was completely inhibited by TonEBP haploinsufficiency. Activity of the NF-κB pathway was increased by hypertonicity and this induction was prevented by expression of dominant-negative TonEBP. Using the specific NF-κB pathway inhibitor SM7368, we found that NF-κB activity was required for hypertonic induction of IL6, TNF, and NOS2, but not TauT or CCL2.

**DISCUSSION:** TonEBP is required to maintain levels of pro-inflammatory genes in NP cells under hypertonic conditions. Haploinsufficiency of TonEBP prevents this response in a whole organ culture model. In the case of CCL2, TonEBP regulates hypertonicity inducibility via a highly conserved TonEBP binding site in the proximal gene promoter. In NP cells, hypertonicity promotes activity of the NF-κB pathway in a TonEBP-dependent manner. Activity of this pathway is required for hypertonic induction of only some pro-inflammatory TonEBP target genes.

**SIGNIFICANCE:** Our results are compelling in light of the fact that the TonEBP targets identified here have all been associated with intervertebral disc degeneration (5-7). However, we do not believe that osmotic fluctuations stemming from healthy loading of the disc would lead to an inflammatory cascade in vivo. Rather, we hypothesize that hypertonic induction of these genes via TonEBP is part of an adaptive response of these cells to their osmotically variable environment and that tight regulation of these targets is essential. Thus, deregulation of TonEBP signaling in this setting poses a serious threat to disc health.


**ACKNOWLEDGEMENTS:** This work is supported by grants from the National Institutes of Health AR055655, AR064733, and T32 AR052273.

**IMAGES AND TABLES:**

**Figure 1.** (A) Representation of wildtype (WT) and TonE-mutated CCL2 promoter reporter constructs. Activity of WT and TonE mutant CCL2 promoter constructs measured in response to (B) hypertonicity or (C) TonEBP overexpression. NaCl, 110 mM NaCl added to regular culture media. Activity of WT promoter is induced by both stimuli while activity of the mutant promoter is unaffected.

**Figure 2.** (A) Schematic of ex vivo intervertebral disc organ culture model. (B) CCL2, (C) IL6, (D) TNF, and (E) NOS2 were induced by hypertonicity in discs from WT mice but not from haploinsufficient TonEBP−/− mice.
TGF-β1 induces a contractile CD146+ phenotype of human annulus fibrosus cells with affinity to collagen scaffold for annulus fibrosus repair

Rose G. Long1,2,3, Tomoko Nakai3,4, Daisuke Sakai1,3, Lorin M. Benneker1,4, James C. Iatridis3,4, Mauro Alini1,5, Sibylle Grad1,5, Zhen Li1,5
1AO Research Institute, Davos, Switzerland
2Leni & Peter W. May Department of Orthopaedics, Icahn School of Medicine at Mount Sinai, New York, NY
3Tokai University School of Medicine, Isehara, Japan
4Inselspital, University of Bern, Bern, Switzerland
5Collaborative Research Partner Annulus Fibrosus Rupture Program of AO Foundation, Davos, Switzerland

Disclosures: Authors have no disclosures.

INTRODUCTION: Lower back pain affects up to 70% of people in their lifetimes [1] and is often coincident with annulus fibrosus (AF) defects [2,3]. The healthy intervertebral disc (IVD) is hypovascular and has low cellularity resulting in low regenerative potential and minimal healing of AF defects. It is a research priority to develop strategies to enhance AF healing. During normal wound healing, fibroblasts contract and secrete TGF-β1 [4,5], which increases contraction of fibroblasts [6]. CD146+ AF cells have been shown to induce murine AF cells to the CD146+ contractile phenotype [8]. We hypothesize that TGF-β1 can induce human AF cells to a CD146+ phenotype with increased contractility and that this may be a useful cell to deliver for AF repair. This study evaluated human AF cells in 2D and 3D culture models to determine: 1) if exogenous TGFβ1 can induce higher CD146 expression and develop greater contractility of human AF cells; and 2) if different hydrogel/scaffold carriers can maintain a CD146+ phenotype in 3D culture.

METHODS: For all studies AF cells were isolated from human trauma donors (n=4 biologic replicates; M, aged 34, 37, 49 & 55, Pfirrmann Grade 2) with ethical board approval and expanded (αMEM, 10% FBS, P/S). All experiments including expansion were performed in hypoxia (2% O2). For the CD146 induction study, AF cells in 2D culture were treated with Basal media (αMEM, 5% FBS, 1% ITS+) or TGFβ1 media (Basal + 5 ng/mL TGFβ1) for 4 days. CD146 was measured using antiCD146 antibody and flow cytometry analysis (n = 3). After 4 days, cells were lysed with TRI reagent and gene expression using real time PCR measured levels of CD146, SM22a, COL1, COL2, aggrecan (ACAN), versican (VCAN), elastin (ELN), scleraxis (SCX) and Mohawk (MHK). The cell contractility study (n = 4/donor) assessed gel area immediately and 16 hours after seeding in 3D culture using a seeding density of 1.5×10⁶ cells/mL in a 1.81 mg/mL type I collagen gel. The carrier study had 5 groups (n = 6/donor), including collagen, fibrin or polyurethane (PolyU) scaffolds (200,000 cells/gel or scaffold) and polystyrene (PS) 2D plastic with and without 5 ng/mL TGFβ1 as positive and negative controls. Cells underwent a 4 day pre-treatment with TGFβ1 media before they were seeded in their scaffold and cultured for an additional 7 days without TGFβ1 in high glucose DMEM, 1% P/S, 2% FBS, 1% ITS+, 50 μg/mL ascorbate 2 phosphate and 1% non-essential amino acids. 5μM ε-aminocaproic acid was also added to the fibrin samples to prevent degradation. Collagen and fibrin gels were 30 μL per gel and PolyU scaffolds were 3 mm in diameter, 4 mm in depth (pore size 90-300 μm). Samples were collected for gene expression analyses at Day 1 and 7. Student’s t-tests assessed differences in area and gene expression between Basal and TGFβ1 media. One-way ANOVA assess differences in gene expression for 5 groups in the carrier study. p &lt; 0.05 was significant.

RESULTS: Human AF cells treated with TGFβ1 had greater CD146 staining than cells in Basal media (p = 0.004, Figure 1). TGFβ1 induced human AF cells to up-regulate CD146 (p = 0.01), SCX (p = 0.10) and SM22a (p = 0.06). TGFβ1 stimulated AF cells had greater contractility than cells in Basal media (p &lt; 0.001, Figure 2). For the carrier study, AF cells had similar gene expression at Day 1 following TGFβ1 pre-treatment for all 5 groups. After 7 days of culture without, human AF cells encapsulated in collagen maintained similar CD146, SCX and SM22a gene expression as cells on 2D PS with TGFβ1. In contrast, the expression of these genes was decreased in cells in fibrin, PolyU or PS without TGFβ1 (p &lt; 0.05, Figure 3).

DISCUSSION: Human AF cells treated with TGFβ1 showed increased CD146 expression at the gene and protein level and increased contractility. TGFβ1 stimulated AF cells also had higher SM22a and SCX gene expression; SM22a is an actin crosslinker protein that may be active in contraction. SCX is a transcription factor which is a tendon marker [9] and may also be a marker for the contractile AF phenotype induced by TGFβ1. Together we believe that CD146, SM22a and SCX may be relevant markers indicating a functional contractile phenotype of AF cells. Contractility was measured as a predictor of wound healing since fibroblasts contract as they differentiate into protomiofibroblasts during wound healing [4]. Thus, TGFβ1 induced AF cells with higher contractility may facilitate sealing of annulus defects after injury. In 3D culture, the CD146+ phenotype of AF cells was best maintained within the collagen gel, indicating that collagen is the most suitable carrier for human AF cells. Nevertheless, sustained TGFβ1 presentation may be helpful to retain or enhance the phenotype over longer times in 3D culture. This study demonstrated a novel AF cell contractile phenotype in human cells, relevant markers for identification of this phenotype, and evaluated carriers for eventual cell delivery and translation. Future investigations will evaluate if delivery of these cells can promote AF repair in pre-clinical models.

SIGNIFICANCE: CD146+ AF cells are a possible cell source for intervertebral disc repair. Developing a genotype and functional phenotype of these cells is a vital step to delivery and in vivo studies. Delivery of CD146+ AF cells may enhance the regenerative capacity of the IVD by promoting AF healing.


ACKNOWLEDGEMENTS: Supported by ROI AR0185797 (ICI) from NIH/NIAMS, NIGMS-funded Integrated Pharmacological Sciences Training Program T32 GM062754, AO Foundation and Whitaker International Program.
Effects of Hypoxia and TGF-β Exposure during Monolayer Expansion on the Survival and Matrix Producing Capacity of Mesenchymal Stem Cells

Sun H. Peck\textsuperscript{1,2}, Justin R. Bendigo\textsuperscript{1,2}, Sarah E. Gullibrand\textsuperscript{1,2}, John W. Tobias\textsuperscript{1,2}, George R. Dodge\textsuperscript{1,2}, Robert L. Mauk\textsuperscript{1,2}, Neil R. Malhotra\textsuperscript{1,2}, Lachlan J. Smith\textsuperscript{1,2}

\textsuperscript{1}Philadelphia VA Medical Center, Philadelphia, PA; \textsuperscript{2}University of Pennsylvania, Philadelphia, PA

Disclosures: SHP (N), JRB (N), SEG (N), JWT (N), GRD (N), RLM (N), NRM (N), LJS (N)

Introduction: Degeneration of the intervertebral discs is implicated as a major cause of lower back pain \cite{1}. There is a need for treatment options that not only alleviate symptoms but also reconstitute native tissue structure and mechanical function within the disc. Over the past several years, application of mesenchymal stem cells (MSCs) for disc regeneration, particularly for the nucleus pulposus (NP), has received considerable attention. Previous studies have shown that MSCs are capable of undergoing differentiation into a NP-like phenotype under certain culture conditions \cite{2-4}; however, a key challenge to successful application of MSCs for NP regeneration is the heterogeneous nature of the intervertebral disc, characterized by low nutrition and oxygen tension, both of which may negatively impact the survival and biosynthetic properties of MSCs \cite{5}. The objective of this study was to investigate whether exposing MSCs to hypoxia during monolayer expansion enhances subsequent survival and regenerative potential in the nutrient and oxygen poor NP environment.

Furthermore, we investigated whether priming MSCs towards an NP-like phenotype by exposing them to TGF-β3 during monolayer expansion enhances subsequent regenerative potential.

Methods: Cell Isolation and Expansion: Bone marrow-derived MSCs were isolated from 3 juvenile (<6 months of age) bovine femurs and tibia, pooled, and expanded to confluence through a single initial passage in monolayer in normoxia (21\% O\textsubscript{2}) and basal medium (DMEM (4.5 g/L glucose) and 10\% FBS). The cells were then passaged and expanded in basal medium in one of four different conditions for 1 week: 1. Normoxia (21\% O\textsubscript{2}; standard MSC expansion conditions); 2. Normoxia\textsuperscript{+}TGF-β3 (10 ng/mL); 3. Hypoxia (2\% O\textsubscript{2}); 4. Hypoxia\textsuperscript{+}TGF-β3 (10 ng/mL). Pellet Culture: After the monolayer expansion protocol described above, cells were passaged and cultured in pellets (250,000 cells/pellet) in a simulated NP-like environment (hypoxia (2\% O\textsubscript{2}) and chemically defined media with low glucose (1 g/L) DMEM and no growth factors). After 2 weeks of culture, pellets were harvested and either fixed in formalin and processed for paraffin histology (n=2) or analyzed for biochemical composition (n=5). For histology, sections were stained with Alcian blue (glycosaminoglycans, GAG) or picrosirius red (collagen). For analysis of biochemical composition, DNA, GAG, and collagen contents were quantified using the Picogreen (Thermo Fisher), dimethylmethylene blue, or hydroxyproline assays respectively. DNA was analyzed per pellet, and GAG and collagen were normalized to DNA. Significant differences (p<0.05) between groups were established using 2-way ANOVA with Bonferroni post-hoc tests (p<0.05).

Microarray Analysis: Bone MSCs were isolated and expanded under the four conditions described above, with cells from 3 different donor animals maintained as distinct biological replicates. Cells were harvested, high quality RNA (RIN>9) was isolated from each sample, and global gene expression was measured using the WTPlus Bovine Gene Chip (Affymetrix GeneChip system). Gene expression data were normalized using Robust Multi-array Average. Significance differences in gene expression were determined using 3-way mixed model ANOVA (p<0.05; adjusted for false discovery rate).

Results: Pellet Culture: DNA content for pellets with MSCs expanded in hypoxia, both with and without TGF-β3, was significantly higher than for those with MSCs expanded in normoxia, both with and without TGF-β3 (Fig 1A). DNA content was lowest for pellets with MSCs expanded in normoxia with TGF-β3 and highest for pellets with MSCs expanded in hypoxia with TGF-β3. There was no significant effect of monolayer expansion condition on pellet GAG content (normalized to DNA, Fig 1B). Collagen content exhibited the opposite trend to DNA and was highest for pellets with MSCs expanded in normoxia with TGF-β3 (p<0.05 vs both normoxia without TGF-β3 and hypoxia with TGF-β3, Fig 1C). Histological results supported these findings (Figs 1D and E), where pellets with MSCs expanded under hypoxia, with and without TGF-β3, were larger than those with MSCs expanded under normoxia, suggesting higher cell numbers. Microarray Analysis: Principal component analysis (PCA, Fig 2A) indicated significant effects of MSC donor on the global gene expression in response to each expansion condition. The effects of altering oxygen tension (without TGF-β3) during monolayer expansion on MSC gene expression were moderate. MSCs expanded under hypoxia exhibited differential expression of genes implicated in the cell stress response (BAGALT6: galactosyltransferase; LPL: lipoprotein lipase; NGF: nerve growth factor; PK: pyruvate kinase) compared to normoxia expanded MSCs (Fig 2B). Exposure to TGF-β3 during monolayer expansion resulted in the greatest effects on global gene expression, irrespective of oxygen tension. In particular, there were significant effects on expression of genes involved in growth and inflammation, including those of the TGF-β, NFκB, and caspase activation pathways (Fig 2C).

Discussion: The results of this study suggest that exposure to hypoxia during monolayer expansion leads to improved survival (higher DNA content) when these cells are subsequently cultured in simulated NP-like conditions with limited oxygen and nutrition. Interestingly, exposure to hypoxia during monolayer expansion had no significant impact on the subsequent matrix (GAG or collagen) producing capacity of MSCs in the absence of TGF-β3. In contrast, exposure to TGF-β3 under normoxic conditions during expansion significantly inhibited subsequent MSC survival and boosted collagen production on a per cell basis with no effect on GAG. This may suggest induction of a post-mitotic and pro-fibrotic phenotype, which may be detrimental to the capacity of MSCs to regenerate NP tissue. Microarray results support this view, with TGF-β3 exerting significant effects on signaling pathways that regulate fibrosis and inflammation, which eclipsed any beneficial effects of hypoxia alone. Ongoing work will seek to verify these findings, by determining the type of collagen (1 or II) being produced and measuring levels of pro-inflammatory factors in the culture media. Finally, microarray results highlighted the significant effects of donor on the response of MSCs to environmental stimuli, potentially due to variations in age and sex, the impact of which should be considered during future translational studies.

Significance: The results of this study demonstrate that alterations in monolayer expansion environment significantly impact the survival and matrix producing capacity of MSCs and provide a foundation for optimizing the regenerative capacity of these cells in the intervertebral disc.

References: \cite{1} Mirza+ Spine, 2007. \cite{2} Smith+ Tiss Eng A, 2013. \cite{3} Perglio+ Spine J, 2013. \cite{4} Gupt+ Tiss Eng A, 2011. \cite{5} Farrell+ OAC, 2014

Acknowledgements: Department of Veteran’s Affairs, Penn Center for Musculoskeletal Disorders.

Figure 1. Composition of MSC pellets after monolayer expansion in different oxygen and TGF-β3 conditions. A. DNA content, B. GAG per DNA, and C. Collagen per DNA. D. Alcian blue staining for GAG. E. Picrosirius red staining for collagen. N=5; \textit{*}p<0.05; scale bar = 0.2 mm.

Figure 2. Microarray results. A. Principal component analysis (PCA) plot. Lines connect all samples from a single animal. B. Effects of hypoxia on gene expression in the absence of TGF-β3. C. Effects of TGF-β3 on growth and inflammation pathway gene expression. N=3; all p<0.05.
HIF-1α-Carbonic Anhydrase Axis Controls Acid-Base Metabolism in Hypoxic Nucleus Pulposus Cells

Elizabeth S. Silagi1, Zachary Schoepflin1, Erin Seifert1, Ernestina Schipani1, Irving M. Shapiro1, Makarand V. Risbud1
Departments of 1Orthopaedic Surgery and 2Pathology, Anatomy and Cell Biology, Thomas Jefferson University, Philadelphia PA
1Department of Orthopaedic Surgery, University of Michigan Medical School, Ann Arbor, Michigan.

Disclosures: None for all authors.

INTRODUCTION: The health and integrity of the avascular and hypoxic nucleus pulposus (NP) plays a crucial role in the development of intervertebral disc degeneration. NP cells generate energy primarily through anaerobic glycolysis and tightly regulate the concentration of metabolites and glycolytic end-products. Although the intracellular pH of NP cells is lower than the physiological pH of most tissues, they are sensitive to acidic environment. Culturing NP cells at pH between 6.6-6.3 inhibits the synthesis of sulfated-GAGs while permitting the production of MMPs. Therefore, compromised acid-base homeostasis in NP tissue would favor the breakdown of the disc extracellular matrix. Besides limited evidence of Na+/H+ exchange in pH regulation, little is known about how NP cells buffer their intracellular and extracellular pH. In other cell types, it has been shown that carbonic anhydrases are important members of a large network of pH sensors responsible for the regulation of CO2, H2O, H+, HCO3−, and lactate concentrations. It is known that CA9 is induced by hypoxia in a HIF-1α dependent manner in other tissues. Likewise, HIF-1α is implied in controlling CA12 expression in NP cells, however, in vivo evidence and mechanistic insights of this regulation are lacking. Since NP cells adapt to their unique hypoxic environment through the essential activities of the HIF homologues, which are regulated in a unique fashion in the NP, we hypothesize that HIF-1α/CA axis plays an important role in maintaining metabolic function and phenotype of NP cells by buffering acid-base equilibrium.

METHODS: Expression levels were evaluated using qRT-PCR, Western blot, and fluorescent immunohistological techniques. Loss of function studies were performed with LV-shHIF-1α and NP-specific HIF-1α knockout mice generated by the strategy previously reported by Merceron et al. 2014. The nucleotide sequence and chromosomal location of the 2kb proximal promoter of rat Car9 and Car12 were found using the UCSC Table Browser, putative HRE consensus sequences (5'-[A/G]CGTG-3') were determined using the JASPAR Core Database, and Multiz alignment of HRE motifs was performed using the Ensembl Lastz Database. Binding of HIF-1α to putative HREs was detected using genomic chromatin immunoprecipitation and real-time qRT-PCR analysis with primer pairs for putative HRE sites. The Seahorse XF24 instrument was used to measure extracellular acidification rate (ECAR) and O2 consumption rate (OCR), as reported by Csordás et al. 2013. All experiments were performed in triplicate at minimum, and data are presented as mean ± S.E. Differences between groups were analyzed by the Student’s t test and one-way ANOVA; p<0.05.

RESULTS: Real-time qRT-PCR and Western blot analysis confirmed expression of Car9 and Car12 in both NP and AF tissues. Furthermore, intervertebral disc sections were immunolabeled with antibodies against CAIX and CA XII to elucidate their tissue localization in vivo. CAIX and CA XII are predominantly located in the NP with some expression in the AF and endplates. Given the robust expression of Car9 and Car12 in the NP tissue, we investigated the effect of oxygen tension on their mRNA and protein levels. Car9 and Car12 mRNA showed significant hypoxic induction, likewise, CAIX and CA XII protein expression significantly increased by hypoxia (Fig. 1). We performed bioinformatic analysis on the Car9 and Car12 promoters to identify predicted HREs. Next, we experimentally validated HIF-1α binding to one of the predicted HRE regions on the Car9 and Car12 promoters, respectively, with genomic ChIP. We also confirmed that Car9 and Car12 mRNA and protein expression was significantly reduced by HIF-1α silencing under both normoxia and hypoxia. To further support this observation, we investigated the effect of HIF-1α deletion on Car9 and Car12 expression in vivo using an NP-specific HIF-1α knockout mouse. We observed that the expression of both CAIX and CA XII was notably reduced in the HIF-1α mutant mice compared to heterozygous and wild-type controls (Fig. 2). To elucidate the role of Car9 and Car12 in NP cell metabolism and acid-base buffering we measured changes in ECAR and OCR after treating NP cells with specific CA inhibitors. Cells treated with Car9 and Car12 inhibitors experienced a significant decrease in ECAR. It was also evident that their mitochondrial OCR was unaffected by CA inhibition.

DISCUSSION: The detailed molecular mechanisms that control expression of carbonic anhydrase isoforms in hypoxic NP cells is not yet elucidated, despite their critical contribution in buffering intracellular and extracellular pH. In this study, using both in vitro loss of function approaches and generating mice with targeted deletion of HIF-1α in the NP, we showed a mechanism of Car9 and Car12 regulation by HIF-1α. Our results clearly showed that HIF-1α binds to conserved HRE regions in the proximal promoters of Car9 and Car12 in both normoxia and hypoxia. Notably, inhibition of specific CA enzymatic activity compromised metabolic function of NP cells, underscoring the importance of these molecules in NP physiology. Relevant to NP cell function, our studies showed that inhibition of Car9 and Car12 leads to unaltered mitochondrial OCR with a concomitant decrease in ECAR, suggesting diminished availability of extracellular proton. It is therefore imperative to consider the significance of these CA isoforms in controlling pH and glycolytic metabolism in NP cells.

SIGNIFICANCE: Millions of Americans suffer from low back pain that is closely linked to degeneration of the intervertebral discs of the spine. The goal of this study is to understand how cells in the discs survive in a hypoxic environment, and how disruption of critical metabolic systems within these cells can contribute to the pathogenesis of age-related disc disease.

ACKNOWLEDGEMENTS: Work supported by NIH grants R01-AR064733, R01-AR055655, and T32-AR052273.

IMAGES AND TABLES:

Figure 1: CAIX expression is hypoxia-inducible in NP cells.

Figure 2: Mice with NP-specific deletion of HIF-1α show decreased expression of CAIX in NP tissue.

Fig. 2) Representative immunofluorescence images of CAIX expression in E15.5 control (HIF-1αf/f) and HIF-1α mutant (Foxa2Cre+; HIF-1αf/f) littermate mice imaged at 20x magnification.
Region-Specific Orthotropic Growth of the Pediatric Thoracic Spine through Finite Element Methods

John Dougherty, James Peters, Sriram Balasubramanian
Drexel University, Philadelphia, PA

Disclosures:
The authors declare that there are no conflicts of interest.

INTRODUCTION: Adolescent Idiopathic Scoliosis (AIS) is a three-dimensional deformity characterized by a progressive lateral curvature and axial rotation of the spine combined with structural changes in the rib cage, and affects five to nine million children ages 10-18 years in the U.S. Currently, the most common intervention to correct AIS deformity is spinal fusion. However, this method restricts the patient's future growth at the levels of instrumentation. Knowledge of the growth remaining in the spine is critical for predicting progression and surgical planning. Finite element (FE) methods have previously been used to create computational models of the growing spine. However, these models have been limited to using a singular growth strain on only the vertebral bodies for the entire spine. The objectives of this study are to apply region-specific growth strain data to a pediatric FE model of the osteoligamentous thoracic spine.

METHODS: Based on our previous work, region-specific growth strains showed differences not only in the direction of growth, but also with age, gender, and level of the spine (Peters et al. 2015). To apply these strains, an approach was taken similar to Fok et al. 2010 using thermal loads. The growth strains were applied for the various vertebral features such as facets, pedicles etc. A previously validated hexahedral meshed 10 year-old male thoracic spine model was used for this analysis. Elements were manually selected in Hypermesh to comprise each region at each level of the spine. A uniform thermal load was applied on the model using ANSYS 17.0. New thermal expansion coefficient values (α) in the X, Y and Z directions were calculated to account for the orthotropic growth. These were done through a combination of Hookes's law for orthotropic materials as well as Fok's thermal growth equations (Table 1). An iterative process was used to compute thermal growth. Each vertebrae was angled differently at each level, and orthotropic properties in ANSYS are uniquely based on each element's orientation, leading to discrepancies even after using the equations. Factor errors were used to scale the α-values, controlling the growth in each direction to improve accuracy. This process was repeated until growth strains (ε) were within 5% of the published literature.

RESULTS: The ε-values from one year of growth in the FE thoracic spine model were compared with the regional normative ε-values computed using linear regression equations for 10 and 11 year-old male subjects from T1-T12 (Table 2). All ε-values have errors below 5%.

DISCUSSION: Using region-specific strain data from retrospective CT scans, new methods were developed to generate comprehensive growth in the thoracic spine through FE analysis. Orthotropic thermal expansion allowed for improved control of the output strains within regions, allowing for a more accurate representation of spine growth. Though this analysis was for a template pediatric FE model over a one-year period of time, this strain data exists for ages 1-19 years, for both males and females. Such a method can be utilized for all patient-specific geometries and can be translated for any FE growth simulation.

SIGNIFICANCE: Our FE model demonstrates new methods to accurately apply region-specific normative growth, which will be expanded to account for the growth of scoliotic spines and allow clinicians to better understand the spinal curvature progression of AIS with patient-specific models. These advanced methods would aid in evaluating deformity-specific interventions and with preoperative planning for AIS patient-specific correction.


TABLES:

Table 1. Orthotropic Thermal Growth Equations

<table>
<thead>
<tr>
<th>εx = (x0 - x)/(x0 - x)</th>
<th>εy = (y0 - y)/(y0 - y)</th>
<th>εz = (z0 - z)/(z0 - z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hooke's Law</td>
<td>ε = (H0 - H)/(H0 - H)</td>
<td>ε = (L0 - L)/(L0 - L)</td>
</tr>
<tr>
<td>Thermal Growth (Fok et al. 2010)</td>
<td>ε = (T0 - T)/(T0 - T)</td>
<td>ε = (A0 - A)/(A0 - A)</td>
</tr>
<tr>
<td>Combination for Orthotropic Thermal Growth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>αT = (T0 - T)/(T0 - T)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Growth data for 10 year-old male from the FE model. FE calculated ε-values from one year of growth are in the first row.

The percent error with normative ε-values from retrospective CT scans (Peters et al. 2015) are shown in the second row.

<table>
<thead>
<tr>
<th>Facets Height</th>
<th>Left Pedicle Height</th>
<th>Right Pedicle Height</th>
<th>Left Pedicle Width</th>
<th>Right Pedicle Width</th>
<th>Spineous Process Length</th>
<th>Left Transverse Process Width</th>
<th>Right Transverse Process Width</th>
<th>Vertebral Body Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>T2</td>
<td>T3</td>
<td>T4</td>
<td>T5</td>
<td>T6</td>
<td>T7</td>
<td>T8</td>
<td>T9</td>
</tr>
<tr>
<td>Facets Height</td>
<td>0.0387</td>
<td>0.0365</td>
<td>0.0352</td>
<td>0.0398</td>
<td>0.0375</td>
<td>0.0399</td>
<td>0.0412</td>
<td>0.0399</td>
</tr>
<tr>
<td>Left Pedicle Height</td>
<td>0.0472</td>
<td>0.0479</td>
<td>0.0439</td>
<td>0.0455</td>
<td>0.0453</td>
<td>0.0455</td>
<td>0.0489</td>
<td>0.0458</td>
</tr>
<tr>
<td>Right Pedicle Height</td>
<td>0.0518</td>
<td>0.0496</td>
<td>0.0433</td>
<td>0.0446</td>
<td>0.0465</td>
<td>0.0479</td>
<td>0.0498</td>
<td>0.0491</td>
</tr>
<tr>
<td>Left Pedicle Width</td>
<td>0.0384</td>
<td>0.0339</td>
<td>0.0324</td>
<td>0.0302</td>
<td>0.0279</td>
<td>0.0313</td>
<td>0.0347</td>
<td>0.0293</td>
</tr>
<tr>
<td>Right Pedicle Width</td>
<td>0.0360</td>
<td>0.0353</td>
<td>0.0300</td>
<td>0.0283</td>
<td>0.0299</td>
<td>0.0328</td>
<td>0.0319</td>
<td>0.0341</td>
</tr>
<tr>
<td>Spineous Process Length</td>
<td>0.0404</td>
<td>0.0424</td>
<td>0.0407</td>
<td>0.0410</td>
<td>0.0402</td>
<td>0.0386</td>
<td>0.0426</td>
<td>0.0421</td>
</tr>
<tr>
<td>Left Transverse Process Width</td>
<td>0.0451</td>
<td>0.0411</td>
<td>0.0347</td>
<td>0.0373</td>
<td>0.0448</td>
<td>0.0425</td>
<td>0.0414</td>
<td>0.0343</td>
</tr>
<tr>
<td>Right Transverse Process Width</td>
<td>0.0451</td>
<td>0.0411</td>
<td>0.0347</td>
<td>0.0373</td>
<td>0.0448</td>
<td>0.0425</td>
<td>0.0414</td>
<td>0.0343</td>
</tr>
<tr>
<td>Vertebral Body Height</td>
<td>0.0643</td>
<td>0.0696</td>
<td>0.0608</td>
<td>0.0603</td>
<td>0.0607</td>
<td>0.0610</td>
<td>0.0615</td>
<td>0.0585</td>
</tr>
</tbody>
</table>
Normalcy of Vertebral Morphology for Lenke-types 1, 2, and 5 Two-Years Following Spinal Fusion Surgery

James R. Peters1, Robert M. Campbell Jr.2, Sriram Balasubramanian3
1 Drexel University, Philadelphia, PA, 2 The Children’s Hospital of Philadelphia, Philadelphia PA

Disclosures: James R. Peters (N), Robert M. Campbell Jr. (N), Sriram Balasubramanian (N)

Adolescent idiopathic scoliosis (AIS) is a highly variable, complex, three-dimensional (3D) spine deformity affecting approximately 3% of adolescents’ ages 10–16 years. Lenke-types 1 (main thoracic), 2 (double thoracic), and 5 (thoracolumbar/lumbar) account for approximately 83% of all AIS deformities that require surgical intervention, primarily posterior pedicle screw-based fusion. Previous work has shown evidence for significant remodeling of the vertebrae for these Lenke-types two years following spinal fusion; however, it is still not known if the resulting geometry can be considered normal. Therefore, the objective of this work was to assess the normalcy of vertebral morphology for subjects with Lenke-types (LT) 1, 2, and 5 curvatures two years following spinal fusion surgery.

EOS (EOS Imaging, France) generated 3D reconstructions of the thoracic and lumbar spines (T1-L5) from 12 female LT 1 subjects, 13 LT 2 subjects (10 female, 3 male) and 10 female LT 5 subjects (average age 16.57 ± 1.94 years) were retrospectively obtained two years following posterior pedicle screw-based spinal fusion surgery. The LT, lumbar modifiers, sagittal modifiers and Cobb angles were clinically evaluated prior to surgery. A skeletally normal age- and sex-matched cohort was selected from 100 subjects with retrospective chest computed tomography (CT) scan reconstructions (35 subjects: average age 16.3 ± 1.43 years), and 102 subjects with abdominal CT scan reconstructions (35 subjects: average age 16.3 ± 1.76 years) using the linear programming methods of Jonker and Volgenant (1987). All normative subjects were between the 5th and 95th percentiles in height, weight and BMI.

For each subject, 3D models of the thoracic and lumbar vertebrae were analyzed using previously reported techniques to record the positions of 30 landmark points and 30 geometric measurements from the surface morphology of each vertebra (Figure 1). For the LTs, due to the presence of multiple curves and their differences in curve sidedness, bilateral measurements were reassigned to either the convex (v) or concave (c) sides of the curve. For the normative subjects, right was matched with convex and left with concave. To control for differences in the overall size of the subject’s vertebrae, ratios of complementary measurements (e.g. anterior per posterior, superior per inferior and convex per concave) were computed and used in the statistical analyses. Angle measurements were compared directly. To account for the differences in apical levels between subjects, the data were compared between the corresponding apices and ± five vertebral levels for LT 1 and ± three vertebral levels for LTs 2 and 5. Data from the matched normative subjects were similarly shifted.

At each vertebral level, permutation tests with 10,000 replications were used to compare the ratio and angle data between the Lenke-types and skeletally normal subjects. To account for multiple hypotheses testing, the false discovery rate control as described by Benjamini and Hochberg (1995) was used to find a suitable significance cutoff. All analyses were performed using MATLAB 2014b (The MathWorks, Natick, MA).

Significant differences (p ≤ 0.0228) were found between the LTs and normative subjects for all tested parameters; however, these differences were sporadic and rarely found near the apex with a few notable exceptions. Parameters like convex per concave vertebral body height ratios, and convex per concave pedicle height, and width ratios were very similar between the LTs and normal subjects. On the other hand, measurements for spinal canal width per depth ratios, convex per concave interfacet height ratios, and superior per inferior interfacet width ratios showed a marked difference from the normal values across all three LTs (Figure 2).

These findings further support the idea of vertebral remodeling following spinal fusion surgery for the vertebral bodies and pedicles. While other structures like the spinal canal and facets, despite showing significant changes in geometry two years after surgery, were still seen to be significantly dissimilar from the normal values. With the advent of newer growth-friendly methods to treat AIS deformity, further studies are needed to better understand growth modulation of the.

Figure 1. Thoracic and lumbar vertebral measurements

Figure 2. (a) Lenke-type 1 convex per concave vertebral body height ratios. (b) Lenke-type 1 convex per concave pedicle height ratios. (c) Lenke-type 1 convex per concave interfacet height ratios. Lenke-types shown in black and normal subjects shown in grey.
Disc Height Loss, But Not Pro-Inflammatory Cytokine or Substance P Expression, Predicts Intervertebral Disc Degeneration-Related Pain in a Rat Model

Thomas W. Evashwick-Rogler(1), Alon Lai(1), Jonathan M. Salandra(2), Devina Purmessur(2), Branko Skovrlj(3), Beth A. Winkelstein(2), Samuel K. Cho(3,5), Andrew C. Hecht(1,3b), James C. Iatridis(4)

1Leni & Peter W. May Department of Orthopaedics, Icahn School of Medicine at Mount Sinai, New York, NY, USA, 2Philadelphia College of Osteopathic Medicine, Philadelphia, PA, USA, 3Department of Biomedical Engineering, The Ohio State University, Columbus, OH, USA, 4North Jersey Spine Group, Wayne, NJ, USA, 5Spine Pain Research Lab, University of Pennsylvania, Philadelphia, PA, USA

INTRODUCTION: Intervertebral disc (IVD) degeneration is commonly associated with back pain, the leading cause of global disability, although the relationship between IVD degeneration and pain is complex and poorly understood. The development of an animal model for painful IVD degeneration with similarities to the human condition is a research priority in order to better elucidate the pathophysiology of IVD-related pain and for screening therapeutics.

We previously developed a rat lumbar IVD injury model and found anterior annular puncture and injection of phosphate-buffered saline (PBS) and tumor necrosis factor-α (TNF-α) of three adjacent IVDs induced acute and chronic painful behaviors and IVD degeneration without evidence for herniation [1]. While this model demonstratedRepeatable pain behaviors, the causes of pain remain unclear. Therefore, the purpose of this study was to identify mechanisms of painful behaviors in this rat IVD injury model by using a multiple regression model to analyze relationships between IVD height loss, IVD degeneration, intradiscal pro-inflammatory cytokines, and substance P (SubP) in dorsal root ganglion (DRG). Intradiscal pro-inflammatory cytokines were measured since TNF-α and interleukins (ILs) can induce IVD catabolism and upregulate neuropeptides in DRG to induce nociception [2].

METHODS: Experimental procedures were approved by IACUC. Thirty skeletally mature (4-5 months old) Sprague-Dawley rats were randomly divided into lumbar IVD injury (n=24) or sham (n=6). IVDs with different severities of injuries were induced by either a shallow or deep puncture and injection with PBS (2.5μL) or TNF-α (0.25ng in 2.5μL PBS) injections. Anterior puncture and injections of three adjacent lumbar IVDs (L2/3, L3/4, L4/5) occurred as described [1]. Deep punctures simulated complete annular tears whereas shallow punctures simulated mild annular injuries without nucleus pulposus involvement. Pain and IVD height were assessed weekly after surgery via mechanical hindpaw hyperalgesia testing using calibrated von Frey filaments (0.4-26g) and by x-rays, respectively. Six weeks after surgery, rats were euthanized, and lumbar spine motion segments and DRG were harvested. Lumbar spine segments and DRG were fixed, decalcified, embedded in paraffin, and sectioned at 5μm. Severity of IVD degeneration was assessed using Safranin-O/fast-green/hematoxylin staining with a semi-quantitative scale [3,4], adapted for the rat. Intradiscal inflammation status was assessed using immunohistochemistry against TNF-α, IL-1β, and IL-6. In the DRG, immunohistochemistry was performed against SubP, and the percentage of immunopositive neurons was obtained. Neuron diameters were measured allowing for identification of the small and medium diameter peptidergic nociceptive neurons [5]. For statistical analyses, paw withdrawal threshold and IVD height at each week were normalized to pre-surgery values. SPSS software was used to perform a linear step-wise regression with an inclusion and exclusion criteria based on variance with a p<0.05. GraphPad Prism 7.00 was used to determine between-group differences using one- and two-way ANOVAs as appropriate with p<0.05 considered significant.

RESULTS: Multivariate stepwise regression determined that normalized IVD height was the only significant contributor to normalized paw withdrawal threshold (β=-2.041, 95% CI=0.982-3.100, p=0.001) (Table 1). Also, a significant correlation was observed between normalized IVD height and normalized paw withdrawal threshold (Figure 1), further demonstrating the relationship between these factors. It is now clear that IVD height loss was also correlated with intradiscal TNF-α, IL-1β, and IL-6 immunopositive labeling (Table 2). Lastly, TNF-α injection induced significantly more IVD degeneration than PBS injection (p=0.0001) and sham (p<0.0001). No significant differences in IVD height were found between TNF-α and PBS injection, and both groups also had paw withdrawal thresholds that were not different by a one-way ANOVA; however, both groups were significantly different than sham [1].

DISCUSSION: In this IVD injury model progressive IVD height loss predicted increased pain behavior. Increased intradiscal pro-inflammatory cytokine expression from annular injury was related to IVD height loss but, interestingly, not primarily responsible for the observed nociceptive responses in this animal model. The strong relationship between the observed pain behavior with IVD height loss without any evidence of IVD herniation on histology or MRI suggests the possibility of spinal nerve compression via foraminal stenosis or mechanical instability. The observed pain behavior was not associated with increased SubP in the class of DRG neurons probed, consistent with the lack of difference in pain behavior between PBS and TNF-α injections. By analyzing the neurons according to size, we were able to increase precision in detecting possible changes in neuropeptide expression, as nociceptive neuropeptides, including SubP, are produced by the smaller Aδ and C fiber neurons [6]. However, neuromodulator expression may have increased transiently after injury to sensitize nociceptive neurons and then returned to baseline levels at this 6-week time point still resulting in the observed hyperalgesia. Additionally, some evidence exists that pro-inflammatory cytokine expression increases only transiently after IVD puncture injury [7], so a shorter end point would be useful and is currently under investigation. Other causal factors, such as neurovascular ingrowth into the IVD and an increased neural firing rate, cannot be excluded with the current analysis and should be considered for future studies. In this rat model, decreasing IVD height was the only factor predictive of painful IVD degeneration, suggesting early interventions to restore IVD height and biomechanics are interventions warranting future investigation.

SIGNIFICANCE: Improved understanding of underlying causes of IVD-degeneration related pain will help inform future therapeutic strategies. This rat model of IVD degeneration develops increased pain behaviors and has many similarities with the human condition.


ACKNOWLEDGEMENTS: This work was supported NIAMS/NIH (R01AR064157). We thank Damien M. Laudier for technical assistance on histology.

Table 1. Multivariate stepwise regression analysis to identify sources of painful behaviors.

<table>
<thead>
<tr>
<th>Source</th>
<th>β</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normalized disc height</td>
<td>2.041</td>
<td>0.001**</td>
</tr>
<tr>
<td>Disc degeneration score</td>
<td>-0.209</td>
<td>0.515</td>
</tr>
<tr>
<td>% TNF-α immunopositivity</td>
<td>0.241</td>
<td>0.428</td>
</tr>
<tr>
<td>% IL-1β immunopositivity</td>
<td>-0.069</td>
<td>0.852</td>
</tr>
<tr>
<td>% IL-6 immunopositivity</td>
<td>0.171</td>
<td>0.508</td>
</tr>
<tr>
<td>% ncl. DRG SP immunopositivity</td>
<td>0.142</td>
<td>0.575</td>
</tr>
</tbody>
</table>

Figure 1. IVD height is correlated to paw withdrawal threshold.

Table 2. Linear correlation analysis between IVD height and intradiscal pro-inflammatory cytokine levels.

<table>
<thead>
<tr>
<th>Source</th>
<th>r</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intradiscal pro-inflammatory cytokine</td>
<td>-0.513</td>
<td>0.042</td>
</tr>
<tr>
<td>IL-6</td>
<td>-0.499</td>
<td>0.058</td>
</tr>
</tbody>
</table>

INTRODUCTION:
Cervical spondylotic myelopathy (CSM) is a leading cause of spinal cord dysfunction; it is a slowly progressive disease that is thought to be associated with irreversible neurological changes. Cervical sagittal balance has been shown to be an important parameter for surgical reconstruction. There is limited data establishing whether sagittal alignment of the cervical spine is correlated with improvements in health-related outcomes after surgery.

METHODS:
Standardized outcome measures including the Neck Disability Index (NDI), Short-Form 12 Physical and Mental Component Scores (SF12 PCS & MCS), modified JOA (mJOA) scores and neck and arm pain levels were prospectively collected. To account for differences at presentation, recovery ratios (RR) were calculated as the percentage difference between pre- and post-operative values. Baseline radiographic parameters including the C2-7 angles in neutral (C27N), flexion (C27F) and extension (C27E) positions, C2-7 Sagittal Vertebral Alignment (C27SVA), C7 slope (C7S) and Ishihara index were analyzed. C2-7 range of motion (ROM) was calculated as the difference between flexion and extension C2-7 angles.

RESULTS:
A total of 235 patients met inclusion criteria and baseline data were collected on 232 (99%). Baseline outcome measures were compared to those at one year or more recent (334-559 days). At least one follow-up outcome was available for 157 patients (68%) and at least one radiographic parameter was available for 172 (73%). Average follow-up was 18±5.4 months and average age at time of surgery was 58±12 years.

Myelopathy improvement (higher mJOA) correlated significantly with changes in extension C27 angle (R -0.36, p=0.014) and C27 range of motion (R -0.42, p=0.005); mJOA also improved with changes in the C7 slope (R -0.26, p=0.042), suggesting a compensatory component to the initial presentation.

Arm pain improved significantly with changes in the flexion C27 angle (R 0.27, p=0.030) and C7 slope (R 0.23, p=0.019).

There were no significant correlations between any radiographic parameter and NDI or SF-12 (PCS or MCS), including change and RR calculations.

There were no significant differences in radiographic parameters between those that reached MCID NDI (>15 point change) and those that did not.

DISCUSSION:
These results suggest that baseline sagittal plane alignment is not well correlated with postoperative health-related outcomes (SF-12, NDI) in patients with cervical myelopathy. Radiographic parameters including range of motion appear to be related to some extent to pain and myelopathy scores and not to quality of life measures. Surgical treatment improved pain and myelopathy (measured by mJOA) regardless of radiographic parameters.

SIGNIFICANCE:
Predictors of desirable outcomes after surgical treatment for myelopathy are of great interest to clinicians treating this common and disabling disorder. This study helps to establish baseline examination factors relevant to surgical outcomes.

IMAGES AND TABLES:

Figure 1: Change in mJOA versus baseline range of motion

<table>
<thead>
<tr>
<th>ΔPCS</th>
<th>p</th>
<th>ΔMCS</th>
<th>p</th>
<th>ΔNDI</th>
<th>p</th>
<th>ΔArm</th>
<th>p</th>
<th>ΔNeck</th>
<th>p</th>
<th>ΔmJOA</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>C27A Neu</td>
<td>-0.099 0.94</td>
<td>-0.146 0.21</td>
<td>0.033 0.84</td>
<td>0.045 0.70</td>
<td>-0.069 0.55</td>
<td>0.070 0.42</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C27A Ext</td>
<td>-0.047 0.60</td>
<td>-0.124 0.27</td>
<td>0.025 0.81</td>
<td>0.050 0.19</td>
<td>-0.122 0.38</td>
<td>-0.387 0.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C27A Flex</td>
<td>-0.044 0.71</td>
<td>-0.097 0.76</td>
<td>-0.039 0.75</td>
<td>0.271 0.03</td>
<td>0.114 0.37</td>
<td>-0.010 0.95</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C27 ROM</td>
<td>0.053 0.66</td>
<td>-0.109 0.36</td>
<td>0.081 0.52</td>
<td>0.225 0.08</td>
<td>-0.209 0.10</td>
<td>-0.422 0.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C27 SVA</td>
<td>-0.056 0.56</td>
<td>-0.115 0.23</td>
<td>-0.030 0.76</td>
<td>0.100 0.32</td>
<td>-0.014 0.89</td>
<td>-0.211 0.10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C7 Slope</td>
<td>-0.109 0.26</td>
<td>-0.108 0.26</td>
<td>0.047 0.64</td>
<td>0.232 0.02</td>
<td>0.001 0.99</td>
<td>-0.259 0.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ishihara</td>
<td>0.015 0.89</td>
<td>-0.130 0.24</td>
<td>0.040 0.73</td>
<td>0.067 0.57</td>
<td>0.006 0.96</td>
<td>-0.172 0.26</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 2: Pearson correlations between changes in outcomes and radiographic parameters

<table>
<thead>
<tr>
<th>ΔPCSp</th>
<th>ΔMCSp</th>
<th>ΔNDIp</th>
<th>ΔArm p</th>
<th>ΔNeck p</th>
<th>ΔmJOAp</th>
</tr>
</thead>
<tbody>
<tr>
<td>C27A Neu</td>
<td>-0.007 0.95</td>
<td>-0.168 0.13</td>
<td>0.035 0.76</td>
<td>-0.026 0.82</td>
<td>-0.052 0.67</td>
</tr>
<tr>
<td>C27A Ext</td>
<td>-0.040 0.73</td>
<td>-0.142 0.21</td>
<td>0.050 0.80</td>
<td>-0.042 0.71</td>
<td>-0.066 0.60</td>
</tr>
<tr>
<td>C27A Flex</td>
<td>-0.064 0.59</td>
<td>-0.058 0.62</td>
<td>0.066 0.60</td>
<td>-0.052 0.68</td>
<td>-0.070 0.59</td>
</tr>
<tr>
<td>C27 ROM</td>
<td>0.070 0.56</td>
<td>-0.111 0.35</td>
<td>-0.064 0.61</td>
<td>0.005 0.97</td>
<td>-0.006 0.96</td>
</tr>
<tr>
<td>C27 SVA</td>
<td>-0.069 0.48</td>
<td>-0.142 0.14</td>
<td>0.010 0.92</td>
<td>-0.050 0.62</td>
<td>-0.096 0.35</td>
</tr>
<tr>
<td>C7 Slope</td>
<td>-0.125 0.20</td>
<td>-0.133 0.17</td>
<td>0.013 0.88</td>
<td>-0.060 0.55</td>
<td>-0.155 0.13</td>
</tr>
<tr>
<td>Ishihara</td>
<td>-0.006 0.96</td>
<td>-0.181 0.10</td>
<td>0.036 0.76</td>
<td>0.004 0.97</td>
<td>-0.105 0.39</td>
</tr>
</tbody>
</table>

Figure 3: Pearson correlations between outcome recovery ratios and radiographic parameters
High Mobility Group Box-1 Promotes Pro-inflammatory Signaling in Human Nucleus Pulposus Cells via Toll-Like Receptor 4

Bhranti S. Shah1, Tiago Fernandes1, D. Olivier Alipui2, Kathryn T. Weber2, M. Chris Overby2, Mitchell Levine2, Nadeen O. Chahine1,2
1The Feinstein Institute for Medical Research, Northwell Health, Manhasset, NY, 2 Hofstra Northwell School of Medicine, Hempstead, NY

Disclosure: The authors have nothing to disclose.

INTRODUCTION:
Intervertebral disc (IVD) degeneration is characterized by the production of inflammatory cytokines, breakdown of extracellular matrix (ECM) and altered cell phenotype. Evidence suggests that a number of endogenous damage-associated molecule patterns (DAMPs) within the IVD serve as primary mediators of disc degeneration. These DAMPs within the IVD include components of ECM fragments, and the nuclear protein, high mobility group box 1 (HMGB1). Stimulation with HMGB1 induces pro-inflammatory cytokines, such as TNF-α, IL-1α, IL-1β, IL-6, IL-8, macrophage inflammatory protein (MIP)-1α, MIP-1β, or MIP-2 in human and murine monocytes, macrophages and dendritic cells. We have previously shown that intradiscal injections of LPS, an inflammatory stimulant, into rat caudal discs results in moderate disc degeneration and elevated HMGB1 protein levels. Grubber et al have shown that HMGB1 gene expression is substantially increased in more degenerated Thompson grade V discs compared to less degenerated grade I/II discs and in herniated surgical specimens compared to control discs. Hence the goal of this study is to identify the inflammatory response of human nucleus pulposus (NP) cells to HMGB1 stimulation and to identify the receptors contributing to inflammatory signaling induced by HMGB1. We specifically evaluated the contributions of toll like receptor (TLR)-4, TLR-2, and receptor for advanced glycation endproducts (RAGE), all of which have been implicated as binding receptors of HMGB1.

METHODS:
HMGB1 levels in degenerate discs: IVD tissue was obtained from a total of 17 patients undergoing spinal surgery in accordance with the IRB approved practices. IVD MRI of each subject at level of surgery was graded using Pfirrmann grade (Grade I-V), and where grouped to ‘early’ (Grade I-II), ‘intermediate’ (Grade III), or ‘advanced’ (Grade IV-V) stages of severity. HMGB1 stimulation: Human NP cells were commercially procured and expanded by culturing in DMEM/F12 complete media. After overnight incubation, the cells were incubated with different doses of HMGB1 diluted in complete media for 24h (recombinant mouse HMGB1 generously provided by Dr. Kevin Tracey). After 24h, the cells were lysed to harvest RNA and the supernatant cell medium was collected for cytokine release analysis (ELISA). Signaling inhibition studies: Human NP cells were pre-incubated with different doses of TAK-242 (TLR4 inhibitor), O-vanillin (TLR2 inhibitor), or FPS-ZM1 (RAGE inhibitor) diluted in complete media for 1 hour. After 1 hour, the pre-incubation treatment medium was removed and the cells were treated with 2 µg/mL recombinant HMGB1 along with the receptor inhibitors in complete media for 24 hours. RNA was harvested from the cells for gene expression analysis (IL-6, MMP-1) and the supernatant cell medium was collected for cytokine release.

RESULTS:
HMGB1 gene expression levels were found to be significantly increased from patients with increasing severity grades. Expression in ‘advanced’ severity group was 2.25-fold greater (p=0.022) compared with ‘intermediate’ and ‘early’ grade severity (Fig 1A). In vitro, we tested the potential of HMGB1 to promote pro-inflammatory signaling in human NP cells. Our results show that HMGB1 induced significant and dose-dependent increases in the levels of IL-6 (Fig 1B) and MMP-1 (data not shown) at the gene and protein release levels (not shown) Next, we evaluated the contribution of HMGB1 binding receptors to measured responses by inhibiting TLR4, TLR2 or RAGE using small molecule inhibitors. Our results show inhibition of TLR4 with TAK-242, a TLR4 signaling inhibitor, significantly reduced IL-6 (Fig 2A) and MMP-1 gene expression (not shown) and protein release (not shown) after 24h treatment. Interestingly, inhibition of TLR2 and RAGE using small molecule inhibitor treatment of human NP cells did not have a significant effect on pro-inflammatory signaling (Fig. 2B, C).

DISCUSSION:
The goal of this study is to identify the role of HMGB1, a known DAMP and potent mediator of chronic inflammation in IVD degeneration. Our findings demonstrate that HMGB1 gene expression levels are significantly elevated in advanced in comparison to early and intermediate degeneration human disc samples. Mechanistically, we show that exogenous HMGB1 stimulation of healthy NP cells not only upregulated gene expression of pro-inflammatory cytokines, IL-6 and MMP1 but also induced their production and secretion in a dose-dependent manner in short term NP cell cultures. In the disc, an elevated level of IL-6 has been shown to reduce expression of IVD ECM proteins, aggrecan and collagen type II and is associated with the neurological symptoms and progression of DD. As in osteoarthritis, MMPs, specifically MMP1 plays a central role in orchestrating ECM breakdown in discs of patients with IVD degeneration. In order to identify the receptors contributing to HMGB1 signaling, we blocked three major receptors, TLR4, TLR2 and RAGE to perturb HMGB1 activity in NP cells. By selectively disrupting the HMGB1-TLR4 axis signal transduction, we completely mitigated HMGB1-induced expression of IL-6 and MMP1. On the other hand, we saw either partial or no inhibition of cytokine-inducing HMGB1 function upon TLR2 and RAGE inhibition, respectively. In conclusion, our study establishes HMGB1 as a targetable DAMP that mediates release of pro-inflammatory cytokines in NP cells, via a mechanism that predominantly depends on TLR4 signaling.

SIGNIFICANCE:
Our findings demonstrate that the nuclear protein HMGB1 acts as a mediator of inflammation in human IVD when released extracellularly, and identifies the receptor responsible for pro-inflammatory activity of HMGB1 in NP cells.

REFERENCES:

ACKNOWLEDGMENTS: NSF CAREER 1151605, NIH R01AR069668, New York State Department of Health (ECRIP)
Neuropeptide Y promoter variant correlates with decreased pain at baseline in older adults with mild to moderate lumbar spinal stenosis but a blunted functional response to exercise-based treatment protocols

Sara Ernst PhD, Wan Huang M D PhD, Kris Gongaware DC, Nam Vo PhD, Michael Schneider DC PhD, Gwendolyn Sowa MD PhD

**Introduction:** Lumbar spinal stenosis (LSS) is a debilitating disorder of the lumbar spine. Currently there is a limited capacity for predicting individual response to non-surgical treatments of LSS. Recent advances in pain research have identified several genetic polymorphisms and blood biomarkers associated with inter-individual symptom and treatment-response variability. One such gene, Neuropeptide Y (NPY), encodes a neuronal protein which may confer resilience in coping with pain.

**Objective:** Explore the association between an NPY gene promoter polymorphism (rs16147), plasma Pro-NPY protein levels, baseline symptom severity and disability, and treatment response.

**Methods:** Patients with diagnostic imaging and clinical symptoms consistent with LSS were recruited from a larger study which randomized patients age 60+ years into one of three treatment groups: usual medical care (UMC, 3x/6 weeks), community-based group exercise (GE, 2x/week), or clinic-based individualized exercise and manual therapy (IEMT, 2x/week). Patient data collected at baseline included blood pressure, BMI, demographics, depression score (PROMIS depression short form), back and leg pain (visual analog scale), Swiss Spinal Stenosis questionnaire (SSS) including symptom severity and physical disability subscales, and performance on a self-paced walking test (SPWT). Saliva and blood samples were obtained for polymorphism genotyping (NPY rs16147) and baseline biomarker analysis (Pro-NPY ELISA). Patients received treatment for 6 weeks and another blood sample, SSS questionnaire, and SPWT data were collected at an 8 week follow-up visit.

**Results:** NPY genotyping was completed for 177 patients (TT n=45; CT n=92; CC n=40). 161 of these completed treatment (UMC n=59; GE n=49; IEMT n=53) and 120 patients gave blood for biomarker analysis (UMC n=46; GE n=28; IEMT n=46). Plasma Pro-NPY trended toward higher levels in individuals with 1 or 2 copies of the variant allele, CT/CC (initial visit mean ± SD: TT = 45.8 ±23.1; CT/CC =57.0 ±59.0 pg/ml, p=0.18; follow-up: TT=41.3 ±22.2; CT/CC =55.8 ±45.0, p=0.042). CT/CC males exhibit lower baseline symptom severity compared with TT individuals (mean SSS-symptom severity subscale: TT=21.4 ±4.7; CT/CC =19.0 ±4.6, p=0.032) and males with higher levels of plasma Pro-NPY correlate with lower SSS scores of symptom severity and physical disability (r²=0.142, p=0.008). No such trend was observed in female subjects. Although experiencing fewer symptoms at baseline, CT/CC individuals have 3.1 times the odds of non-responder status on SPWT following treatment (p=0.03). This difference is most obvious in the Group Exercise and Manual Therapy treatment groups (Odds Ratio =6.7, p=0.05).

**Conclusion:** Our data demonstrate that a polymorphism in the NPY gene promoter, previously correlated with increased expression of NPY, correlates with reduced symptom severity in male LSS patients but may predict worse functional response to exercise-based treatment interventions. These data may suggest a role for NPY genotyping in personalizing treatment plans for male patients with mild to moderate LSS.
Identification of the Compositional Traits and Permeabilities of the Cartilage Endplate that are Required for Nutrient Transport and Disc Cell Survival

J. Wong, A. Ouyang, S. Sampson, J.C. Lotz, A.J. Fields
University of California, San Francisco, CA United States

Disclosures: J. Wong (None); A. Ouyang (None); S. Sampson (None); J.C. Lotz (None); A.J. Fields (None)

Introduction: Low back pain is the leading cause of disability and is closely linked to disc degeneration. Intradiscal biologic therapy is a promising strategy for managing disc degeneration. However, the poor nutrient environment of the disc is now recognized as an obstacle for biologic therapies that increase disc cellularity or matrix synthesis rates [1, 2]. One reason for the poor nutrient environment could be low cartilage endplate (CEP) permeability, since nutrients and metabolites entering and exiting the nucleus pulposus must diffuse across the CEP. The goals of the study were to: 1) identify critical levels of CEP permeability needed to support cell densities associated with healthy discs; and 2) discover compositional characteristics of the CEP matrix that diminish its permeability and thereby hinder nutrient transport and disc cell function. To do this, we first used diffusion chambers to test how human CEPs with a wide range of permeability impact nutrient transport and disc cell function. Then we probed the molecular components of the CEP with Fourier Transform Infrared Spectroscopy (FTIR) imaging to determine differences in the amount and spatial distribution of molecular markers that distinguish permeable CEPs from impermeable CEPs.

Methods: Tissues: Twelve intact human CEPs bordering the nucleus pulposus were harvested from six cadaveric lumbar spines (age range: 38–66 years old; mean age: 56 ± 10 years). Permeability: To determine the permeability of the CEP samples, we measured the diffusivity of a small fluorescent tracer (sodium fluorescein, 376 Da; 0.1 mg/ml in PBS) in the CEPs using fluorescence recovery after photobleaching. Nutrient Transport: Diffusion chambers were used to identify critical levels of CEP permeability needed for disc cell survival. The chambers consisted of two glass plates held apart by spacers; bovine nucleus pulposus cells cultured in the center of the chambers were separated from their nutrient source (low-glucose DMEM with 6% FCS) at the open sides of the chambers by the CEP samples. After incubating the chambers (48 hr; 21%/5% O2/CO2), cells were stained with a Live/Dead assay, and the viable distance was measured with a fluorescence microscope. We performed this procedure using two cell densities: 4 million cells/ml, which is the average cell density of an adult disc [3], and 8 million cells/ml. CEP Composition: The same CEPs were cryosectioned (thickness 7 µm) perpendicular to the surface of the CEP. Sections were mounted onto BaF2 windows and air-dried. FTIR images were acquired with a spectral resolution of 4 cm⁻¹ and pixel size of 6.25µm (Perkin Elmer Spotlight 400) and averaged for three adjacent CEP sections. We measured the depth-wise distribution of the following parameters: collagen content (peak area of 1595–1710 cm⁻¹), mineral/matrix ratio (ratio of phosphate’s 895–1215 cm⁻¹ P-O stretch peak area to collagen’s Amide I peak area), and collagen maturity (absorbance ratio at 1660 cm⁻¹ and 1690 cm⁻¹ peaks [4]). Statistics: Results (Mean ± SD) were compared using t-tests.

Results: Diffusion chambers revealed that physiologic fluctuations in CEP permeability had a significant effect on disc cell viability, and that low CEP permeability hinders transport regardless of cell density (Fig. A). Solute diffusivity varied nearly 4-fold between the CEPs studied, and chambers with CEPs that had low diffusivity had a significantly shorter viable distance (p < 0.01). As expected, increasing cell density in the chambers shortened the viable distance; however, this effect depended on CEP permeability. Specifically, for CEPs with diffusivities <60 µm²/s, there was no change in viable distance, which suggests that these CEPs may not allow for sufficient nutrient transport to satisfy cell nutrient demands. FTIR imaging showed significant differences in composition between nutrient transport chambers with low vs. high diffusivity (Fig. B-D). Compared to CEPs with high diffusivity (>80 µm²/s), those with low diffusivity (<40 µm²/s) had higher collagen content (p < 0.001), greater mineral/matrix ratios (p ≤ 0.01), and lower collagen maturity (p < 0.001) at all depths (Table). Discussion: Our study set out to: 1) identify critical levels of CEP permeability needed for disc cell survival; and 2) discover compositional characteristics of the CEP that diminish its permeability and hinder nutrient transport. Results demonstrated that nutrient diffusion across the CEP is insufficient to meet the metabolic demands of disc cells when solute diffusivity in the CEP is less than 60 µm²/s, and that CEPs with low solute diffusivity have higher collagen content, higher mineral/matrix ratios, and lower collagen maturity. Previous work using diffusion chambers showed that increasing disc cell density shortens the viable distance because doing so raises nutrient demands [5]. Here we found this effect depends on CEP permeability. Namely, when solute diffusivity in the CEP was <60 µm²/s, viable distance was insensitive to cell density. This suggests that the limiting factor in these cases is low nutrient transport across the CEP and not the high nutrient demand of the disc cells. One implication of these findings is that patients with low CEP permeability may be poor candidates for biologic therapies intended to regenerate the nucleus pulposus. Instead, enhancing CEP permeability may be required. To determine compositional traits that distinguish the CEPs with low vs. high permeability and which could thus serve as possible targets for treatments to enhance CEP permeability, we performed FTIR imaging of the CEPs. Our finding that low solute diffusivity was associated with a greater amount of collagen, more mineral, and lower collagen maturity suggests that these characteristics may physically block nutrient diffusion. For example, collagen fibers can resist tissue swelling and hinder solute uptake [6]; mineral is highly impermeable; and lack of enzymatic crosslinks, which are increased in mature tissue, could destabilize the collagen network. Finally, unlike the zonal variations observed in articular cartilage [7], endplate cartilage composition was constant through the depth of the CEP, which implies that treatment strategies to enhance CEP permeability may need to consider the entire CEP.

Significance: The poor nutrient environment of the disc may hinder the success of intradiscal biologic therapies. Our findings are significant because they: 1) show that low CEP permeability may be a limiting factor for intradiscal therapy; and 2) identify compositional deficits in the CEP that prevent adequate nutrient diffusion and which thus represent targets for novel treatments to enhance CEP permeability.


Acknowledgements: This study was supported by UCSF Pathways to Discovery (JW), REAC (AJF), NIH AR063705 (JCL), and NIH AR066262 (AJF).

References:

Figure: A. Viable distance in the diffusion chambers depended on cell density (nutrient demand) and CEP permeability (nutrient transport). Overlap in the relationships for CEPs with low diffusivity (<60 µm²/s) suggests that nutrient transport is insufficient to meet cell demands. B-D. Representative FTIR data and images for CEPs with high and low solute diffusivity. Compared to CEPs with high solute diffusivity, those with low solute diffusivity had greater collagen content, greater mineral/matrix ratios, and lower collagen maturity across the CEP. Envelopes indicate ± 1SD for n = 3 adjacent sections per CEP.

Table: Compared to CEPs with high solute diffusivity, those with low solute diffusivity had significantly greater collagen content, greater mineral/matrix ratios, and lower collagen maturity. Compositional measurements did not vary significantly with depth. n = 3 CEPs per group.
Changes in Muscle Mass and Bone Morphology of the Spine and Lower Limb after Spinal Chord Injury and Treadmill Training
Shania Shaji¹, Gabrielle Gehron¹, Brittany King², Jaclyn Witko², Dr. Jennifer Kadlowec², Dr. Anita Singh¹
¹: Biomedical Engg, Widener University, Chester, PA ²: Mechanical Engg, Rowan University, Glassboro, NJ

Introduction: Approximately 12,000 people are the victims of spinal cord injuries (SCIs) every year¹. Common side effects include paralysis below the point of injury, and osteoporosis² resulting in lowered bone stiffness and failure loads. Bone and Muscle loss is a common condition that accompanies SCI. After Body Weight Supported Treadmill Training (BWSTT), some functional recovery accompanied by bone and muscle mass restoration can occur. This study investigates changes in the strength of the lower limb and spine and the accompanying muscle mass restoration after SCI and BWSTT.

Materials and Methods: 19 female Sprague-Dawley rats were divided into three groups: Group 1: Control. Group 2: SCI/Ctsn. Group 3: SCI/Ctsn+BWSTT. Animals in Groups 2 and 3 received moderate contusion SCI. One week after injury, animals in Group 3 started treadmill training at 1000 steps per day, 5 days per week, for 8 weeks. During BWSTT, a robotic arm supported 75% body weight while a treadmill running at 7cm/s forced (Fig 1) assisted locomotion. At the end of the study, the right Soleus muscles was harvested, weighted and calculated against the total body weight. Also, the lumbar L5 and tibial bones were harvested. The harvested tibiae bone was subjected to a 3 point bending test at a constant displacement rate of 5 mm/min and the ultimate failure load was calculated for each specimen. The lumbar vertebra was compressed using the same machine at 2 mm/min rate until the maximum compressive load was determined (Fig. 2). The methods in this study were in accordance with those approved by the Institutional Animal Care and Use Committee.

Results and Discussion: No significant difference in the mechanical behavior of bones was observed for any group (Fig. 3). However, BWSTT led to significant improvement in the soleus muscle mass when compared to no training animals. We attribute no changes in the bone properties after BWSTT to the high 75% BWS during training. Training with lower body weight support might result in significant improvement in the biomechanical properties of the bones below the level of injury. Future studies are needed to investigate the effects of BWS and bone strength in various SCI models.

Conclusions: SCI has significant effects on the muscle mass and bone loss and BWSTT leads to some restoration of muscle mass but have no effect on the bone mechanical properties in both the tibia and the lumbar spine.

Acknowledgements: New Jersey State Commission Grant #CSCR14ERG001, Bridgette Saverine, Lindsay Stoy, Sarah Townsend.

Load-sharing of the Intervertebral Discs During Standing Determined by Open-Upright Magnetic Resonance Imaging

Christian J. Weber1, Ching-Ting Huang2, Linda R. Van Dillen1,4, Simon Y. Tang1,4
1Biomedical Engineering; 2Movement Science; 3Physical Therapy; 4Orthopaedic Surgery
Washington University in St. Louis, St. Louis, MO

DISCLOSURES: None

INTRODUCTION: Low back pain (LBP) is a function-limiting condition that affects up to 80% of the population at least once in their lifetime. The estimated healthcare costs exceed $100 billion a year [1]. The intervertebral disc (IVD) is a suspected pain generator due to its load-bearing nature and susceptibility to degeneration [2]. Currently, Magnetic Resonance Imaging (MRI) is the most commonly utilized clinical technique to visualize and assess the IVD [3]. The Open Upright® MRI systems allow an individual to stand during imaging thus allowing the examination of the spine in a loaded state. The objective of this study was to examine the changes in the spine between supine and standing by measuring changes in the intervertebral (IV) angles.

METHODS: Twelve back-healthy people were recruited (age 18-35, 3 female, BMI <30). Images of the lumbar spine (L1-S1) were obtained in supine (unloaded) and standing (loaded) using a 0.6 T Open Upright® MRI system. A 3-plane localizer was used to acquire sagittal T2 weighted images (repetition time = 610 ms, echo time = 17 ms, field of view = 24 cm, acquisition matrix = 210 x 210, slice thickness = 3 mm, gap = 0 mm, scan duration = 2 min) [4]. Initially, the participant stepped into the scanner and a pillow was placed behind the head. The MRI table then was moved to a horizontal position. The participant remained in supine and scans were acquired after 10 minutes. The MRI table then was moved back into vertical (standing) and the pillow was removed. The participant then was imaged in standing. From the acquired images in both positions, the IV angles were measured for the L1/L2 through L5/S1 vertebral levels. The IV angle was calculated as the angle created by a vector formed between the anterior and posterior edge of the inferior endplate and a vector formed between the anterior and posterior edge of the superior endplate at a given vertebral level that intersects at the posterior midpoint of the disc. For each participant, the change in IV angle from supine to standing was calculated for each vertebral level (L1-S1). The mean and standard deviation of percent change in lumbar IV angle across levels then was calculated. For each participant, the percent change in IV angle for each vertebral level also was calculated and plotted. This study was conducted with the approval from the Human Research Protection Office at Washington University School of Medicine.

RESULTS: Figure 1 provides data for 12 back-healthy (denoted as S1…S12) participants. Individual participant data are displayed. The change in IV angle from supine to standing is expressed as a percentage, with positive values indicating an increase in IV angle (increased posterior loading), and negative values indicating a decrease in IV angle (decreased posterior loading). For each participant, the mean and standard deviation for the percent change of all of the lumbar IV angles, as well as the percent change for each individual vertebral level (L1/L2-L5/S1) are presented. The plots show the variability in the percent change in IV angles within and across participants. Within a participant some IV angles show much larger changes relative to the other levels. Across participants some (S1 - S5) display low variability in the percent change in IV angle across levels, while others (S6 -S12) display high variability in percent change in IV angle across levels.

DISCUSSION: Compared to supine, standing imposes increased loads on the spine, and these loads are transmitted through the IVD. As a result of the change in loading, the IVDs adapt and distribute these loads through changes in their morphology. We use the IV angle here to quantify the morphological changes of the IVDs at each vertebral level. Positive increases in the IV angle indicate increased loading on the posterior elements of the vertebral unit. Thus, the distribution of each participant’s percent change in IV angles provides insight as to how loads are shared and distributed across the participant’s lumbar spine between supine and standing. A small standard deviation of the percent change in the IV angles suggests that each of the lumbar IVDs under go similar changes, while a large standard deviation suggests an uneven distribution in the loading on individual IVDs. The variability in IV angles both within and across participants may be particularly revealing for back-healthy participants. Those IVDs with large percent changes in IV angle that lie outside of the participant’s standard deviation may be susceptible to fatigue damage given the large changes in loading. Since there is data to suggest that back-healthy individuals can develop low back symptoms in prolonged standing [5], these ‘at-risk’ IVDs that are sustaining elevated posterior loading could be potential pain generators in these individuals. Further analyses are required to examine whether these IV angle changes are associated with the stresses and strains within the IVDs.

SIGNIFICANCE: This work illustrates the potential importance of studying the differences exhibited in IVDs within an individual between supine and standing. The assessment of the IV when loaded may provide important insights into the mechanisms for the development and treatment of low back pain.


ACKNOWLEDGEMENTS: This work was supported by the Washington University Musculoskeletal Research Center (NIH P30 AR057235) NIH ST32EB018266, and NIH K01AR069116.

FIGURE 1: The percent change of the IV angle from supine to standing for each vertebral level and the mean IV angle percent change with standard deviation bars are plotted for each participant (S1-S12). A positive change represents a decrease in angle (increase in posterior loading), while a negative change represents an increase in angle (decrease in posterior loading). Using this approach, the distribution of loads across vertebral levels can be identified in individuals.
Predicting Intradiscal Pressure in the Cervical Spine Based on Disc Height

Forbes E. Howington, Kevin M. Bell
Department of Orthopaedic Surgery, University of Pittsburgh, Pittsburgh, PA

Disclosures: The authors have nothing to disclose.

INTRODUCTION: The majority of studies examining the intradiscal pressure (IDP) within the spine have focused on the lumbar spine. These studies have consistently shown that pressure within the disc increases as compressive load is added, and that the pressure is dependent on the posture. Only a single study, performed by Hattori et al., has examined in-vivo IDP within the cervical spine. The greatest pressures have been found in maximum extension, and the next highest have been found in maximum flexion. Degeneration of the disc was found to have implications on the pattern of IDP vs. flexion angle, and the position in which maximum and minimum pressures were found was not consistent across all subjects. Because of the ethical barriers to any further study of the in-vivo IDP within the cervical spine, recent work has been limited to investigation of IDP in-vitro. In looking at the IDP in-vitro, it is important to take into account not only the pressure generated from the relative positions of the vertebrae, but also the compressive loading. In-vitro, this compression is applied by the weight of the head and postural muscles. Follower loading, developed by Patwardhan et al., was developed to mimic this compressive load in-vitro. A previous study has looked at the effects of follower load on IDP as a moment is applied to the cervical spine, which is valuable but difficult to translate to the in-vitro scenario. Therefore, this study instead focused on the relationship of IDP and disc height across a flexion/extension motion path with the ultimate goal of predicting IDP based on the relative positions of the vertebrae.

METHODS: The in-vitro data was collected under a CORID approved protocol. Ten cadaveric spines (50.9 years ± 7.7) were evaluated using a robotic testing system in which the specimens were moved through flexion/extension with and without 100N of follower load applied. Intradiscal pressure was recorded C4-C5 as well as C5-C6 with a 500 PSI miniature pressure transducer (Precision Measurement Company, Model 060). Vertebral body motion was tracked using a five-camera VICON tracking system. The transducers were synchronized with the motion tracking system such that pressure data could be matched up to positional data for each vertebra C3-C7. CT scans of each specimen were used to create 3D models of each vertebra of interest using commercial software ( Mimics software, Materialise, Leuven, Belgium). These models were then loaded into a custom software suite which allowed for the interactive placement of anatomical markers used to define the boundaries of the discs. The software then applied the previously collected positional data to the 3D models, as shown in Figure 1. This allowed for direct measurement of the anterior and posterior disc height over the course of the flexion/extension motion. From this data a correlation was made between the intradiscal pressure and the difference between the anterior and posterior disc height (Figure 2). Both anterior and posterior disc heights were standardized to their respective heights in the static position. The r² values for an exponential fit of the IDP vs. anterior-posterior disc height were calculated per specimen, and the r² values and corresponding best fits for all samples were averaged across each disc under each loading condition in both flexion and extension.

RESULTS: Shown in table 1 are the average best-fit curves for the corresponding vertebrae and motion. The fit was exponential such that in practice the curves had the form e^{AxB}, where A is the slope and B is the intercept. In every instance, the magnitude of the slope of the curve was decreased when follower load was added relative to the pure moment loading of the specimens. Table 2 demonstrates the average r² values of the fits applied to each specimen at every joint for both flexion and extension. In 3 out of 4 instances the addition of follower load had a large negative impact on the average r² value. In table 3 the average IDP of the specimens while in a static position (no flexion or extension) is shown. In both discs the pressure was increased by more than 40 PSI due to the addition of 100 N of follower load.

<table>
<thead>
<tr>
<th>Table 1: IDP vs. Average Disc Height Regression. Equation in form e^{AxB}</th>
<th>Table 2: Average r² values for fits to corresponding motion segments</th>
<th>Table 3: Average IDP (PSI) in static position</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Extension</strong></td>
<td><strong>Slope</strong></td>
<td><strong>Intercept</strong></td>
</tr>
<tr>
<td>C4-C5</td>
<td>4.34</td>
<td>2.66</td>
</tr>
<tr>
<td>C5-C6</td>
<td>1.68</td>
<td>1.50</td>
</tr>
<tr>
<td>C4-C5</td>
<td>0.95</td>
<td>3.23</td>
</tr>
<tr>
<td>C5-C6</td>
<td>0.62</td>
<td>3.71</td>
</tr>
</tbody>
</table>

DISCUSSION: These findings demonstrate that changes in disc height correspond to an exponential increase in IDP. Physiologically this outcome is reasonable. As the disc is further compressed, the stiffness of the annulus rapidly increases as the limits of its elastic deformation are reached. If disc height was a perfect indicator of intradiscal pressure, then there would be no difference in the curves generated by pure moment loading and pure moment + follower loading of the specimen. The decrease of the fit’s slope in all motion segments indicates that there is less change in intradiscal pressure per the same change in disc height when follower loading is added. There are several possible explanations for this. It was previously shown that the application of follower load to the joint changes the disc height at every flexion angle when compared to pure moment loading of the joint. Due to this reduction in disc height, it may be the case that there are bony interactions occurring. It is especially conceivable that this may occur at the facet joints. This would lead to a reduction in intradiscal pressure due to the pressure being offloaded onto the facet joints instead of being transmitted through the discs. Future studies should focus on finding methods of calculating IDP that takes into account variables such as the sex, age, and disc health (initial disc height) of the specimen. In addition, it may be beneficial to determine the IDP based on the volume of the disc rather than based off the difference in anterior and posterior disc heights. This may allow for the relative neutral-zone found in each specimen to be more accurately taken into account, as it may be the case that the disc pressure begins its exponential growth following some percent reduction in volume.

SIGNIFICANCE: This work provides a method of determining the intradiscal pressure in-vivo, which is currently not feasible. Because the only information necessary for these calculations is the anterior/posterior disc height in the static position and at each flexion/extension angle of interest, there already exists a large body of in-vivo data to which it can be readily applied.


ACKNOWLEDGEMENTS: The Albert B. Ferguson, Jr. MD Orthopaedic Fund of The Pittsburgh Foundation and the Dean’s Summer Research Project of the University of Pittsburgh School of Medicine are gratefully acknowledged.

Figure 1: Motion tracking of 3D vertebral model displaying CT defined marker beads (green) and anatomically defined disc boundaries (red)

Figure 2: IDP vs. anterior minus posterior disc height for a single specimen in C4-C5. Separate best-fit curves were calculated for flexion and extension, as shown.
Development of an In Vivo Model of Neonatal Intervertebral Disc Injury and Regeneration

Olivia M. Torre¹, Grace E. Mosley¹, Rohit Das¹, Alice H. Huang¹, James C. Iatridis¹
¹Leni & Peter W. May Department of Orthopaedics, Icahn School of Medicine at Mount Sinai, New York, NY, USA

INTRODUCTION: Back pain is a leading cause of global disability that inflicts tremendous socio-economic burden [1], and is commonly associated with intervertebral disc (IVD) degeneration. Painful IVD degeneration commonly involves herniation and other structural defects [2]. Disectomy is the gold standard treatment when conservative care fails, yet the annulus fibrosus (AF) defect is not repaired and recurrent herniation and degeneration related pain is a risk [3] due to the poor healing capacity of the IVD. One key challenge for developing new cell-based strategies that promote AF healing is the paucity of regenerative models for the AF. To address this limitation, we turned to the neonatal mouse as a model of mammalian regeneration, which has been established for diverse tissues, such as the heart [4], cochlear hair cells [5], and tendon [6]. To test the capacity of neonatal AF for reparative repair, the objectives of this study were to: 1) develop a novel, reproducible model of IVD injury in neonates using the Scleraxis-GFP (ScxGFP) reporter to identify AF, and 2) determine intrinsic AF healing capacity (composition and structure) following neonatal and adult injury.

METHODS: Needle puncture injuries were induced in dorsal-lateral regions of caudal IVDs of neonatal (postnatal day P5, n=10) and adult (4-5 month, n=3) ScxGFP mice [7]. Needle diameters (90% IVD height) were chosen based on previous studies in adult mice [8]. Due to the small size of neonatal IVDs, ScxGFP expression in AF cells was used to visualize neonatal IVDs and generate reproducible injuries (Fig 1). Punctures were created in neonates and adults using syringe needle tips (31G & 26G, respectively) to a depth of 50% of dorsal-ventral width. For all animals, 2-3 IVDs were punctured per tail (marked by India Ink), separated by uninjured internal control IVDs, and whole tails were harvested at 3 and 28 days post-injury. Picrosirius red/alcian blue staining followed by polarized light imaging was performed to determine changes in collagen structure and composition. Collagen intensity was quantified using ImageJ and statistical analysis by Student’s t-test with significance set at p<0.05.

RESULTS: A reproducible needle puncture injury technique was developed in neonatal and adult caudal IVDs in ScxGFP reporter mice. At day 3, punctured neonatal and adult IVDs showed consistent structural damage, including decreased IVD height, obvious AF disruption along the needle track, and complete loss of the nucleus pulposus (NP) (Fig 2). Neonatal AF at day 3 showed early signs of provisional matrix accumulation within the needle track (Fig 2), in contrast to the adult AF needle track, which was completely devoid of tissue at day 3 (not shown). Consistent with previous studies, adult AF at day 28 showed degenerative changes with little evidence of self-repair after injury. The adult needle track remained distinct and devoid of tissue at day 28, and the AF tissue adjacent to the puncture track exhibited structural changes indicative of fibrotic scarring (disorganized and non-uniform collagen intensity) (Fig 3). In contrast, the puncture tracks in neonatal IVDs were completely healed by day 28, with intense collagen-rich matrix, improved fiber density, and alignment (though lamellar structure was not perfectly restored at this timepoint). Staining intensity analysis of brightfield images verified qualitative observations with neonatal AF regions exhibiting similar collagen intensity 28 days post-injury, while adult AF tissue adjacent to the puncture remained abnormal compared to uninjured levels (p=0.01, not shown). NP tissue was not restored in neonates or adults, although the NP space was occupied by fibrotic tissue at day 28.

DISCUSSION: In this study we demonstrated the regenerative potential of the neonatal mouse AF following puncture injury (indicated by improved collagen deposition and organization compared to adults). Using the neonatal mouse model, we previously established a model of regenerative healing in tendon, with full functional restoration after transection injury [6]. Genetic lineage tracing further identified a novel cellular mechanism of tendon cell activation, recruitment, and differentiation into the injury site, a process that does not occur after adult tendon injury [9]. Although the AF is very similar to tendon/ligament (these tissues share embryonic origin [10] and key markers), the AF is subject to complex mechanical demands that are reflected in its unique structure. The current study tested whether our findings in tendon regeneration can be extended to the AF. Ongoing work will utilize lineage tracing tools to identify cellular mechanisms of AF regeneration (i.e., stem cell recruitment, intrinsic AF recruitment, or transdifferentiation of neighboring supporting cells), establish whether the lamellar structure, inner and outer AF zones, and mechanical function is restored, and identify molecular pathways underlying regenerative and non-regenerative repair. One limitation in the current injury model is that the severe defect to the neonatal AF results in complete loss of NP, which does not regenerate post-injury, suggesting this puncture model may not be useful for whole IVD regeneration. The remarkable capacity of the neonatal AF to repair even in this relatively severe condition highlights the promise of this model for AF-specific questions. Future work may develop novel defect models that result in retention of some NP tissue, more akin to what would be observed clinically in herniated or degenerated IVDs.

SIGNIFICANCE: Current attempts for AF repair have failed due to high reherniation rates of implants, which could maintain or worsen clinical symptoms, meriting a paradigm shift in approach for IVD repair and regeneration. This novel neonatal mouse model of AF injury has potential to identify cell populations and molecular factors responsible for regenerative healing and may eventually inform therapies to modify fibrotic healing responses and promote reparative repair in adult IVDs.


ACKNOWLEDGMENTS: This work was supported by R01 AR064157 (JCI) and R01 AR069537 (AHH) from NIH/NIAMS. We are grateful to Dr. Ronen Schweitzer for the ScxGFP mice and Dr. Peter Taub for training on surgical procedures.
Engineered Endplates Enhance the In Vivo Performance of a Replacement Disc-Like Angle Ply Structure (DAPS)

Sarah E. Gullbrand,1 John T. Martin,1 Beth G. Ashinsky,1 Dong Hwa Kim,1 Lachlan J. Smith,1 Dawn M. Elliott,2 Harvey E. Smith,1 Robert L. Mauck1

1 University of Pennsylvania, Philadelphia, PA and Philadelphia VA Medical Center, Philadelphia, PA, 2 University of Delaware, Newark, DE

Disclosures: None

Introduction: Intervertebral disc degeneration involves a progressive cascade of cellular, compositional and structural changes.1 Surgical treatment of disc degeneration is most commonly achieved via fusion of the degenerated motion segment, which does not restore native disc structure or function, and may exacerbate degeneration of adjacent discs.2 For the treatment of advanced degeneration, total disc arthroplasty with a cellular, engineered replacement is a promising alternative to fusion; a viable, functional substitute may restore normal mechanics to the degenerated spine. To that end, our lab has created disc-like angle ply structures (DAPS) that mimic the structure and function of the native disc by combining an electrospun nanofibrous annulus fibrosus (AF) with a hydrogel nucleus pulposus (NP).3 We have previously shown that while the DAPS are mechanically functional following in vivo implantation in the rat caudal disc space, the constructs do not integrate with the adjacent vertebral bodies and exhibit progressive reductions in MRI T2 signal and NP proteoglycan content.4 Here, we report on the in vivo performance of an endplate DAPS (eDAPS) implant that was designed to improve construct integration and promote retention of implant composition via the addition of acellular porous polymer endplates.

Methods: eDAPS Fabrication and Culture: DAPS sized for the rat caudal disc space were fabricated by concentrically wrapping aligned, angled strips of electrop spun polycaprolactone (PCL) nanofibers to form the AF region, and filling the center with a hyaluronic acid hydrogel to form the NP region. Both regions were seeded with bovine disc cells (2x10^6 cells/AF and 6x10^5 cells/NP) and cultured separately for two weeks in chemically defined media containing TGF-β3. After two weeks of culture, the AF and the NP regions were combined, and acellular porous PCL endplates, (4 mm diameter, 1.5 mm high) fabricated via salt leaching, were apposed to each side of the DAPS to form the eDAPS construct (acellular construct viewed by µCT, Figure 1A). The eDAPS were cultured for an additional three weeks for a total of 5 weeks preculture. Implantation Surgery: Athymic male r 2

Results: The NP and AF T2 relaxation times of the eDAPS were superphysiologic 7 days after implantation into the rat caudal disc space; the T2 values decreased from 7 to 17 days post-implantation. AF T2 values remained superphysiologic up to 35 days in vivo (data not shown), while NP T2 values at 17 and 35 days in vivo were not different from the NP T2 of native rat tail discs (Fig 1B). The maintenance of NP T2 signal corresponded with robust Alcian blue staining in the NP region of the eDAPS at 35 days post-implantation. DAPI staining illustrated infiltration of the acellular PCL foam endplate from the AF and NP regions of the eDAPS, in addition to infiltration of native cells from the adjacent vertebral body (Fig 1D). DAPI staining also indicated sustained cellularity of the AF and NP regions of the eDAPS from 7 days to 35 days in vivo. After 35 days in vivo, GAG content was highest in the NP region of the eDAPS (0.27% ± 0.01%/ww), followed by the AF region (0.11% ± 0.01%/ww) and EP region (0.03% ± 0.006%/ww). The toe and linear region moduli of the eDAPS implanted motion segments were not significantly different from native discs. However, the transition and maximal strains were significantly higher in the eDAPS implanted motion segments compared to native (Fig 1C).

Discussion: Overall, the addition of engineered endplates improved integration and maintenance of DAPS matrix composition in vivo. This is in contrast with our previous findings, in which DAPS implanted without endplates were characterized by a lack of integration with adjacent vertebral bodies and progressive loss of NP T2 signal and proteoglycan content.4 The improved in vivo performance of the eDAPS may be due in part to the PCL endplates serving as a barrier to the harsh native environment. eDAPS toe and linear region moduli were similar to that of native tissue, indicating the potential of this engineered implant for functional restoration of motion segment mechanics. Ongoing work will investigate longer durations of in vivo implantation, as well as remobilization strategies to further enhance integration and in vivo maintenance.

Significance: Current surgical strategies for disc degeneration do not restore native structure and function to the spine. A biologic total disc replacement that better integrates with surrounding tissue (while maintaining composition and mechanical function in the native tissue) will significantly improve the standard of care for patients with low back pain.


Acknowledgments: This work was supported by the Department of Veterans’ Affairs and the Penn Center for Musculoskeletal Disorders.
FLASH MRI for Potential Clinical Diagnostics of Cartilaginous Endplate Discontinuities

KD Meadows¹, AA Claeson¹, JF DeLucca¹, EJ Vresilovic², DM Elliott¹
¹University of Delaware, Newark, DE, ²Pennsylvania State University, Hershey, PA

Introduction
The cartilaginous endplate (CEP) is important for both nutrient transfer and mechanics of the intervertebral disc, but with degeneration, both CEP permeability¹ and tensile modulus decrease.² The thickness of the CEP varies with location, and the transitions between varying thicknesses are typically smooth. However, CEP discontinuities and abrupt changes in thickness are sometimes present. It is unclear whether these CEP discontinuities occur as a result of disc degeneration or, conversely, if CEP discontinuities instead contribute to disc degeneration, perhaps via altering permeability and nutrition to the nucleus pulposus (NP). There is also potential that CEP disruptions are related to low back pain, although this will require future clinical studies. The ability to image the CEP in vivo provides an opportunity to study CEP discontinuities in the context of disc degeneration and low back pain. Here we present findings regarding CEP discontinuities across lumbar levels and the degenerative spectrum using an MRI sequence that is designed to be easily translated to a clinical setting.

Methods
Using a 3T Siemens scanner, an MRI FLASH sequence was developed to enhance the contrast of the CEP compared to the NP and the annulus fibrosis (AF) based on the sequence designed by Moon et al.³ Sagittal plane images were captured of intact lumbar spines using two Siemens large flex coils at a resolution of 0.4 x 0.4 x 2.5 mm³ in under 2 min. Imaging parameters were as follows: TR = 9 ms, TE = 3.6 ms, flip angle 20°. Four intact cadaveric lumbar spines (L1-S1) were imaged via this method yielding 20 discs (5 from each spine) for examination of CEP discontinuities at the mid-sagittal slice (Figure 1). Additionally, 15 discs from a previous study⁴ were also examined. Discontinuities were identified as a loss of signal or an abrupt change in shape within the CEP. The total sample set (n=35 discs) ranged in degenerative grade from 1-4 based on the Pfirrmann grading scale. The effect of degenerative grade and spine level on number of CEP discontinuities were compared using one way ANOVA and post-hoc Tukey tests with significance set at p < 0.05. Statistical analyses were performed for the pooled sample set and also for superior and inferior CEP.

Results
Total discontinuities by grade had a trend where higher degenerative grade discs had a greater number of discontinuities (p=0.07, Figure 2). Within this comparison, significance was reached when number of superior endplate discontinuities were compared by grade (p=0.04). Post-hoc Tukey test showed that grade 4 discs had a significant increase in number of superior CEP discontinuities when compared to grade 2 discs (p<0.05). Spine level did not influence the number of CEP discontinuities.

Discussion
In this study we successfully developed a clinically translatable FLASH MRI sequence that provides high contrast between CEP, NP, and AF while also finding that the number of CEP discontinuities increases with degenerative grade in the disc. We have improved the sequence previously used in vivo by our group⁵ to a 50% thinner slice while still keeping scan time short such that a subject could lay still for the duration of the scan. Cadaver spines provide a good proof of concept that the scan can achieve high contrast between the CEP, AF, and NP. In future work this scan will be applied in vivo, and the CEP evaluated for a potential role in degeneration and low back pain.

Significance
FLASH MRI provides sufficient CEP contrast for evaluation of CEP changes that can be imaged in vivo in order to relate discontinuities to degeneration and low back pain.

References

Acknowledgements
NIH R01AR05005
Injectable Scaffolds with Bioadhesive Properties for the Regeneration of the Annulus Fibrosus and Nucleus Pulposus of the Intervertebral Disc

Thomas Christiani, Tyler Christy, Kyle Grotenboer, Mark Dittmar, Frantzeska Giginis, Jenna Scully, Karl Dyer, Jennifer Kadlowec, Cristina Ihole, Andrea Jennifer Vernengo
Rowan University: 201 Mullica Hill Road, Glassboro, NJ 08028

BACKGROUND: Repairing intervertebral disc (IVD) tissue, such as the nucleus pulposus (NP) and annulus fibrosus (AF), requires a space filling adhesive that exhibits cellular biocompatibility. A novel hydrogel composed of poly (N-isopropylacrylamide) grafted with chondroitin sulfate (PNIPAAm-g-CS) was synthesized and blended with alginate microparticles. The thermally sensitive PNIPAAm forms a miscible solution with water below its lower critical solution temperature (LCST) at approximately 32°C. When heated above the LCST to 37°C, the polymer becomes hydrophobic, expels water from its matrix, and forms a compact gel embedded with localized microparticles, which enhances adhesion to the tissue. In this work, mechanical properties of the hydrogel composite were assessed ex vivo using porcine IVD. The resistance to extrusion, biomechanical restoration, and cellular viability of the injectable implant were examined.

METHODS: 5% (w/v) PNIPAAm-g-CS in PBS blended with alginate microparticles at 50 mg/mL was used to replace both NP and AF tissue. The composite was dyed blue to distinguish the implant from the surrounding porcine tissue. Adult lumbar porcine spinal motion segments (n = 5) were casted in polyurethane and used for biomechanical testing. Motion segments were defrosted for at least 24 hours at 4°C and preheated to 37°C in saline. The following stages were mechanically tested on the IVD in sequential order: intact, annular injury, nucleotomy, mechanically induced degeneration, and hydrogel injection. An annular injury was created with a 2 mm punch and NP tissue was aspirated with a vacuum-syringe setup. During each stage, segments were subjected to 10 cycles of 20 lb. in tension and 200 lb. in compression at 0.1 Hz. Mechanical degeneration was induced by subjecting the IVD specimen to 50 cycles of compression from 0 lb. to 400 lb. at 0.1 Hz. Resistance to extrusion was observed qualitatively, and range of motion (ROM) was calculated using the last cycle of each stage. Motion segments were also mechanically tested in a lateral bending motion using a custom-built apparatus. The motion segment was subjected to an upward and downward vertical 10 lb. load, applied 1 inch from the axis of rotation, for 10 cycles at 0.2 Hz, resulting in ±1.2 N-m of torque (n=1). Rotational displacement was recorded using a video camera recording at 30 fps and ROM was calculated. 3T3 fibroblast cells (3T3s) were suspended in various formulations at a density of 10^5 cells/ml to assess the cellular viability using an Alamar Blue reagent (n=6). 3T3s were plated at a density of 50,000 cells/mL and grown in low glucose DMEM with 10% FBS, 1% glutaMax, 100 U/mL penicillin, 100 U/mL streptomycin, and 0.25 mg/mL fungizone. PNIPAAm-g-CS and PNIPAAm-g-CS with small microparticles (20 ± 5 µm) or large microparticles (100 ± 37 µm) were blended with PNIPAAm-g-CS at concentrations of 25 and 50 mg/mL. The percent reduction of each seeded hydrogel disc was calculated.

RESULTS: The adhesive was successfully implanted within the nuclear cavity and annular defect (Figure 1). Range of motion relative to the intact condition was calculated for each IVD specimen (Figure 2). Creating the 2 mm annulus injury resulted in a negligible increase in ROM (p<0.05). Approximately 44 ± 7% of the total mass of NP was removed during nucleotomy, thus causing ROM to significantly increase (p<0.05). Mechanical degeneration induced through excessive compressive fatigue caused ROM to increase, yet not significantly (p>0.05), compared to the nucleated condition. Injection of the hydrogel composite restored the ROM relative to the intact condition (p<0.05). Implant extrusion through the annular defect was also not observed during cyclical compressive loading. The hydrogel composite was also successful in resisting extrusion and restoring biomechanics in a lateral bending motion (Figure 3). After 2 days, there were significant differences in cellular reduction (p<0.05) amongst all samples, except between PNIPAAm-g-CS with small microparticles at 25 mg/mL and with large microparticles at 50 mg/mL (p=0.05) (Figure 4).

DISCUSSION: ROM was successfully restored within normal physiological loading compared to the intact condition, suggesting potential biomechanical restoration. Implantation of the bioadhesive was successfully administered to the NP cavity and annular defect and extrusion of the composite was not observed during cyclic compression. Restoration mechanics are highly dependent on the mass of NP tissue removed, mass of hydrogel composite injected, and disc size and anatomy. Further mechanical tests must be performed in lateral bending to confirm the biomechanical restoration and resistance to extrusion. Small microparticle size (20 ± 5 µm) and high microparticle concentration (50 mg/mL) seem to negatively impact cellular metabolism. These results suggest that high microparticle surface area to volume ratios can lead to decreased cell performance and proliferation. Microparticle formulations will be assessed for their long-term viability with 3T3s and adipose-derived mesenchymal stem cells will be used in the future for differentiation studies.

SIGNIFICANCE: This study addresses the important need to develop an implantable construct for the replacement of the NP and AF for IVD tissue engineering. An injectable, hydrogel scaffold will allow for minimally invasive implantation in situ and restoration of biomechanical function of the spinal motion segment.

ACKNOWLEDGEMENTS: Research reported in this publication was supported by the National Institute of Arthritis and Musculoskeletal and Skin Diseases and the National Institute of Biomedical Imaging and Bioengineering of the National Institutes of Health under Award Number 1R15 AR 063920-01. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.
Injectable Cellulosic Hydrogels as Nucleus Pulposus Replacements: Assessment of Herniation Risk, Fatigue Behavior, and In Vivo Biocompatibility

Huizi Anna Lin1, Devika M. Varma1, Warren W. Hon2, Michelle A. Cruz2, Philip R. Nasser2, James C. Iatridis1,2, Steven B. Nicoll1

1Department of Biomedical Engineering, The City College of New York, New York, NY, USA
2Leni & Peter W. May Department of Orthopaedics, Icahn School of Medicine at Mount Sinai, New York, NY, USA

INTRODUCTION: Intervertebral disc degeneration is strongly linked to low back pain, which has a lifetime prevalence of 80% and an associated annual cost of $100-200 billion [1]. Nucleotomy, which involves removal of the nucleus pulposus (NP) tissue, is a conventional treatment for disc herniation and early disc degeneration. Although nucleotomy may provide pain alleviation, it alters disc biomechanics and may further disease progression, thus creating the need for NP replacements [2]. While NP replacement hydrogels have been widely investigated, preformed implants are invasive and have a high risk of herniation. To address these limitations, we recently developed a novel injectable cellulosic hydrogel system with the capability of undergoing in situ gelation and restoring compressive disc biomechanics [3]. Successful translation of this NP replacement biomaterial also requires retention in the disc under rigorous loading conditions and high biocompatibility. Therefore, the objectives of this study were to assess the herniation risk, fatigue behavior, and in vivo biocompatibility of cellulosic hydrogels for NP replacement.

METHODS: Polymer synthesis: Methacyrilation of carboxymethylcellulose (CMC) and methylcellulose (MC) polymer was performed as previously described [4]. Hydrogel preparation: Modified CMC and MC, dissolved in PBS at 3% (w/v) macromer concentration each, were mixed with redox initiators ammonium persulfate (20 mM) and tetramethylethylenediamine (20 mM) in dual-barrel syringes and injected via a 20G needle. Motion segment preparation: Vertebral-disc-vertebra motion segments were isolated from skeletally mature bovine tails and divided into four groups: (1) Intact, (2) Herniation – in which a cruciate incision was created in the posterolateral region of the annulus and the NP was disrupted, (3) Nucleotomy – in which approximately 0.15 – 0.20 g of NP was removed, and (4) Repair – in which CMC-MC solutions were injected into the NP void of nucleotomized specimens. Failure testing (n = 12): Herniation risk was assessed through failure testing using a MTS Bionix Servohydraulic Test system, with specimens loaded to failure at 5° posterior flexion in displacement-control mode (Fig 1A). Failure strength was calculated as load at failure point normalized by the disc area and subsidence to failure indicated displacement at failure point. Fatigue testing (n = 3): Fatigue loading protocol, similar to prior studies [5,6], consisted of cyclic compression between 50N and 300N at 1 Hz and at an offset of 20 mm to induce a physiological bending moment of 6 Nm, with the loading indenter cyclically rotating from -135° to +135° from the axis opposite of the incision site (Fig 2A & 2B). In vivo biocompatibility (n = 5): Sterilized CMC-MC solutions were injected into the subcutaneous dorsum of male Sprague Dawley rats at 12 weeks, 4 weeks, and 1 week before gel extraction (Fig 3A). Hematoxylin and Eosin (H&E) staining of extracted hydrogels was performed (Fig 3B) and thicknesses of the fibrous capsules were measured. Statistical analysis: One-way ANOVA with Tukey’s post-hoc test was used to compare values of ultimate strength, subsidence to failure, and cycles to failure. Repeated measures ANOVA was used to compare fibrous capsule thicknesses. Significance was set at p < 0.05. Data are mean ± SD.

RESULTS: Failure testing: All intact samples failed by endplate fracture whereas all other samples failed by nuclear extrusion (Fig 1B). Failure strengths (Fig 1C) of Herniation specimens (2.2 ± 2.0 MPa) and Nucleotomy specimens (7.0 ± 4.1 MPa) were significantly lower than that of the Intact group (13.0 ± 3.9 MPa). Values for the CMC-MC Repair group (9.7 ± 2.1 MPa) were significantly higher than those of the Herniation group, but not significantly different from the Intact or Nucleotomy group. Subsidence to failure (Fig 1D) patterns were similar to those of failure strength (Intact: 4.0 ± 0.9 mm; Herniation: 1.5 ± 0.7 mm; Nucleotomy: 2.8 ± 1.0 mm; Repair: 3.7 ± 0.6 mm). Fatigue testing: With the applied physiological bending moment, Intact specimens did not fail within 20,000 cycles. Values for cycles to failure for the Herniation group and the CMC-MC Repair group were significantly higher than that of the Herniation group, but not significantly different from each other (Fig 2C). In vivo biocompatibility: H&E staining revealed a fibrous capsule around the injected CMC-MC hydrogels with thickness < 150 μm and no significant differences in thickness were observed over 12 weeks (Week 1: 123.9 ± 43.8 μm; Week 4: 79.7 ± 37.9 μm; Week 12: 101.0 ± 32.9 μm). A high density of macrophages at the gel surface detected at Week 1 (Fig 3B) subsided by Week 12 (Fig 3C), with the formation of a more aligned fibrous capsule.

DISCUSSION: This study assessed the translational potential of a novel cellulosic hydrogel for NP replacements. Failure and preliminary fatigue testing demonstrated that AF injury renders discs highly susceptible to herniation. Nucleotomy and CMC-MC repair had similar performance, significantly decreasing herniation risk and improving cyclic endurance compared to the Herniation group; and CMC-MC has the added benefit of restoring disc height and compressive biomechanical behaviors [3]. Ongoing fatigue testing experiments will include additional samples and evaluation of disc mechanics throughout cyclic loading. In vivo biocompatibility testing indicated that CMC-MC gels were biocompatible, as shown by the lack of major adverse reactions and fibrous capsule thickness comparable to those of other polymeric hydrogels used for biomedical applications [7]. In addition, the CMC-MC hydrogels can be expected to exhibit a milder biological response in the epidural space due to the immunoprivileged nature of the NP [8], but this should be confirmed in a large animal model in future studies. In conclusion, CMC-MC hydrogels do not present an increased risk of reherniation compared to standard nucleotomy procedures and exhibit high biocompatibility, while providing the previously reported benefits of restoration of disc height and compressive biomechanical behavior [3].

SIGNIFICANCE: CMC-MC hydrogels are a promising candidate for nucleus pulposus replacements to treat early disc degeneration. Current studies demonstrate improved mechanical performance compared with nucleotomy, and CMC-MC hydrogels may eventually serve as carriers for regenerative cells and therapeutic factors for biological repair of degenerated discs.

ACKNOWLEDGEMENTS: Funding: NIAMS/NIH R01 AR057397. Kevin Seon of City College for technical assistance.


Figure 1. (A) Schematic of failure testing. (B) Representative load-displacement curves of intact and herniated samples. (C) Failure strength of specimens, and (D) subsidence to failure of specimens. Red circle indicates point of failure. * significantly different from intact group. # significantly different from Herniation group.

Figure 2. (A) Representative subfailure compressive and cyclic loading points. (B) Cyclic failure. Red circle indicates AF failure. # significantly different from Herniation group.

Figure 3. (A) Photograph of CMC-MC gel in subcutaneous space. (B) H&E staining at Week 1, and (C) H&E staining at Week 12.
Poster Presentations
Novel Techniques for the Evaluation of Physical Activity in a Large Animal Intervertebral Disc Degeneration Model

Justin R. Bendigo, Sarah E. Gullbrand, Brendan D. Stoeckl, Zosia Zawacki, Thomas P. Schaer, Harvey E. Smith, Robert L. Mauck, Neil R. Malhotra, Feini Qu, Laclan J. Smith

1University of Pennsylvania, Philadelphia, PA, 2Philadelphia VA Medical Center, Philadelphia, PA

Disclosures: JRB (N), SEG (N), BDS (4-Animotion LLC), ZZ (N), TPS (N), HES (N), RLM (N), NRM (N), FQ (4-Animotion LLC), LJS (N)

**Introduction:** Intervertebral disc degeneration (IDD) is a progressive, age-related condition that leads to structural and mechanical failure of the disc. This deterioration is commonly associated with low back pain (LBP). Therefore, pain is the most clinically significant characteristic of IDD, and the ideal animal model should recapitulate pain and functional impairment in addition to structural and mechanical alterations to the disc. Our group previously developed a large animal goat model of IDD that effectively recapitulates the structural and mechanical changes that occur with degeneration [1]; however, intervertebral disc degeneration in sheep or goats does not result in clinically perceptible pain, even at very advanced stages. Various methods currently exist to evaluate activity and pain in small animal models, including: the LABORAS platform, which measures vibration/force for position and behavior tracking [2]; hindpaw withdrawal in response to mechanical (von Frey Test) and thermal (Hargreaves Test) hyperalgesia signifying increased pain sensitivity [3]; and the Rotarod Test, which uses a rotating rod to measure balance and activity endurance [3]. These techniques are not readily translatable to large animal models. An objective tool to assess functional change that is consequent to painful degeneration would be invaluable to evaluation of therapeutics in a preclinical animal model. The objective of this study was to develop and validate two novel techniques for quantifying physical activity in an established caprine model of disc degeneration.

**Methods:** Two male large frame goats, ~2 years of age, were housed together in a 3-sided barn. IDD was induced at 4 lumbar levels per animal via intratracheal injection of 1U chondroitinase ABC. Our previous work showed that this insult results in moderate to severe degeneration of the disc after 12 weeks, as assessed via MRI, disc height, and histology [1]. Over this 12 week period, two methods of activity monitoring were investigated. **Overhead Video-Based Motion Tracking:** A GoPro HERO4 camera recording in SuperView mode was mounted to the barn ceiling to capture live images of the entire pen. Video was recorded for 1 hour per day when humans were not present to capture unprovoked activity. Motion was tracked for one goat in MATLAB using the DLTdv5 texture tracking program [4], which tracks a manually selected monochromatic texture region of interest — in this case the goat’s body (Fig 1A). The center of this region for each video frame was then output to Excel as x-y coordinates, and the average x-y position was rounded to the nearest whole number. The distance formula was used to calculate change in position between each 1-second increment, and these values were summed over the hour-long video to yield total activity. Activity was monitored during 2 pre-operative weeks to establish baseline activity and from 1-12 weeks following induction of disc degeneration. Daily activity measurements were binned into two-week periods for analysis. Differences between time points were assessed via unpaired Student’s t-tests compared to pre-op activity. To test for inter-observer reliability of the video tracking software, pre-op videos were tracked by two observers, and the activity levels were compared via unpaired Student’s t-test.

**Step-Count Quantification using a Custom Wearable Device:** Step count was also measured on a daily basis in a separate goat to characterize activity. A custom built wearable device [5] consisting of a sensor board with gyroscope, accelerometer, and magnetometer; microcontroller; radio; data logger; and lithium polymer battery was attached to the right forelimb proximal to the carpus (Fig 1B). A neodymium magnet was attached distal to the carpus. Discrete steps were identified by local maxima in the magnetic field strength, which occurred with carpal flexion during ambulation. Data from the device was uploaded to a computer each day over a period of 4 weeks prior to surgery, and for 12 weeks following surgical induction of disc degeneration. MATLAB was used to count the number of steps in a 30-minute window each day. Prolonged periods of elevated magnetic field strength — indicating that the goat was lying down — were excluded from the analysis. As with the video tracking data, activity was binned into two-week periods for analysis. Differences between time points were assessed via unpaired Student’s t-tests compared to pre-op activity.

**Results:** **Overhead Video-Based Motion Tracking:** No significant difference in pre-op activity level was found between observers (Fig 1A). There was a significant increase (p<0.05) in activity from 1-6 weeks post-operative compared to pre-op baseline, followed by a return to baseline activity from 3-12 weeks post-op (Fig 2B). **Step-Count Quantification using a Custom Wearable Device:** A significant reduction (p<0.05) in activity 1-2 and 5-6 weeks post-op was observed compared to the pre-op baseline, with 7-12 weeks post-op also trending towards decreased activity (p=0.0614 at 11-12 weeks) (Fig 2C).

**Discussion:** We developed two novel, independent methods for quantifying large animal activity in a model of lumbar disc degeneration and demonstrated that both methods are able to detect changes in activity over time. While activity levels differed between the two goats immediately post-surgery, both tracking methods show a long-term trend towards returning to or below baseline. Ongoing work will further validate these methodologies to explore and optimize relationships between disc degeneration and functional parameters in large animals. Concurrently, we are assessing biomarker signatures such as serum inflammatory markers and immunohistochemistry for nociceptive nerve fibers. Recently, NIH leaders called for improved transparency and reproducibility in animal models [6, 7]. Our activity monitor methodologies described here combined with competent physical examination will offer a platform for improved in vivo assessment when using large animal models. Other applications for the wearable device include tracking limb movement during augmentation of orthopedic hardware in fragility fractures or tracking three dimensional head and neck kinematics in future work involving goats undergoing cervical total disc replacement.

**Significance:** Use of these novel activity monitoring techniques in large animal models of musculoskeletal disease will enhance the clinical relevance of these models by improving scientific rigor and model fidelity resulting in a more predictable translation to human clinical use.


**Acknowledgments:** This study was supported by the Department of Veteran’s Affairs, the Montague Research Award, and the Penn Vet Preclinical Translational Core.
TonEBP-COX-2 Axis Promotes Nucleus Pulposus Cell Survival Under Hyperosmotic Conditions

Hyowon Choi1, Weera Chaiyamongkol1,2, Alexandra C. Doolittle1, Zariel I. Johnson1, Shilpa S. Gogate1, Zachary R. Schoepflin1, Irving M. Shapiro1, Makarand V. Risbud1

1Thomas Jefferson University, Philadelphia, PA, 2Prince of Songkla University, Songkhla, Thailand

INTRODUCTION: The nucleus pulposus (NP) of intervertebral disc experiences dynamic changes in tissue osmolarity as a result of diurnal loading of the spine. TonEBP/NFAT5 is a transcription factor that is critical in osmoregulation as well as survival of NP cells in the hyperosmotic milieu. However, the detailed mechanisms of TonEBP-dependent osmoprotection in the NP has not been fully elucidated. COX-2 has been reported to have cytoprotective role in renal epithelial cells that experience extreme osmotic gradients. The goal of this study was to investigate whether TonEBP regulated COX-2 expression in the NP, and if COX-2 contributed to hypertonic cell survival.

METHODS: Rat NP cells were cultured in either isotonic or hypertonic medium with or without calcium chelator, BAPTA, or calcineurin inhibitor, FK-506/cyclosporin A, and the COX-2 mRNA and protein levels as well as its promoter activity were measured. In order to determine the regulatory mechanism of COX-2 expression, NP cells were treated with various MAPK inhibitors under hypertonic condition, and COX-2 expression was measured. The involvement of TonEBP in modulating hypertonic expression of COX-2 was assessed by lentiviral ShRNA-mediated stable knockdowns of TonEBP in NP cells, and by ex vivo disc organ culture study using haploinsufficient TonEBP+/− mice. Finally, cell viability was measured after inhibiting COX-2 activity using Celecoxib under hypertonic conditions. All measurements were performed in at least triplicate. Data is presented as the mean ± SE. Differences between groups were assessed by student’s t-test. p < 0.05 was considered statistically significant.

RESULTS: Under hypertonic conditions, NP cells upregulated COX-2 mRNA and protein expression. This induction was abolished by BAPTA treatment, but unaltered by FK-506/cyclosporin A. When p38/MAPK pathway was inhibited, hypertonic induction of COX-2 was suppressed. Stable silencing of TonEBP in NP cells resulted in inability to induce COX-2 expression under hyperosmolar condition. Likewise, TonEBP+/− mouse intervertebral discs were not able to upregulate COX-2 in response to hypertonic stimuli. Mechanistically, COX-2 promoter activity increased with hypertonicity as well as TonEBP over-expression, and decreased with DN-TonEBP. Importantly, inhibition of COX-2 activity with Celecoxib under hyperosmolar conditions resulted in significantly reduced cell viability.

DISCUSSION: The results demonstrated that COX-2 expression levels were modulated by hyperosmolarity in NP cells. Interestingly, this process was mediated by intracellular calcium signaling, independent of calcineurin pathway. In addition, hypertonic activation of p38/MAPK signaling pathway played a role in controlling COX-2 expression. Since both calcium and p38 signal through TonEBP, it was not surprising that in vitro, and ex vivo studies showed that hypertonic induction of COX-2 was controlled by TonEBP. Importantly, under hyperosmotic condition, COX-2 activity was required for the NP cell survival.

SIGNIFICANCE: Our study showed for the first time that hypertonic induction of COX-2 was controlled by TonEBP, and that COX-2 promoted survival and adaptation of cells to their physiologically hyperosmotic microenvironment.

ACKNOWLEDGEMENTS: This work was supported by NIH grants: AR064733, and AR055655.

IMAGES AND TABLES:

Figure 1. (A) Real-time RT-PCR analysis showing induction of COX-2 mRNA under hypertonic conditions. (B) Western blot showing increased COX-2 protein levels in response to hyperosmotic stimuli. (n=3); * p<0.05.

Figure 2. NP cell viability measured by MTT assay demonstrating significant reduction in cell viability with COX-2 inhibition under hypertonic condition. (n=4); * p<0.05.
Cell-Seeded Adhesive Biomaterial for Repair of Annulus Fibrosus Defects in Intervertebral Discs
Michelle A. Cruz1, Warren W. Hom1, Robert Merrill1, Olivia M. Torre1, Huizi A. Lin2, Philip Nasser3, Andrew C. Hecht3, Svenja Illien-Junger4, James C. Iatridis4

1 Leni & Peter W. May Department of Orthopaedics, Icahn School of Medicine at Mount Sinai, New York, NY United States
2Biomedical Engineering Department, The City College of New York, New York, NY, United States

INTRODUCTION: Intervertebral disc (IVD) degeneration with herniation is a primary cause for neck/arm and back/leg pain1. After conservative care has failed, microdiscectomy surgery is most common treatment to remove herniated nucleus pulposus and annulus fibrosus (AF) fragments. Microdiscectomy does not repair the AF defect and reherniation and recurrent pain can occur at the operated level2. Cell therapies for IVD repair show promise in early clinical trials with capacity to increase cellularity, modulate inflammation, and promote anabolism3. Important design objectives for AF repair are therefore to deliver cells in a carrier that prevents leakage and unwanted off-target effects of cells2, while also providing biomechanical benefits of sealing AF defects with little herniation risk. This 3-part study evaluated cell-seeded Genipin-crosslinked Fibrin (FibGen) to develop a cell-seeded adhesive for repair of AF defects using hydrogel mechanics tests, in situ failure biomechanical tests, and cell culture screening studies. The goal of this study was to determine optimal fibrin and genipin concentrations that meet mechanical design requirements for AF repair while also promoting cell survival and extracellular matrix production.

METHODS: Final concentrations of fibrin were 140, 70, or 35 mg/mL and final concentrations of genipin were 6, 2, or 1 mg/mL to create 9 FibGen formulations (i.e., FxGy where x & y are respective concentrations). All FibGen formulations had final thrombin concentration of 28 U/mL Part 1: Hydrogel mechanical testing (n=4-8): Young’s modulus and complex shear modulus were determined by unconfined compression tests from 0-15% strain (0.2% strain/sec) and by frequency sweep tests in pure shear, respectively (Figure 1A) for 9 FibGen formulations. Part 2: In situ failure biomechanics tests used bovine caudal motion segments (n=5-9) that were injured with a 4 mm posterolateral biopsy punch defect and repaired with 4 selected FibGen formulations. Motion segments were compressed to failure (2 mm/min) under a 5° posterolateral flexion (Figure 2A). Part 3: Cell culture screening tests evaluated 4 FibGen formulations for 7 days. Bovine AF cells were mixed in fibrinogen prior to gelation at a concentration of 20 x 10⁶ cells/mL. Gels were cultured in hypoxia (5% O2) in low glucose DMEM (10% FBS). Percent cell viability (n=6-8) was assessed by confocal microscopy (Calcine AM and DAPI). DNA content was quantified (Quantifluor, Promega, Wisconsin, USA) using papain-digested FibGen (n=4). Gene expression of anabolic AF phenotype markers including Aggrecan and collagen 1, 5 and 12 were analyzed by TaqMan qPCR (n=4-6). RNA was isolated with Trizol (Life Technologies, New York USA), cDNA was synthesized (Superscript Vilo cDNA synthesis Kit, Invitrogen), and mRNA at day 7 was quantified relative to day 1. One-way (formulation) and two-way (formulation and time) ANOVA determined statistical differences using p<0.05 as significant.

RESULTS: Mechanical characterization of FibGen formulations determined that both fibrin and genipin concentration as well as the interaction between them significantly affected Young’s compressive and simple shear moduli (Figure 1B, C). During in situ failure testing, all repair groups and injured groups were significantly different from intact motion segments. F140G6 had significantly greater failure strength and subsidence to failure than injured, although the mean for F140G1 was also greater than injured and had a trend towards significance (p=0.15) (Figure 2B, C). Cell culture screening tests showed all formulations had high cell viability, which increased with lower fibrin and genipin concentrations. F35G1 had significantly higher viability (95%) than F140G6 (85%; Figure 3A). DNA content measurements demonstrated similar effects with significantly greater day 1 DNA content at lower concentrations, indicating that loss of viability likely occurred with seeding (Figure 3B). F35G1 also had a non-significant decrease in DNA with time. F70G1 and F35G1 gels had significantly increased gene expression of AF phenotypic markers including Aggrecan, Col 1, Col 5 and Col 12 compared to Intact levels. F35G1 and F70G1 formulations had the best cell culture screening performance with significantly greater cell viability and AF gene expression values than other formulations. Overall, F70G1 appeared to be the formulation that best balanced mechanical and biological performance which provided a supportive environment for cell viability and matrix production while nearly matching AF tissue properties and having relatively lower failure. Ongoing studies will measure the long-term mechanical and biological performance of cell-seeded FibGen gels in culture with the eventual goal of advancing towards organ culture and eventual in vivo assessments.

SIGNIFICANCE: Development of injectable AF repair and IVD tissue regeneration techniques has the potential to improve current treatments for IVD herniation. This study developed an injectable, cell-seeded adhesive biomaterial with formulations optimized to seal AF defects, reduce reherniation risk, and promote AF matrix production.


ACKNOWLEDGMENTS: We gratefully acknowledge Dr. Steven Nicoll for important discussion and technical contributions. Funded by NIH grant R01AR057397.
Effect of Multiaxial Mechanical Loading on Creep Recovery in Human Intervertebral Discs
John DeLucca¹, Dhara Amin², Edward Vresilovic¹, John Costi², Dawn Elliott¹
¹University of Delaware, Newark, DE
²Flinders University, Adelaide, Australia
³Pennsylvania State University Bone and Joint Institute, Hershey, PA

Introduction
The intervertebral disc is subjected to large dynamic and multiaxial loading conditions during daily activity. As such, the 6 degree-of-freedom (6DOF) mechanics of the disc have been studied using experiments of multiple frequency sweeps and that incorporate each DOF in sequence. However, the hydrated and viscoelastic nature of the intervertebral disc makes it sensitive to loading history [1]. Consequently, the objective of this study was to determine the creep recovery of the disc following each DOF in a 6DOF testing sequence. It was hypothesized that loading conditions which result in the most volume change in the disc (e.g. compression, bending, and flexion) will result in the largest required recovery displacement. Determining the effects of loading history is critical to designing disc implants that can recapitulate the native mechanics of the disc.

Methods
Non degenerate (grades 2 and 3) bone-disc-bone segments (n=9) with posterior elements removed were dissected. Each disc was thawed overnight (16 hr) under 50 N load in 0.15 M PBS with protease inhibitors at room temperature. After potting in PMMA, samples were set up in a 6DOF hexapod robot [2] and preloaded to inaxial compression over 12 hr at 37°C in 0.15 M PBS with protease inhibitors. Preload force was calculated as 0.2 MPa x 0.84 x WAP x WLAT [3] where WAP and WLAT are the anterior-posterior and lateral widths, respectively, of the disc. Pilot testing evaluated the effect of 0.1 MPa, 0.2 MPa, and 0.5 MPa compressive preload on creep during test and confirmed 0.2 MPa as an appropriate preload to reduce creep during testing. After preload, 6DOF mechanical testing was performed under a 0.2 MPa follower load in the following order: lateral shear (Tx) (±0.6 mm), anterior-posterior shear (Ty) (±0.6 mm), axial rotation (Rz) (±3°), lateral bending (Ry) (±3°), flexion/extension (Rx) (±3°), and axial compression (Tz) (0.2 MPa to 1.1 MPa). For each DOF testing consisted of a sweep of 5 cycles of 0.5 Hz, 5 cycles of 0.05 Hz, and 5 cycles of 0.005 Hz. Testing was performed under hybrid load/position control such that the primary axis of applied loading was position controlled while off-axis forces and moments were minimized via real-time load control [3]. Once frequency sweeps were completed for a given DOF, the disc was allowed to recover axially at the follower load, 0.2 MPa. The axial displacement during testing was compared across DOFs and the recovery profiles after each DOF were compared by determining the displacement during recovery.

Results
The non-compression DOFs all resulted in disc elongation during testing (Fig. 1). Excluding axial compression, axial rotation resulted in the largest disc displacement during testing (0.36 mm) while flexion produced the smallest disc elongation during testing (0.02 mm). Recovery for each DOF returned to the continuous creep curve for the 0.2 MPa compressive follower load. Compression loading required the largest recovery displacement (0.16 mm) and took the longest time to recover (19.6 min). Anterior-posterior shear required the smallest displacement to achieve recovery (-0.02 mm) and was the fastest to recover (0.26 min). Translation and rotation recovery patterns were paired to axis of loading. For example, lateral shear and lateral bending required similar recovery profiles while flexion and anterior-posterior shear recovered with similar profiles.

Discussion
This study sought to determine the creep recovery behavior after each DOF in a 6DOF loading sequence. Unsurprisingly, since the axial compression DOF results in the largest volume change in the disc it required the largest recovery in axial displacement. Surprisingly, flexion and its associated shear translation resulted in the smallest axial recovery, suggesting volume change due to fluid flow in flexion is not prominent. The creep displacement and axial recovery profiles in this study will be useful in designing experiments to quantify multiaxial disc mechanics and design more physiological disc implants.

Acknowledgements
NIH R01AR050052 and Whitaker International Summer Fellowship

Approaches to Understanding the Genetics of Vertebral Malformations

Philip F. Giampietro¹, Ulrich Broeckel², Robert D. Blank², Cathy L. Raggio³ and Michael Pickart⁴

¹ Drexel University College of Medicine, Philadelphia, PA ² Medical College of Wisconsin, Milwaukee, WI ³ Hospital for Special Surgery, NY ⁴ Concordia University Mequon, WI

Introduction: Vertebral malformations (VM) in humans are associated with significant health problems including congenital scoliosis (CS). Difficulty of prognostication is generally proportional to VM complexity. Capacity to associate genes with different prognoses will improve prediction of clinical outcome and inform patient care.

Methods: Patients with VM and family members were admitted under an IRB approved protocol. Sanger and whole exome sequence analysis were performed on DNA specimens obtained from leukocytes.

Results: Sequence variants in PAX1, DLL3, WNT3A and T (Brachyury) associated with decreased penetrance have previously been identified by our group in patients with VMs and were seen at low frequency or not detected in healthy controls. A missense T(Brachyury) mutation c.1013C>T was identified in 3 patients with VM with clinical features corresponding to Klippel Feil syndrome (patient 1), Sacral agenesis (patient 2), CS associated with cervical and thoracic VM, Sprengel anomaly and an omovertebral bone (patient 3). The same mutation was also identified in a patient with sacral agenesis in a separate cohort (Papapetrou C, et al. J Med Genet. 1999). Homozygous mutations (c.796A>G) in T(brachyury) have been identified in three consanguineous families in which affected individuals have sacral agenesis, persistent notochordal canal and abnormal ossification of vertebral bodies. This mutation results in altered expression of T(Brachyury) targeted genes related to ossification and axial skeletal development. (Postma AV, et al. J Med Genet 2014). We previously conducted WES analysis on one family (with autosomal dominant VM phenotypes using Agilent Sure Select hybridization-based exome capture methodology which targets an approximately 50Mb protein-coding genomic sequence. Salient clinical features of “Family 1” in Fig. 1 (Giampietro PF et al. Am J Med Genet 2014) sequenced by WES, included an affected father and daughters with phenotypic features consistent with Klippel-Feil spectrum including cervical segmentation defects, cleft palate, Sprengel deformity and sensorineural hearing loss. (18) WES of this family revealed 4 stop gain/loss and 178 single nucleotide variants (SNVs). Utilizing a WES approach, POLR1D (mutation: exon2:c.T332C:p.L111P) emerged as a biologically relevant gene. Haploinsufficiency of POLR1D has previously been associated with altered ribosomal levels supporting development of neural crest-derived craniofacial structures.

Discussion: Use of whole exome sequence technologies can identify candidate novel genes for vertebral malformations. Proof of pathogenicity of mutations identified on sequencing platforms, genetic heterogeneity and penetrance of identified mutations in family members and possible tissue mosaicism, represent obstacles that need to be overcome in order to provide optimal genetic counseling for family members. Utilization of animal models such as zebrafish would be of value in achieving this.

Significance: The capacity of this WES/zebrafish pipeline model to discern causal variants has important clinical implications relative to provision of genetic counseling to families of individuals affected by VMs and development of therapeutic strategies for families who have a child with a VM.
Effect of Dynamic Loading on the Transport of Charged Antibiotics in Human Intervertebral Discs

Weiyong Gu1, Qiaoqiao Zhu1, Na Li1, Mark D. Brown2
University of Miami, 1Dept. of Mechanical & Aerospace Engineering, 2Dept. of Orthopaedics, Miami, FL.

Disclosures: Weiyong Gu (N), Qiaoqiao Zhu (N), Na Li (N), Mark D. Brown (N).

Introduction
Antibiotics are broadly used in the perioperative prophylaxis and treatment of intervertebral disc (IVD) infections. Most of the antibiotics are more or less electrically charged [1, 2]. However, very little quantitative information exists in the literature on the kinetics of charged antibiotic transport in the disc, especially in the discs under dynamic loading (e.g., under diurnal loading). This information however is crucial for understanding the mechanism of charged antibiotics transport in the disc. Thus the objective of this study was to quantitatively analyze the transport process of various charged antibiotics into human lumbar IVDs under dynamic loading conditions.

Methods
Transport of charged and uncharged antibiotics in a human lumbar disc under dynamic loading was analyzed using a 3D finite element model. The valence (z) of antibiotics varied from z=+2 (positively charged) to z=-2 (negatively charged). A non-charged antibiotic (z=0) was used as a control. Cases of different charged antibiotics with constant antibiotic concentrations at disc boundary under dynamic compression (Fig. 1A) were simulated.

Results
It was found that after several loading cycles, for positively charged antibiotics (e.g., z=+2), the concentrations were higher in the disc with dynamic compression; while for non-charged or negatively charged ones (e.g., z=0), the concentrations were lower in the disc with the dynamic compression compared to those without the dynamic compression (Fig. 1B-D).

Discussion
Dynamic compression of IVD increases tissue fixed charge density, reduce water content (or tissue pore size) and disc thickness (data not shown). These changes will affect the transport of antibiotics into the IVD, as shown in our results. The interactions among mechanical, chemical and electrical effects within the disc are very complicated and result in the nonlinear effect of dynamic loading on transport of charged antibiotics in the discs. It appears that dynamic compression has, in general, a beneficial effect on positively charged antibiotics while a negative effect on the transport of negatively charged ones, except for the initial loading phase. This indicates that appropriate physical activity is generally helpful for treating disc infections with positively charged antibiotics, except at the initial loading period. This study provide a new insight into transport of charged antibiotics into the disc under dynamic loading conditions.

Significance
This is the first study to quantitatively investigate the effect of dynamic loading on the transport of charged antibiotics in the IVD. This study can be used to provide quantitative guidance for selecting antibiotics and for treating disc infections under various loading regimen.

Acknowledgement
This study was supported in part by a research grant from the NIH (AR066240) and by the Ratcliffe Foundation gift to Miami Center for Orthopedic Research and Education (Miami CORE). There is no conflict of interest.

Reference
Scale Up of Disc-Like Angle Ply Structures (DAPS) for Total Disc Replacement in Preclinical Animal Models

Sarah E. Gullbrand,1 Beth G. Ashinsky,1 Dong Hwa Kim,1 John T. Martin,1 Dawn M. Elliott,2 Lachlan J. Smith,1 Robert L. Mauck1, Harvey E. Smith1

1University of Pennsylvania, Philadelphia, PA; 2Philadelphia VA Medical Center, Philadelphia, PA, 3University of Delaware, Newark, DE

Disclosures: None

Introduction: Replacement of the disc with a viable, tissue engineered construct that mimics native disc structure and function is an attractive alternative to standard fusion or mechanical arthroplasty for the treatment of disc degeneration. Significant progress has been made by many groups towards advancing disc tissue engineering from in vitro culture to in vivo evaluation in animal models.1,2 Our group has developed disc-like angle ply structures (DAPS) sized for the rat caudal disc space that achieve near native composition and mechanical function with in vitro culture.3 However, the anatomical size of the rat caudal disc is an order of magnitude smaller than that of human or large animal discs suitable for preclinical studies. In order to translate the DAPS technology towards clinical use, successful fabrication and maturation of these constructs at larger size scales is of critical importance. The purpose of this study was to evaluate cell viability and matrix production of medium (rabbit lumbar disc scale) and large (goat/human cervical disc scale) sized DAPS constructs over 5 weeks of in vitro culture and following subcutaneous implantation.

Methods: DAPS Fabrication and Culture: DAPS were fabricated in two sizes – medium (n=5, 3mm high x 10mm diameter, NP diameter = 5mm) and large (n=5, 6mm high x 20mm diameter, NP diameter = 10mm). The AF region of the DAPS was fabricated by electrospinning aligned sheets of 250-300 µm thick poly(e-caprolactone) (PCL), and cutting the sheets into strips at a 30 degree angle. The strips were hydrated, coated with fibronectin and seeded with bovine AF cells (3,333 cells/mm²). Following 1 week of culture in chemically defined media with TGF-β3, strips were coupled at opposing fiber angles (+/−30 degrees), and wound using a custom mold to form the circular AF region. The NP region of the DAPS was fabricated by seeding bovine NP cells in a 2% agarose hydrogel (20 million cells/ml), and culturing for 2 weeks in chemically defined media with TGF-β3 prior to combining with the AF region. The combined DAPS were cultured for an additional 3 weeks on an orbital shaker for 5 weeks total culture duration. Five additional NP hydrogels of each size were cultured separately on an orbital shaker, without combining with the AF region, for either 2.5 weeks or 5 weeks. Viability, Metabolic Activity and Biochemistry: Following 5 weeks of culture, 3 DAPS of each size were bisected for analysis. GAG content of one half-DAPS was quantified via the DMMB assay and normalized to sample wet weight. GAG content for medium and large DAPS were compared to prior data obtained for small, rat size DAPS.3 From the remaining half DAPS, cell viability of the NP region was assessed via live-dead staining, and metabolic activity of the AF region was assessed via the MTT assay. Histology: 2 DAPS from each group were processed through paraffin, sectioned, and stained with Alcian blue (glycosaminoglycans) and picrosirius red (collagen). Subcutaneous Implantation: Large DAPS (n=3) were fabricated with a clinically applicable hyaluronic acid hydrogel for the NP region, pre-cultured for 5 weeks and implanted subcutaneously in athymic rats for 2 weeks. NP viability and AF metabolic activity were assessed via live-dead staining and MTT assay, respectively, and matrix assessed via histology.

Results: Medium and large size DAPS were successfully fabricated and grown in culture for up to 5 weeks (Fig 1A). Cell viabilities in the NP region were ~88% and ~89% for large and medium DAPS, respectively, and were not significantly different from one another (p=0.75, Fig 1B). Furthermore, the percent viability of the NP region of full DAPS was not different than the viability region of NP hydrogels cultured independent of the AF region. Normalized MTT absorbance of the AF region was not different between medium and large DAPS after 5 weeks culture (p=0.12, Fig 1C). GAG content in the NP region of the DAPS after 5 weeks culture was significantly higher in small constructs compared to large constructs, while NP GAG content was not different between medium and large constructs. Culturing the NP hydrogel inside the AF region did not have a significant effect on GAG content compared to NP hydrogels culture without the AF. GAG content of the AF region of the DAPS was significantly higher for small DAPS, but was not different between medium and large DAPS (Fig 2A). Histology revealed proteoglycans distributed throughout the NP region of the large DAPS, with more intense Alcian blue staining at the NP periphery (Fig 2B). Matrix was distributed more evenly throughout the AF region, and had more collagen than proteoglycan at this stage of maturation, consistent with the small DAPS. Following 2 weeks subcutaneous implantation, viability and metabolic activity were preserved in both the NP and AF regions, and abundant collagen and proteoglycan matrix was evident throughout the DAPS (Fig 3).

Discussion: Through these studies we have demonstrated the feasibility of scaling up DAPS for total disc replacement to clinically relevant size scales. Despite the increases in height and diameter, large DAPS had similar viability and matrix production to the medium DAPS, although large DAPS were outperformed by the small DAPS. Matrix production of large DAPS could be further improved via the inclusion of nutrient channels, as has been utilized in an cartilage tissue engineering, or via culture in a bioreactor.4-5 Viability and matrix production in the large DAPS was maintained in vivo in a subcutaneous implantation model. Ongoing work is assessing the viability, biochemistry and mechanical properties of the DAPS at these size scales with longer durations of in vitro culture and evaluating the in vivo performance of the large size DAPS in a goat cervical disc replacement model.

Significance: We have successfully fabricated tissue-engineered discs at multiple size scales, including sizing comparable to that of the human cervical disc. Evaluation of the DAPS in large animal models will facilitate the clinical translation of these constructs for the treatment of end stage disc degeneration.


Acknowledgments: This work was supported by the Department of Veterans’ Affairs and the Penn Center for Musculoskeletal Disorders.
Stem Cell Seeded Injectable Hydrogels for Intervertebral Disc Regeneration in a Preclinical Animal Model
Sarah E. Gullbrand1, Thomas P. Schaer1, Justin R. Bendigo1,2, Prateek Agarwal1, Zosia Zawacki1, George R. Dodge1,2, Edward J. Vresilovic3, Dawn M. Elliott4, Robert L. Mauck1,2, Neil R. Malhotra1, Lachlan J. Smith1,2
1University of Pennsylvania, Philadelphia, PA, 2Philadelphia VA Medical Center, Philadelphia, PA, 3Penn State Hershey Bone and Joint Institute, Pennsylvania State University Hershey, PA, 4University of Delaware, Newark, DE

Disclosures: None

Introduction: Intervertebral disc degeneration is a progressive cascade that leads to structural and mechanical failure of the disc, and is frequently associated with low back pain. For early or mid-stage disc degeneration, there is considerable interest in employing biologic therapeutics — including stem cell, hydrogel, and/or growth factor injections — to stimulate tissue regeneration.1,2 We previously developed a large animal model of disc degeneration in the goat lumbar spine, in which moderate degeneration is achieved 12 weeks following intradiscal injection of 1U chondroitinase ABC (ChABC).1,2 Our group has also shown that a triple interpenetrating network hydrogel composed of dextran, chitosan and teleostean (DCT) mimics nucleus pulposus (NP) mechanical properties and acts as an effective carrier for mesenchymal stem cells (MSCs).3,4 The purpose of this study was to undertake a clinically relevant investigation to establish the feasibility and efficacy of a combined stem cell and DCT hydrogel intradiscal injection to regenerate the disc and restore mechanical properties using a large animal model.

Methods: Ex Vivo Studies: Goat lumbar spine motion segments from a previous animal cohort were utilized to assess the capacity of the DCT hydrogel to normalize disc mechanics. Ex vivo motion segments (n=5) that had previously been degenerated via 1U ChABC in vivo for 12 weeks were mechanically tested in tension-compression (20 cycles, -230N to +115N) prior to, and then following, intradiscal injection of the DCT hydrogel. Force and optical displacement data were analyzed in MATLAB to quantify compressive and neutral zone (NZ) modulus, NZ range of motion (ROM) and total ROM; differences between groups were assessed via one-way ANOVA and Tukey’s post-hoc tests. In Vivo Studies: Surgery was performed on 3 male large frame goats to induce disc degeneration at four levels of the lumbar spine (n=12 discs total) via intradiscal injection of 1U ChABC.1 Following progressive degeneration for 12 weeks, a second surgery was performed to deliver the combined cell and hydrogel therapeutic. Four therapeutic groups were randomized to the 12 degenerated lumbar discs: DCT hydrogel alone, allogeneic MSCs + hydrogel, allogeneic MSCs preconditioned in hypoxic culture (2% O2 for 1 week in monolayer culture) + hydrogel, allogeneic NP cells + hydrogel. Cells were suspended in the hydrogel at a density of 10 million cells/mL, and injected into the disc via a 22G needle (200 - 600µL gel per level). The hydrogel was labelled with radiopaque zirconia nanoparticles (30 wt%). Lateral lumbar spine radiographs were taken immediately before and after each surgical procedure, as well as every 4 weeks during degeneration, and weekly following therapeutic delivery. Disc height index (DHI) was quantified and normalized to pre-operative values. Two weeks following therapeutic delivery, animals were euthanized and the lumbar spines harvested. µCT scans were obtained at 3T for quantitative MRI T2 and T1p mapping. DHI and MRI data were analyzed via two-way ANOVA. Following MRI, high resolution µCT was performed for each motion segment to assess the presence and distribution of the injected hydrogel in the disc. Samples were then fixed, decalcified, processed through paraffin and sectioned for histological analyses.

Results: Ex Vivo Studies: ROM was significantly increased and NZ modulus significantly decreased in degenerative discs compared to intact, healthy discs. Following injection of the DCT hydrogel, mechanical properties of the degenerated motion segments were not significantly different from controls (Figure 1). In Vivo Studies: The radiopaque gel was easily detectable in the disc space on post-op plain radiographs (Figure 2A). µCT analysis illustrated that the hydrogel was well-distributed throughout the disc, was present in both the NP and in between layers of the annulus fibrosus, and had not extruded from the disc (Figure 2B). Two-way ANOVA indicated no significant contribution of cell treatment to MRI or DHI outcome measures, thus, data were pooled for analysis. 12 weeks following delivery of 1U ChABC, disc height was reduced a mean 22.7% compared to pre-operative values, consistent with our previous data in this model. A partial recovery of DHI occurred immediately following cell and hydrogel delivery which was maintained at 89.9% of pre-operative DHI 2 weeks following treatment (Figure 3A). The mean NP MRI T2 and T1p values of cell and hydrogel treated discs were not different from healthy control discs, and were significantly higher than 1U ChABC degenerated, untreated discs at 12 weeks (data obtained from a prior animal cohort) (Figure 3B). Histological analyses of disc structure and composition, and the respective contributions of each cell therapy to regeneration, are ongoing.

Discussion: Results from this study illustrate the feasibility of stem cell and hydrogel delivery as a regenerative therapeutic approach using a preclinical animal model. These data illustrate that the hydrogel remains within the disc space over a 2 week period following delivery, and suggests that the DCT hydrogel and/or stem cell injections may acutely restore disc height and MRI signal of degenerate discs to near control levels. When tested ex vivo, the DCT hydrogel restored whole disc mechanics to within the normal range, consistent with our prior studies on human cadaveric discs following partial nucleotomy.2 Ongoing work is assessing cell distribution and survival in the disc space, including the potential benefits of hypoxic MSC conditioning. Future work will explore the regenerative potential of combined cell and hydrogel therapies over longer durations in this preclinical animal model of disc degeneration.

Significance: Using a clinically relevant large animal model, this study illustrates the potential of stem cell-seeded injectable hydrogels as a regenerative therapeutic approach for the treatment of mild to moderate disc degeneration.


Acknowledgments: This study was supported by the Department of Veteran’s Affairs and the Penn Center for Musculoskeletal Disorders. The authors acknowledge Dr Weiliam Chen for providing hydrogel components.
Lactate uptake inhibition alter annulus fibrosus cell bioenergetics

Robert Hartman¹, Prashanti Patil¹, Robert Fisherman¹, Bennett Van Houten¹, James Kang¹, Nam Vo¹, Gwendolyn Sowa¹
¹University of Pittsburgh, Pittsburgh, PA


INTRODUCTION: Intervertebral disc cells reside in an environment that subjects them to low glucose and oxygen and high lactate concentrations. The current prevailing theory posits that lactate accumulation, consequence of NP cell anaerobic respiration, contributes to disc degeneration by reducing disc cell viability and negatively impacting their metabolism. However, disc cells are capable of surviving high lactate milieu for decades, yet the mechanisms by which they do so remains unclear. We propose a new hypothesis that predicts that annulus fibrosus (AF) cells exhibit metabolic flexibility whereby they are able to uptake and utilize lactate as a carbon energy source and regulate extracellular acidity. As a consequence, inhibition of lactate uptake is expected to alter cellular energy metabolism. This study tested whether the addition of exogenous lactate or a lactate uptake inhibitor to AF cells reduced cellular energy metabolism.

METHODS: This study was approved by the University of Pittsburgh IACUC (14073827). AF cells were isolated from lumbar discs of skeletally immature (6-9mo) New Zealand White rabbits (n=3 per group). Cells were cultured at 5% O2, passaged, plated for 48h and treated for 24h with or without 2 mM exogenous lactate (Sigma) and 5 mM α-cyano-4-hydroxycinnamate (CHC) (C2020, Sigma), an inhibitor of the common lactate importer, monocarboxylate transporter (MCT)-1. Following treatment, bioenergetics profiles were measured using a Seahorse XFe 96 extracellular flux analyzer (Seahorse Bioscience) with n=3-5 wells per sample. Oxygen consumption rate (OCR), reflecting oxidative phosphorylation (OXPHOS), and extracellular acidification rate (ECAR), reflecting glycolysis, were measured at basal conditions and subsequent additions of a series of pharmaceutical drugs (mitochondrial Complex V inhibitor, mitochondrial membrane permeabilizer, glycolysis inhibitor, and mitochondrial Complex I inhibitor) to characterize mitochondrial energy for ATP production, reserve capacity—a measure of mitochondrial ability to respond to stress, total respiratory capacity—the maximal OCR less non-mitochondrial OCR, and glycolytic capacity—maximal ECAR less ECAR after inhibiting glycolysis [1]. Mean ± standard deviation with a sample size of n=3.

RESULTS: The addition of exogenous lactate slightly reduced total respiratory capacity but either improved or maintained basal OCR, ATP production, and glycolytic parameters (Figures 1). The addition of lactate uptake inhibitor reduced all bioenergetic parameters relating to both OXPHOS and glycolysis.

DISCUSSION: Exposure of AF cells to exogenous lactate at physiologic concentration (2 mM) lactate produced little effects on AF cell bioenergetics, which may reflect their adaptation to a high-lactate disc environment (2-6 mM) [2]. On the other hand, inhibition of lactate uptake severely suppressed metabolic activity and capacity for energy production, suggesting lactate as an important carbon energy source for AF cells. Ongoing studies are underway to analyze varying doses of lactate as well as potential non-specific effects of the lactate inhibitor CHC.

SIGNIFICANCE: Identifying disc cell metabolic adaptations to the unique environment of the intervertebral disc is critical to elucidating molecular changes during degeneration and developing biological approaches to preserve cell viability and their bioenergetics and matrix synthesis capacity.

A Potential Safe and Novel Dietary Supplementation For Preventing Pain Associated With Disc Degeneration – An In-Vivo Rat Model

Alon Lai1, Thomas W. Ewashick-Rogler1, Jonathan M. Salandra2, Lap Ho3, Damien M. Laudier1, Samuel K. Cho1, Andrew C. Hecht1, Beth A. Winkelstein4, Giulio M. Pasinetti1,3, James C. Iatridis1
1 Icahn School of Medicine at Mount Sinai, New York, NY, 2 Philadelphia College of Osteopathic Medicine, Philadelphia, PA, 3 James J. Peters Veterans Affairs Medical Center, Bronx, NY, 4 University of Pennsylvania, Philadelphia, PA

INTRODUCTION: Low back pain is the leading cause of disability worldwide. Although the causes of back pain are multifactorial and often unclear, intervertebral disc (IVD) degeneration and pro-inflammatory cytokines have been shown to be highly associated. We previously showed that annular puncture and injection injury in multiple lumbar IVDs in a rat model induced painful behaviors as well as IVD height loss, IVD structural failure, and sensitization of dorsal root ganglion (DRG) [1]. Polyphenols are a large and diverse group of naturally occurring compounds with phenol structural units that are present in many plant-derived foods and beverages, and are generally safe for dietary consumption [2]. Polyphenols have been shown to act as anti-inflammatory agents and to mitigate catabolic changes resulting from pro-inflammatory cytokines in IVD cells in-vitro [3, 4], and localized application of the polyphenol resveratrol reduced in radiculopathic pain in-vivo [4]. The objective of this study was to determine if oral consumption of select Bioactive Dietary Polyphenol Preparation (BDPP) would be an effective treatment on the immediate and long-term painful behaviors associated with IVD degeneration induced by annular injury. Because BDPP are considered a safe, mild supplement, the study design included both pre-injury and post-injury treatment groups.

METHODS: All experimental procedures were approved and guided by the IACUC. For the immediate effect (1-week study), thirty-eight healthy, skeletally mature SD rats were used and randomly divided into 5 groups: Naive, Sham, Injury, Injury+BDPP (post-) or Injury+BDPP (pre- & post-). IVD injuries were induced in all rats (except Naive and Sham group) by annular puncturing the L3-4, L4-5 and L5-6 IVDs using 25G needle with a depth of 3mm followed by slow injection of 2.5μL of PBS [1]. BDPP were provided to the rats before injury in Injury+BDPP (pre- & post-) group for 2 weeks and continuously after surgery, and after injury only in Injury+BDPP (post-) group. BDPP is comprised of a select Concord grape juice, a select grape seed polyphenol extract and all trans-resveratrol and all trans-resveratrol. No annular injury was performed in the Sham group, and there was no surgery in the Naive group. For the long-term 6-week effect, we prioritized the pre-injury treatments because of larger effects with twelve rats, randomly assigned to Injury and Injury+BDPP (pre- & post-) groups (n=6). Pain behaviors and IVD degeneration were assessed before surgery and weekly after surgery using hindpaw mechanical hyperalgesia tests and radiograph disc height measurements, respectively. Animals were sacrificed at 1-week or 6-week post-surgery. Lumbar IVDs were harvested for determining morphological changes via safranin-o/fast-green/haematoxylin staining and intradiscal IL-1β levels via Western blot analysis. DRG sensitization was also quantified by immunohistochemical analysis against calcitonin gene related peptide (CGRP). Hindpaw withdrawal threshold and disc height were normalized to pre-surgery and compared using two-way mixed ANOVA, and the differences in IL-1β levels and CGRP immunopositivity were compared using a one-way ANOVA. All statistical tests were performed using SPSS v.20 with p<0.05 being significant.

RESULTS: For the immediate effect, the paw withdrawal thresholds were significantly decreased in the Injury group at 1 week after surgery suggesting increased pain behaviors compared to the Naive group (Figure 1). Paw withdrawal threshold was significantly greater for Sham and Injury+BDPP (pre- & post-) groups (Figure 1) than Injury suggesting reduced pain behaviors in the polyphenol pre-treatment and sham group. The Injury+BDPP (post-) group was significantly lower than Naive but had a higher mean than Injury. IVD morphology and pro-inflammatory cytokine level as well as DRG sensitization are still under investigation. For long-term effects, the paw withdrawal threshold was significantly greater with pre-injury polyphenol treatment than Injury, suggesting reduced pain behaviors. The long-term study also showed there were no significant differences between the Injury and Injury+BDPP (pre- & post-) groups for IVD height with time, or 6-week endpoint measurements of IVD histology (Figure 2), intradiscal pro-inflammatory levels or DRG CGRP immunopositivity suggesting oral polyphenols had little or no effect on IVD degeneration.

DISCUSSION: Polyphenol-rich products are a safe dietary supplementation and our results suggest they may be beneficial for alleviating pain/discomfort associated with IVD injury and degeneration. Results also highlighted that the time of starting polyphenol ingestion is critical, with significant improvements in pain/discomfort when polyphenols were supplied before injury. Beneficial effects of polyphenol were greatly diminished when ingestion started after the injury was suffered, suggestive of a notably smaller or possibly negligible effect. In the 6-week study, the annular injury and PBS injection in the Injury group showed a trend of improvement in pain behaviors with time with no recovery of the IVD height. The long-term study also provided little evidence that polyphenol-rich products promoted IVD repair since there were no observable differences in IVD structure or intradiscal pro-inflammatory cytokine levels for Injury or polyphenol treatment groups. While it remains definitive that increased pain behaviors initiate from IVD injury in this model, the mechanism for acute and chronic pain perception by the animals appears to be more complex than the IVD injury alone, as it is known to be in human IVD injuries. Since the polyphenol did not induce significant changes in DRG sensitization and IVD degeneration, the mechanism for the effect of polyphenols on relieving pain behavior requires further investigation in order to optimize treatments and delivery methods.

SIGNIFICANCE: Polyphenol-rich products are novel conservative and safe dietary supplements and this animal study provides evidence that orally ingested polyphenols may have potential to alleviate pain associated with IVD injury and degeneration.


ACKNOWLEDGEMENTS: This work is supported by NIAMS/NIH grant R01AR064157 (JCI) and in part by the Botanical Center for promotion of Cognitive and psychological resilience P50 AT008661 (GMP).

Figure 1. Polyphenols with pre-surgery treatment alleviated pain behavior induced by annular injury.

Figure 2. Polyphenols could not prevent the IVD degeneration induced by annular injury.
Advanced glycation end-products are associated with the declined mechanical performance of the intervertebral disc in murine models of Type I and Type II diabetes

Jennifer W. Liu 1,2, Adam Abraham 2, & Simon Y. Tang 1,2,3
1Washington University in St. Louis, Dept. of Biomedical Engineering, 2Dept. of Orthopaedic Surgery, 3Dept. of Mechanical Engineering

RESULTS: Type I diabetic mice had blood glucose levels significantly greater than controls starting from 8 weeks of age (p < 0.01), and until 12 weeks of age when they were sacrificed. Type II diabetic mice had elevated blood glucose levels starting from 4 weeks of age (p < 0.001) until 12 weeks of age when they were sacrificed. There were no significant differences in weight for the Type I mice. Type II mice had a significantly higher weight starting from 3 weeks of age (p < 0.01) until sacrifice. Type I and Type II diabetic IVDs had decreased disc height, (p < 0.01) for both, and decreased wet weight, (p < 0.01) for both strains, blood glucose measurements were taken weekly to confirm blood glucose levels over 300 mg/dL, which is deemed diabetic. Lumbar intervertebral discs were dissected from the Type I diabetic mice, Type II diabetic mice, and respective controls (n = 6 each). Wet weight was measured using a laser micrometer, proteoglycan and AGEs content were measured using biochemical fluorescent assays, and mechanical properties were determined using cyclical dynamic compression [6]. A subset of samples were processed for histology and stained for Safranin/Fast Green.

METHODS: All animal experiments were done with approval from the Washington University Animal Studies Committee approval. Type I diabetes was induced using streptozotocin (STZ) injection to disrupt pancreatic beta-islet cells in C57BL/6 mice. 6-week old male mice received daily intraperitoneal injections of 50 mg/kg STZ for 5 days and then observed for 2 weeks. Controls were given injections of PBS. Type II diabetes is induced by a mutation in the leptin receptor Leprdb, where mice homozygous (Leprdb+/+) for the mutation become morbidly obese, hyperglycemic, and diabetic by 4 weeks of age 12-week old mice homozygous for the Leprdb mutation were used to model Type II diabetes, while their mice homozygous littermates were used as controls. For both strains, blood glucose measurements were taken weekly to confirm blood glucose levels over 300 mg/dL, which is deemed diabetic. Lumbar intervertebral discs were dissected from the Type I diabetic mice, Type II diabetic mice, and respective controls (n = 6 each). Wet weight was measured using a laser micrometer, proteoglycan and AGEs content were measured using biochemical fluorescent assays, and mechanical properties were determined using cyclical dynamic compression [6]. A subset of samples were processed for histology and stained for Safranin/Fast Green.

DISCUSSION: The observed mechanical changes in the Type I and Type II diabetic animals are consistent with previous studies connecting increased AGEs with reduced viscoelastic behavior of IVD tissues [7,8]. In this study, we have identified a link between compromised mechanical function and increased AGEs in diabetic discs. Additionally, AGEs was shown to cause changes in IVD matrix composition and alter the structural integrity of the disc as a whole. Taken together, these factors result in functional and mechanical damage and ultimately lead to degeneration of the IVD. Future work will involve the mechanistic investigations of AGEs in diabetes-mediated degeneration of the IVD.

ACKNOWLEDGEMENTS: We would like to acknowledge the Washington University Musculoskeletal Research Center (P30 AR057235), the Washington University Summer Engineering Fellowship, NIH T32AR060719, and NIH R21AR069804.
Which Domains of the NDI Improve Most After Surgery for Cervical Myelopathy?

Paul Millhouse¹, Kristen Nicholson¹, Emily Pflug¹, Ayman Bodair², Hamadi Murphy¹, Barrett Ivory Woods¹, Christie Elizabeth Stawicki¹, Christopher Kepler¹, Alan S Hilibrand¹, Alexander Vaccaro¹, Kristen E Radcliff¹
¹Rothman Institute, Philadelphia, PA, ²Drexel University, Philadelphia, PA

INTRODUCTION:
The neck disability index (NDI) is an easily scored, common, ten-item questionnaire about symptoms relevant to cervical spine pathology: pain intensity, personal care, lifting, reading, headaches, concentration, work, driving, sleeping, and recreation. Initial validation for the NDI considered only "whiplash"-injury patients in an outpatient clinic and was published in the physical therapy literature. However, the NDI is now widely used to evaluate the outcomes of cervical surgery. The purpose of this study was to determine which domains of the NDI improve most after cervical spine surgery for myelopathy and whether improvement in the composite NDI score or specific domains better predicts change in physical function.

METHODS:
Analysis of a prospectively-kept registry of patients treated at a major academic medical center. At baseline standardized outcome measures including the Neck Disability Index (NDI) and the Short-Form 12 Physical Component Scores (SF12 PCS) were collected. Preoperative outcome measures were compared to those at one year after surgery using paired Studentâ€™s t-tests. For this study, each of the ten items was treated separately. Multiple linear regressions were performed using change in SF12 PCS as the dependent variable and change in NDI components as the independent variables.

RESULTS:
Baseline data were collected on 118 patients (mean age 58 years). A total of 66 patients had complete 1 year follow-up data. Each of the ten NDI components significantly improved from baseline (p<0.004). The NDI items with the largest improvements from baseline were: sleeping (-1.5 mean change from baseline), recreation (-1.443), lifting (-1.186), work (-1.043), and pain (-1.014).

Linear regression for change in NDI components versus change in SF12 PCS revealed a significant correlation (r² = 0.407, p<0.001). The only significant (p=0.001) predictor value was change in recreation score (-2.41, 95% CI -3.81, -1.00). "Lifting" was the only other factor with a robust coefficient (-1.21, 95% CI -2.42, 0.00) although this was not significant (p=0.051).

Linear regression for change in the composite NDI and change in PCS was significant (p<0.001), and had a weaker correlation (r² = 0.315).

A linear regression incorporating only "recreation" and "lifting" had an r-squared value of 0.434 (p<0.001).

DISCUSSION:
All domains of the NDI do not improve equally after surgery for myelopathy. The pain subdomain had only a moderate observed improvement and a poor correlation to health related quality of life. Some specific domains correlate more strongly with improvement in health related quality of life than the composite NDI score. Based upon these results, we conclude that the item bank and composite scoring of the NDI are inappropriate for evaluating quality of life in studies of surgically treated cervical spondylotic myelopathy patients.

SIGNIFICANCE:
Newer Value-based reimbursement models emphasize patient-reported outcomes including disease-specific measures. The neck disability index (NDI) is a common tool used when evaluating cervical spine pathology and disability. This study helps to establish benchmarks and evaluate the relevance of the NDI for assessing a group of myelopathic patients treated surgically.
Outcomes after treatment for cervical myelopathy
Paul Millhouse¹, Kristen Nicholson¹, Emily Pflug¹, Ayman Bodair², Barrett Ivory Woods¹, Christie Elizabeth Stawicki¹, Christopher Kepler¹,
Alan S Hilibrand¹, Alexander Vaccaro¹, Kristen E Radcliff¹
¹Rothman Institute, Philadelphia, PA, ²Drexel University, Philadelphia, PA

INTRODUCTION:
Cervical spondylotic myelopathy (CSM) is a leading cause of spinal cord dysfunction. Value-based reimbursement models emphasize patient-reported outcomes. The purpose of this study was to evaluate the patient-reported outcomes for a group of patients at least 1-year after surgical intervention for CSM.

METHODS:
A consecutive series of patients surgically treated for CSM at a single large orthopaedic surgery practice was identified. Patient-reported functional outcomes including Short-Form 12 Physical and Mental Component Scores (SF-12 PCS & MCS), Neck Disability Index (NDI), neck pain, arm pain, and modified Japanese Orthopaedic Association (mJOA) scores were collected prospectively and analyzed. Outcome measures were assessed prior to surgical intervention (baseline) and compared to those at one year or more recent. To account for baseline differences, recovery ratios (RR) were calculated as the percentage difference between pre- and post-operative scores.

RESULTS:
Baseline data were collected on 232 patients (mean age 58 years). Some 171 patients were treated with an anterior fusion (ACDF) (mean 2.4 levels fused), 36 patients with a posterior (PCDF) (mean 4.6 levels fused, mean age 61 years), 15 patients underwent a combined anterior and posterior procedure (mean 3.9 levels fused), and 13 patients were treated with a laminoplasty without interbody fusion (mean age 68 years). There were no significant differences in baseline outcomes between the ACDF and PCDF groups. Three patients had revision surgery within a year and were excluded from follow-up.

At baseline, the myelopathy patients had moderate disability (NDI mean 37 out of 100), poor physical function (PCS mean 33), and average mental health (MCS mean 47). Mean mJOA score was 14.7±2.7. Patients also had high neck (4.5) and arm (5.3) pain on a 10-point numeric scale.

A total of 157 patients had at least one follow-up outcome. There were significant improvements from baseline in PCS (9 points), MCS (4 points), NDI (15 points, RR 43%), arm pain (-3 points, RR 45%), neck pain (-3 points, RR 41%), and mJOA (1 point, RR 39%). Most patient (63%) report an improvement in mJOA; however fewer than half (36%) improved by at least 2 points (MCID). Nearly two-thirds of the patients reported an improvement greater than MCID for PCS (61% >5 points) and arm pain (58% >2 points). The mJOA score decreased (myelopathy worsened) for 13% of the patients while 17% reported lower NDI (increase in disability) at latest follow-up.

DISCUSSION:
These results indicate that patients with cervical myelopathy have substantial baseline impairments in quality of life. Older patients tended to have more severe myelopathy scores at baseline. Surgical intervention improves quality of life, disability, myelopathy symptoms, neck pain, and arm pain for most patients. Value-based reimbursement models need to consider the psychometric properties of instruments and which domains of health are important to the patient when determining quality benchmarks.

SIGNIFICANCE:
Newer Value-based reimbursement models emphasize patient-reported outcomes (PROs) including Quality of life (QoL), disability, pain, and diseasespecific measures. Desirable outcomes after surgical treatment for myelopathy are of great interest to clinicians treating this common and disabling disorder. This study helps to establish standard outcomes for a large group of myelopathic patients treated surgically.

IMAGES AND TABLES:

Figure 1: Follow-up versus baseline functional outcomes

Figure 2: Postoperative outcome improvements versus baseline
Does baseline sagittal balance influence the surgical outcomes of patients with L4/5 degenerative spondylolisthesis?

Paul Millhouse1, Kristen Nicholson1, Laura Steel1, Olivia Horwitz1, Barrett Ivory Woods1, Christie Elizabeth Stawicki1, Christopher Kepler1, Alan S Hilibrand1, Alexander Vaccaro1, Kristen E Radcliff1

1Rothman Institute, Philadelphia, PA, 2Sidney Kimmel Medical College, Philadelphia, PA

INTRODUCTION:
Sagittal balance is an important parameter for multilevel deformity reconstruction. It is unclear whether global parameters affect focal pathology such as L4/5 degenerative spondylolisthesis (DS). This analysis aimed to determine whether preoperative lumbar sagittal alignment influences outcomes of patients who undergo monosegmental surgery.

METHODS:
A consecutive series of patients with L4/5 DS who underwent posterior, monosegmental surgery were identified. Relevant clinical outcome measures including Oswestry Disability Index (ODI), Short-Form 12 Physical and Mental Component Scores (SF-12 PCS & MCS), and back & leg pain were prospectively collected. Baseline radiographic parameters including lumbar lordosis (LL), pelvic incidence (PI), and pelvic tilt (PT) were analyzed by three blinded observers, and sacral slope (SS) and LL-PI mismatch were calculated. Sagittal balance was measured as the L1 axis S1 distance (LASD).

RESULTS:
One hundred twenty two patients were eligible; however appropriate preoperative radiographs were not always available and three patients were excluded for prior lumbar fusions. There were 109 patients for whom radiographic parameters were available. Outcomes were collected at least 1-year after surgery (334 - 802 days) from 101 patients. No strong correlations existed between any radiographic parameters and changes in outcome measures. There were 67 patients that achieved MCID ODI (15 point change) and 29 patients that did not. The patients who did not achieve MCID had more lordosis (59° vs. 52°, p=0.011) and higher SS (42° vs. 37°, p=0.024). There were no differences in LASD, PI, PT, or PI-LL between patients that achieved MCID versus those who did not. In patients with preoperative PI-LL mismatch (>9°) there was no significant difference in change in ODI, PCS, MCS, Back or Leg pain.

DISCUSSION:
These data indicate that preoperative sagittal plane alignment does not affect the improvements in outcomes of patients with L4/5 degenerative spondylolisthesis, suggesting there is a compensatory rather than anatomic component to the presentation. Patients with lumbo pelvic mismatch had the same improvement in outcome as patients who did not. Therefore, surgeons may safely perform limited monosegmental fusion at L4/5 without addressing global alignment in patients with L4/5 degenerative spondylolisthesis.

SIGNIFICANCE:
Radiographic parameters are commonly assessed in diseased populations to aid in surgical planning. Of great interest is whether these parameters correlate with postoperative outcomes. Further, addressing global alignment may lead to a longer and more difficult operation. This study helps to establish benchmarks and evaluate the clinical relevance of tools for assessing a group of patients surgically treated.
Does sagittal balance influence baseline pain in patients with L4/5 degenerative spondylolisthesis?

Paul Millhouse1, Kristen Nicholson1, Laura Steel2, Olivia Horwitz1, Barrett Ivory Woods2, Christie Elizabeth Stawicki3, Christopher Kepler1, Alan S Hilibrand1, Alexander Vaccaro1, Kristen E Radcliff1

1Rothman Institute, Philadelphia, PA, 2Sidney Kimmel Medical College, Philadelphia, PA

INTRODUCTION:
Sagittal balance is an important parameter in multilevel deformity reconstruction. However it is unclear whether global parameters affect focal pathology such as degenerative spondylolisthesis (DS) at the L4/5 level. This analysis aimed to determine whether global lumbar sagittal plane alignment influenced baseline clinical measures.

METHODS:
A consecutive series of patients with L4/5 DS who underwent posterior one-segment surgery were identified. Relevant baseline clinical outcome measures including the Oswestry Disability Index (ODI), Short-Form 12 Physical and Mental Component Scores (SF-12 PCS & MCS), and back & leg pain scores were collected. Baseline radiographic parameters including lumbar lordosis (LL), pelvic incidence (PI), and pelvic tilt (PT) were analyzed by three blinded observers. Sagittal balance was measured as the L1 axis S1 distance (LASD).

RESULTS:
A total 119 patients were eligible, at least one radiographic parameter was available for 109, and there were 101 subjects with outcomes reported at least one year after surgery (mean 548 days). There was no strong (greater than R 0.30) correlation between age or BMI and any radiographic parameter. LL correlated with PI (R 0.45, p<0.001) and SS (R 0.80, p<0.001), and negatively with PT (R -0.32, p<0.001) while PI correlated with PT (R 0.53, p<0.001), SS (R 0.62, p<0.001) and LASD (R 0.39, p<0.001). PI-LL difference correlated with LASD (R 0.50) and PT (R 0.79). There was a correlation between LASD and baseline ODI (R 0.32, p<0.001) and a weak correlation between LASD and baseline back pain (R 0.25, p=0.012). There was no compelling correlation between any other radiographic parameter and any outcome measure. Comparing patients with PI-LL mismatch greater or less than 11, there was a small difference in ODI (52 vs. 45, p=0.043) and PCS (27 vs. 31, p=0.004).

DISCUSSION:
Overall lumbar lordosis, pelvic incidence, sacral slope, or pelvic tilt did not correspond to baseline pain in patients with L4/5 degenerative spondylolisthesis. Preoperative lumbopelvic mismatch corresponded to increased back pain and worse physical function upon presentation. L1 axis S1 distance appears to correspond to overall lumbar sagittal alignment and specifically lumbopelvic mismatch. Lumbar sagittal imbalance, reflected in increased L1 axis S1 distance, correlated to increased back pain and disability upon presentation. In conclusion, global sagittal plane alignment affects pain upon presentation even in the case of patients with a focal, monosegmental problem.

SIGNIFICANCE:
Newer value-based reimbursement models emphasize patient-reported outcomes including pain and disease-specific measures. Radiographic parameters are commonly assessed in diseased populations before and after surgery. This study helps to establish benchmarks and evaluate the clinical relevance of these tools for assessing a group of patients surgically treated.
Outcomes after surgical management for L4/5 degenerative spondylolisthesis
Paul Millhouse¹, Kristen Nicholson¹, Laura Steel², Olivia Horwitz³, Barrett Ivory Woods¹, Christie Elizabeth Stawicki¹, Christopher Kepler¹, Alan S Hilibrand¹, Alexander Vaccaro¹, Kristen E Radcliff¹
¹Rothman Institute, Philadelphia, PA, ²Sidney Kimmel Medical College, Philadelphia, PA

INTRODUCTION:
Patient-reported outcomes after management for degenerative spondylolisthesis (DS) have been well reported in other studies. However, few studies have used prospectively collected data to measure and quantify improvements in pain and health status following surgical management for patients suffering from DS. The purpose of this study was to report the prospectively collected change in outcome measures of a cohort of patients with monosegmental L4/5 DS.

METHODS:
A consecutive series of 112 patients who underwent surgical intervention for treatment of single level degenerative spondylolisthesis at L4/5 was identified. Patients with trauma, tumor or infection were excluded. Patients with previous lumbar surgery were included. Outcome measures including the the Oswestry Low Back Pain Disability Index (ODI) and the Short Form, 12-Item Health Survey, Physical and Mental Component Scores (SF-12 PCS and MCS), back pain and leg pain were collected preoperatively and at least one-year postoperatively. Follow-up outcomes were prospectively collected either at office visits or via telephone interviews.

RESULTS:
There were 101 patients with one year follow-up (90%). Mean improvement versus baseline was statistically significant for all outcome measures (ODI, MCS, PCS, Leg pain, Back pain) (p<0.001). Mean improvement in ODI was 25.5 points (SD 19.0, 53% of baseline ODI). There were 70 patients with ODI improvement greater than 15 points. Ten patients (9.9%) worsened or stayed the same and one patient (1%) had ODI worsen by more than 15 points. Mean improvement in SF-12 MCS was 6.6 (SD 10.2, 14% improvement), and PCS was 9.1 (SD 11.0, 31% improvement). There were 26 patients with worse MCS and 25 patients with worse PCS compared to baseline. VAS leg pain improved by -4.0 (SD 3.7, 57%) and back pain by -4.0 (SD 3.1, 63%). There were 14 and 9 patients with worse or the same leg or back pain, respectively, after surgery. There was no significant difference between instrumented posterolateral fusion and TLIF in outcome improvement.

DISCUSSION:
In this population of patients with L4/5 degenerative spondylolisthesis, there were significant improvements from baseline following surgical intervention. On average, patients improved by more than 50% over their baseline pain status. In this patient group, there was no additional improvement in outcomes at one year with TLIF versus instrumented posterolateral fusion procedures. For select patients, surgeons can safely perform instrumented posterior fusion without the interbody component.

SIGNIFICANCE:
Radiographic parameters are commonly assessed in diseased populations to aid in surgical planning. Of great interest is whether these parameters correlate with postoperative outcomes. Further, patients often wonder how much surgery can and will improve their symptoms. This study helps to establish benchmarks and evaluate the clinical relevance of tools for assessing a group of patients surgically treated.

Table 1: Comparison of baseline and follow-up outcomes

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Baseline</th>
<th>Followup</th>
<th>delta</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ODI</td>
<td>47.7 ± 17.9</td>
<td>21.7 ± 18.9</td>
<td>-25.5 ± 19</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(8.9-90)</td>
<td>(0-78)</td>
<td>(-68-24.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n=112</td>
<td>n=101</td>
<td>n=100</td>
<td></td>
</tr>
<tr>
<td>MCS</td>
<td>47.7 ± 10.6</td>
<td>54.3 ± 9</td>
<td>6.6 ± 10.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(26.2-67.7)</td>
<td>(26.2-68.3)</td>
<td>(-16.6-33.6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n=112</td>
<td>n=100</td>
<td>n=97</td>
<td></td>
</tr>
<tr>
<td>PCS</td>
<td>29.6 ± 7.7</td>
<td>39.1 ± 11.9</td>
<td>9.3 ± 10.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(17.3-53.1)</td>
<td>(17.5-59)</td>
<td>(-14.8-31.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n=112</td>
<td>n=100</td>
<td>n=97</td>
<td></td>
</tr>
<tr>
<td>Leg Pain</td>
<td>6.6 ± 2.6</td>
<td>2.7 ± 3</td>
<td>-4 ± 3.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(0-10)</td>
<td>(0-10)</td>
<td>(-10-7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n=100</td>
<td>n=95</td>
<td>n=83</td>
<td></td>
</tr>
<tr>
<td>Back Pain</td>
<td>6.7 ± 2.7</td>
<td>2.7 ± 2.8</td>
<td>-4 ± 3.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(0-10)</td>
<td>(0-10)</td>
<td>(-10-5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n=102</td>
<td>n=95</td>
<td>n=84</td>
<td></td>
</tr>
</tbody>
</table>
Effects of Inflammation on Cellular Deformation of Nucleus Pulposus Cells: A Biphasic Finite Element Model
Quynhhoa T. Nguyen, Nadeen O. Chahine
Biomechanics and Bioengineering Research Laboratory
Feinstein Institute for Medical Research, Northwell Health System, nchahine@northwell.edu

Disclosure: The authors have nothing to disclose.

INTRODUCTION: Nucleus pulposus is the central region of the disc that is comprised of sparsely distributed cells embedded in a matrix that is rich in proteoglycans and has high water content [1,2]. Disc degeneration is characterized by changes in extracellular matrix properties, including loss of proteoglycans and collagens, degenerative fibrillation, and decreased water content, as well as by elevated levels of inflammatory cytokines that have been implicated in matrix degradation [3,4]. While inflammatory signaling has long been recognized to alter cell metabolism, our recent studies have shown that cellular biomechanical properties and cell morphology are also altered in inflammatory environment [5,6]. Although disc cells are known to be mechanosensitive, the mechanism of cellular mechano-transduction in pathological conditions (e.g. in inflammatory environment) is poorly understood. In the current study, we hypothesized that changes in NP cell biophysical properties induced by inflammatory stimuli would alter the deformational behavior of cells in response to applied deformational loading. To address this, we modeled the multiscale cell deformation behavior using biphasic finite element analysis.

METHOD: To investigate microscopic deformation behavior of nucleus pulposus cells, a multiscale finite element analysis was performed using FEBio software suite, whereby an NP cell was embedded in the center of a cylindrical agarose disk. Agarose was used as a model system because NP cells maintain a rounded morphology in this biomaterial and because its compressive properties are on same order of magnitude as native NP ECM. A constant 5% displacement was applied in unconfined configuration between 2 impermeable platens on top and bottom surfaces of the agarose disk. The agarose disk was modeled as a biphasic matrix with isotropic elastic solid (Young’s modulus, \(E_y = 13.1 \text{kPa}, \) Poisson’s ratio, \(\nu = 0.15\)) and constant hydraulic permeability (\(k_p = 5.14 \times 10^{-14} \text{m}^4/(\text{N.s})\)) [7]. The cell assumed a spherical geometry with 10 \(\mu\)m radius and was modeled as a homogeneous gel, representing the protoplasm (cytoplasm, cytoskeleton, and all enclosed organelles), surrounded by a semi-permeable membrane. The cell protoplasm was also modeled as a biphasic material with isotropic elastic solid and constant hydraulic permeability. Deformation behavior of healthy NP cells (\(E_y = 2\text{kPa}, \) \(\nu = 0.3, \) and \(k_p = 5 \times 10^{-13} \text{m}^4/(\text{N.s})\)) as well as cells exposed to inflammatory stimulation (\(E_y = 1\text{kPa}, \) \(\nu = 0.3, \) and \(k_p = 10 \times 10^{-13} \text{m}^4/(\text{N.s})\)) were analyzed based on prior experimental measures [5]. A layer of thin elements (h = 50 nm) was used to represent the cell membrane (\(E_y = 1\text{kPa}, \) \(\nu = 0.3, \) and \(k_p = 10 \times 10^{-13} \text{m}^4/(\text{N.s})\)). A hexahedral mesh was generated in PreView, nonlinear finite element analysis was performed in FEBio, and post-processing was done in PostView. Average maximal and minimal principal stresses and strains were computed at end of ramp strain (peak) and after equilibrium was reached.

RESULTS: Both at peak ramp and equilibrium, the cell exhibited lower max and min principal strains, but greater principal stresses than the surrounding agarose material (Figs. 1 and 2). Peak principal strains of inflammatory treated cells was comparable to healthy NP cells (Fig. 1A,C). However, the minimum principal strain at equilibrium was markedly greater (~75% increase) in inflammatory conditions compared to control NP cell (Fig. 1D). Compared to normal untreated cell, the magnitude of both minimum and maximum principal stresses of inflammatory treated cell were ~30% lower at equilibrium (Fig. 2B, 2D), whereas comparable principal stress levels were observed at peak ramp (Fig. 2A, 2C).

DISCUSSION: The results of this model show that cells respond differently to an applied compression in an inflammatory environment compared to control condition, mediated by altered biomechanical properties of the cells in inflammation. NP cells that are exposed to inflammatory stimuli have lower stiffness, and hence experience larger compressive deformation (min principal strain) in response to the same macroscopic displacement when compared to untreated cells. Interestingly, we observed that principal stress levels at equilibrium were lower. This may be mediated by the increase in hydraulic permeability due to inflammation. Indeed, preliminary findings suggest that lower fluid pressurization at peak ramp and increased fluid flux at equilibrium may contribute to decreased principal stresses on the cell. Future studies will include analysis of cell deformation in response to macroscopic dynamic loading and validating the model with empirical measurements.

SIGNIFICANCE: This study provides biomechanical analysis of the microscopic deformation behavior of NP cells in response to inflammatory stimuli. This knowledge could be beneficial in understanding the mechanism of mechano-biology of disc cells in healthy and degenerated microenvironments.


ACKNOWLEDGMENTS: NSF CAREER 1151605, NIH R01AR069668.
Rescuing Chondrocyte Hypertrophic Differentiation Potential and Exploring Therapeutic Approaches for Enhancing Bone Formation in Mucopolysaccharidosis VII Dogs

University of Pennsylvania, Philadelphia, PA

Disclosures: SH (N), JLK (N), JRB (N), PO (N), CAF (N), JB (N), NRM (N), EMS (N), MLC (N), LJS (N)

Introduction: The mucopolysaccharidoses (MPS) are genetic, lysosomal storage diseases characterized by deficient activity of enzymes that degrade glycosaminoglycans (GAGs) [1]. MPS VII is characterized by mutations in the β-glucuronidase gene, leading to incomplete digestion and progressive accumulation of three GAG types [2]. MPS VII patients exhibit severe skeletal abnormalities, especially of the spine [3]. Persistent cartilaginous lesions are present in the vertebrae representing failed cartilage-to-bone conversion during postnatal development, which result in progressive kyphoscoliosis and spinal cord compression [4-7]. Using the naturally-occurring MPS VII canine model, we established that impaired hypertrophic differentiation of epiphyseal chondrocytes contributes to failed bone formation during early postnatal development [8], which in turn is associated with decreased Wnt/β-catenin signaling [9]. We also showed that Wnt pathway activation resulted in normalization of chondrocyte differentiation in vitro in MPS VII epiphyseal cartilage [10]. GAGs perform crucial roles in controlling the distribution and availability of Wnts, which are critical regulators of chondrocyte differentiation during endochondral ossification. Thus, we hypothesized that aberrant GAG accumulation in MPS VII contributes directly to impaired chondrocyte function and that in the absence of abnormal GAGs, hypertrophic differential potential could be rescued. To test this hypothesis, we undertook in vitro studies to compare differentiation potential of MPS VII chondrocytes in the presence and absence of their GAG-rich environment. Furthermore, to explore therapeutic approaches to correct MPS VII bone disease, we undertook a preliminary in vivo study in our canine model to establish a dosing regimen and safety profile using lithium, a Wnt pathway agonist, which has been previously shown to enhance bone formation and is approved clinically for other indications [11].

Methods: For this study, we used the naturally-occurring MPS VII canine model that closely mimics the skeletal phenotype of human patients [12]. In Vitro Analysis of GAG Accumulation and Chondrocyte Hypertrophic Differentiation Potential: With IACUC approval, unaffected control and MPS VII dogs (n=4 for each) were euthanized at 9 days-of-age, and lumbar vertebral epiphyseal cartilage was isolated. For monolayer cultures, cartilage was digested with collagenase until cells were released from the extracellular matrix. Isolated chondrocytes were expanded in basal medium (DMEM, 10% FBS, 1% PSF) then cultured in monolayer in either basal or osteogenic media. For explant cultures, epiphyseal cartilage was cultured as whole tissue explants in basal medium. Media was collected at 3, 7, and 14 days for monolayer cultures and at 5 days for explant cultures. Total media GAG content was measured using the dimethylmethylen blue assay and normalized to total cell count. Cells from monolayer cultures and explants were harvested, RNA extracted, and mRNA expression levels of chondrocyte differentiation markers ( Sox9-proliferative; Runx2-prehypertrophic; Col10-hypertrophic) were measured using qPCR. Significant differences between groups (p<0.05) were established using unpaired t-tests. In Vivo Lithium Treatment: To establish dosage needs of lithium, normal control dogs (n=2) were treated with twice daily doses of 5 mg/kg of powdered lithium carbonate packaged into gelatin capsules for 1 week for acclimation, then with twice daily doses of 10 mg/kg for 2 weeks, starting at 15 days-of-age. Dogs were monitored for side effects, and serum lithium levels were measured using a commercial assay (Crystal Chem).

Results: In Vitro Analysis of GAG Accumulation and Chondrocyte Hypertrophic Differentiation: In whole explant culture, MPS VII chondrocytes secreted significantly higher amounts of GAGs into the media compared to controls over time, while isolated chondrocytes showed no differences over 14 days of culture (Fig 1). Likewise, in whole explant culture, MPS VII chondrocytes showed impaired differentiation over time compared to controls, but both control and MPS VII chondrocytes exhibited similar propensity to differentiate over time in isolated cell culture (Fig 2). In Vivo Lithium Treatment: After the initial 1 week acclimation period, both dogs maintained serum lithium levels within the desired therapeutic range (0.2-1.5 mmol/L) over the following 2 weeks (Fig 3). Dogs exhibited a mild tremor which resolved within a few days. No significant adverse side effects from lithium treatments were observed.

Discussion: The results of this study show that MPS VII chondrocytes regain normal hypertrophic differentiation potential upon removal from their GAG-rich environment. Abnormal GAG accumulation in MPS VII epiphyseal cartilage may disrupt extracellular control of secreted growth factors, such as Wnts, which are necessary to initiate and sustain chondrocyte differentiation. We previously showed that activation of the Wnt pathway with exogenous factors can also normalize chondrocyte differentiation in vitro, and taken together, these results indicate that combinatorial therapies that normalize GAG accumulation and activate Wnt signaling may be able to rescue the differentiation potential of resident cells and ultimately normalize bone formation. As a preliminary step, we successfully treated neonatal dogs with lithium, establishing safety and optimizing an oral dosing regimen to sustain therapeutic serum levels. In ongoing in vivo studies, we are examining whether GAG reduction via exogenous enzyme replacement therapy (ERT) and Wnt/β-catenin pathway activation via lithium treatment are able to normalize chondrocyte function and bone formation in MPS VII dogs during postnatal growth.

Significance: MPS VII is associated with debilitating skeletal disease for which there is no treatment. Our results suggest that therapeutic strategies combining GAG reduction (ERT) and inducing endochondral bone formation (lithium) may effectively treat skeletal abnormalities in MPS VII patients.


Acknowledgements: Funding sources: NIH, Penn Orphan Diseases Center, National MPS Society and Penn Center for Musculoskeletal Disorders. The authors thank the staff and students at the Penn Vet School for animal care.
Growth Factor and Extracellular Matrix Expression and Localization during Nucleus Pulposus Formation

Sun H. Peck1, Kendra K. McKee2, Neil R. Malhotra1, Brian D. Harfe2, Lachlan J. Smith1
1University of Pennsylvania, Philadelphia, PA; 2University of Florida, Gainesville, FL

Introduction: Intervertebral disc degeneration is implicated as a major cause of low back pain [1]. Current available treatment options primarily focus on relieving pain rather than regenerating disc tissue, and thus, there is a need for 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 a NP cell-like phenotype to optimize adult disc regeneration. Previously, we established changes in global mRNA expression profiles of resident cells as the notochord transforms into the NP using whole-transcriptome sequencing (RNA-Seq), and found that key signaling pathways that regulate patterning, growth, differentiation, as well as structural extracellular matrix (ECM) molecules, showed significant differential gene expression across this embryonic developmental window [5]. In this study, our objectives were to build on these findings by examining protein expression of growth factors and ECM molecules identified in our RNA-Seq results at key developmental stages as the notochord transforms into the NP.

Methods: For these IACUC approved studies, we used the Shh-cre;ROSA:YFP mouse model [3], where all Sonic Hedgehog (Shh) expressing notochord-derived cells express YFP throughout the life of the mouse (i.e. creates a fate map). We examined two key developmental stages representing the immediate, opposite ends of the notochord to NP transformation: E12.5 (fully formed, intact notochord) and P0 (fully formed spine with distinct disc space). Whole embryos (E12.5) or isolated spines (P0) were fixed in formalin, and processed into paraffin. Midsagittal, 8 µm thick sections were stained with Alcian blue/picrosirius red (ABPR) for GAG and collagen respectively, hematoxylin and eosin (H&E) for cellularity, or immunostained with antibodies specific to proteins-of-interest (ECM: Collagens I, II and VI, and aggrecan; growth factors: Shh, transforming growth factor β1 (TGF-β1), and insulin-like growth factor 1 (IGF-1)) and counterstained with hematoxylin. Staining intensity in the notochord/NP and associated tissues was semi-quantitatively assessed.

Results: At E12.5, there was a discrete notochordal structure with a GAG-rich inner core and outer sheath, both of which were relatively acellular compared to the rest of the notochord (Fig 1). GAG-rich mesenchymal condensations in regions that will form future vertebral bodies were clearly present (Fig 1). At P0, the spine was fully formed with distinct vertebral bodies and disc spaces, including clear boundaries between the anulus fibrosus and the NP (Fig 1). Extracellular matrix components collagens I, II and VI, and aggrecan showed diffuse staining in non-vascular regions (core and sheath) of the E12.5 notochord (Fig 2). At P0, these molecules exhibited intense staining at the outer boundary of the NP. SHH, TGFβ1, and IGF1 all showed cellular expression in the E12.5 notochord. At P0, expression of TGFβ1 and IGF1 by NP cells was heterogeneous (strongly by some cells, weakly by others). SHH expression in the NP was weaker at P0 than at E12.5. In both the E12.5 and P0 samples, positive immunostaining of non-notochord/NP tissues for many of these molecules was also observed. Semi-quantitative scoring of protein localization is presented in Tables 1 and 2.

Discussion: In our previous whole-transcriptomic profiling study, we found a large number of differentially expressed growth factor and ECM genes at P0 compared to E12.5 [5], which are largely reflected on the protein level in our current results. We demonstrated marked changes in protein localization and expression levels between E12.5 and P0. As mRNA and protein levels do not always directly correlate in expression, ongoing work is focused on elucidating regulatory and functional roles of these genes on both the transcriptional and translational levels. The changes observed most likely reflect a switch from patterning (decreased Shh signaling) to growth (increased TGFβ1, IGF1, and ECM structural genes) as the NP develops into a functional, load-bearing tissue. Heterogeneous expression within the NP at P0 suggests that resident cells may be undergoing progressive phenotypic changes to accommodate evolving functional requirements. Interestingly, we also observed staining of non-notochord derived tissue in our studies, which will help to inform future studies exploring the roles of these molecules in embryonic spine development as a whole. Overall, these data support our long-term goal 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 and economic burden. The results from this study further our knowledge and understanding of NP development and serves to inform development of improved cell-based therapeutics for disc regeneration.


Acknowledgements: NIH, University of Pennsylvania Institute on Aging, Penn Center for Musculoskeletal Disorders.

Table 1.

<table>
<thead>
<tr>
<th>Molecule</th>
<th>E12.5</th>
<th>P0</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHH</td>
<td>**</td>
<td>-</td>
</tr>
<tr>
<td>TGFβ1</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>IGF1</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

Summary tables of immunostaining patterns. Table 1. ECM. Table 2. Growth factors. N: notochord; DC: disc condensations; VC: vertebral condensations; VP: vertebral plate; NP: nucleus pulposus; IAF: inner AF; OAF: outer AF; E: epiphysis/growth plate. : absent; *: weak; **: moderate; ***: strong.

Table 2.

<table>
<thead>
<tr>
<th>Molecule</th>
<th>E12.5</th>
<th>P0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen I</td>
<td>++++</td>
<td>++</td>
</tr>
<tr>
<td>Collagen II</td>
<td>++++</td>
<td>+++</td>
</tr>
<tr>
<td>Collagen VI</td>
<td>+++</td>
<td>++</td>
</tr>
</tbody>
</table>

Figure 1. Overall morphology at E12.5 (notochord) and P0 (disc). AF: annulus fibrosus; NP: nucleus pulposus. Scale bar = 100 µm; inset scale bar = 20 µm.

Figure 2. Representative immunostaining of ECM and growth factors at E12.5 and P0. For each pairing, the left image is E12.5, and the right image is P0. Panels in B represent higher magnification images of notochord/NP from A. Scales in A: E12.5 = 50 µm, P0 = 100 µm, and B: E12.5 = 10 µm, P0 = 50 µm.
Normative Morphology and Growth of the Pediatric Lumbar Vertebrae

James R. Peters1, Robert M. Campbell Jr.2, Sriram Balasubramanian1
1 Drexel University, Philadelphia, PA, 2 The Children’s Hospital of Philadelphia, Philadelphia PA

Disclosures: James R. Peters (N), Robert M. Campbell Jr. (N), Sriram Balasubramanian (N)

Current literature on normative pediatric lumbar spine morphology is either restricted to geometric characterization of a small subset of vertebral structures or extensive quantification of pedicle morphology alone and few studies attempt to quantitatively assess the growth of these vertebral structures. Hence, the objectives of this study were to comprehensively quantify the normative morphology and growth of the lumbar vertebrae for pediatric subjects between the ages of 1 and 19 years.

Retrospective, abdominal, computed tomography (CT) scans were obtained from 102 subjects (54 males: average age 10.23 ± 5.41 years, 48 females: average age 10.36 ± 5.64) between the 5th and 95th percentiles in height, weight, and BMI. All CTs were digitally reconstructed using the medical image processing software, MIMICS v.16 (Materialise, Inc., Belgium) and the positions of 30 landmark points and 30 geometric measurements were recorded from the surface morphology of each lumbar vertebra (Figure 1). Nonparametric statistics were used to test for differences in measurements across lumbar levels (Kruskal Wallis test), test for differences between sexes at each lumbar level (Mann-Whitney U test), and to investigate asymmetry (right vs. left, superior vs. inferior) (Wilcoxon Rank-Sign test). To evaluate growth of the lumbar vertebrae, first order, linear equations using age as the independent variable (\( y = \beta_0 + \beta_1t \)) were fit to each measurement at each lumbar level separately for males and females. For each equation the intercept, \( \beta_0 \), is the approximate value of the measurement at birth, the slope, \( \beta_1 \), is the growth rate and \( t \) is the subjects’ age in years. Partial F tests were used to detect differences in growth rates across levels and between sexes. To account for multiple hypotheses testing, the false discovery rate control as described by Benjamini and Hochberg (1995) was used to find a suitable significance cutoff. All analyses were performed using MATLAB 2014b (The Math Works, Natick, MA). Examples of the collected data and growth equations are shown in Figure 2.

Significant differences (p ≤ 0.0134) in measured values across lumbar vertebral levels were found for vertebral body widths and depths, pedicle widths and areas, transverse pedicle angles, spinal canal width and depth, spinous process angles, superior and inferior facet angles, and facet angles for both sexes. Significant differences (p ≤ 0.0134) in measured values were found between sexes for right and left pedicle widths. Significant symmetry differences (p ≤ 0.0134) were found for vertebral body heights, widths, and depths, pedicle heights, widths, and transverse angles, interfacet widths, and facet angles. Significant differences (p ≤ 0.0134) in growth rates across lumbar vertebral levels were found for vertebral body heights, widths, depths, pedicle heights, widths, and areas, spinous process length, intertransverse process width, interfacet heights, and widths, and facet angles. Significant differences (p ≤ 0.0134) in growth rates between sexes were found for vertebral body widths and depths, pedicle widths and areas and intertransverse process width.

These data demonstrate significant structure specific differences in measurements and growth across lumbar vertebral levels and between sexes. Knowledge about the normative geometry and growth of the pediatric lumbar vertebrae can be used to inform the design and placement of medical devices, and pedicle screws and to development analytical and finite element models of the spine. Clinically, the same information, can help determine the type and timing of correctional treatment for spine deformities and to assess treatment efficacy over time.

Figure 1. Lumbar vertebral measurements

Figure 2. (a) Median and interquartile ranges for right pedicle widths. (b) Scatter plot with linear equations and R² values for L1 right pedicle width. (c) Growth rates with 95% confidence intervals for right pedicle width. Males shown in black and females shown in grey.
Arthritic Human Cartilage Molecular Engineering Using Biomimetic Proteoglycans Shows Infiltration throughout the Cartilage Extracellular Matrix Ex Vivo

Evan Phillips, Nicholas Bertha, Brandon Shallap MD, Katsiaryna Prudnikova PhD, Michele Marcolongo PhD, Mary Mulcahey MD
Drexel University, Philadelphia, PA

INTRODUCTION: Facet joint degeneration is a common source for back pain characterized by the deterioration of articular cartilage within the joint. The treatment options for facet joint degeneration are aimed at treating the pain with analgesics, physical therapy, lifestyle changes, and bone fusion surgery if the pain is too debilitating. Several biomolecules are depleted from the degenerative joint such as aggrecan in articular cartilage. We propose to molecularly engineer the damaged cartilage with novel biomimetic proteoglycans (BPGs) with the eventual goal to restore hydration and mechanical function to the cartilage. Aggrecan plays a vital role in the hydration and mechanical properties of articular cartilage and is lost during the early stages of degradation. BPGs mimic the 3-D bottle brush structure and properties of naturally occurring aggrecan and consist of a poly(acrylic acid) (PAA) core with chondroitin sulfate (CS) bristles. As a step toward this objective, here we report the infiltration of BPGs into human osteochondral sections and examine the distribution of BPGs through the cartilage extracellular matrix (ECM).

METHODS: Human osteochondral fragments were obtained from patients that underwent total knee arthroplasty (IRB #1503003490). The osteochondral fragments from the tibial plateau were cut into 9 x 9 mm sections from both load bearing and non-load bearing regions to obtain samples with varying degrees of osteoarthritis (OA). The Collins’ method was utilized to grade the cartilage sections where grade 0 indicates non-osteoarthritic cartilage, grade 1 mild OA, grades 2-3 intermediate OA, and grade 4 severe OA where the cartilage is essentially fully degraded. The human osteochondral sections were suspended in fluorescently labeled CS and BPG solutions such that the articular cartilage surface was submerged while limiting the amount of BPG solution that could enter through the cut lateral sides of the section. BPG10 molecules were synthesized using previously described methods. Briefly, BPG10 consists of a 10kDa PAA core with approximately 8 attached CS chains giving the molecule a molecular weight of approximately 180kDa. BPG10 and CS molecules were fluorescently labeled with DCCH (7-Diethylaminocoumarin-3-Carboxylic acid, Hydrazide) and reconstituted in 1X PBS. The human osteochondral sections were equilibrated in 1X PBS and then immersed in fluorescently labeled 20mg/mL CS and BPG10 solutions for 24 hours with care taken to expose only the articular surface to the solution. The osteochondral sections were then embedded in OCT and cryosectioned. The sections were observed using an Olympus FV1000 confocal microscope through a DAPI filter to detect the DCCH fluorescence.

RESULTS: Both CS (22kDa) and BPG10 (180kDa) passively diffused into the human cartilage for grades 0-3 and dispersed throughout the cartilage ECM as seen in the representative images. The BPG10 and CS molecules tend to aggregate around the chondrocytes within the cartilage, as indicated by higher fluorescence intensity in the images. OA grades 0-1 showed more BPG10 and CS chondrocyte localization than grades 2-3. The localization of BPGs and CS around chondrocytes and the overall diffusion into the cartilage ECM seems to decrease with more degenerative cartilage. Due to the insufficient cartilage in OA grade 4 samples, the diffusion experiments with these samples yielded more variable results.

DISCUSSION: We have demonstrated that BPGs can infiltrate into human articular cartilage with varying degrees of OA, distribute throughout the ECM, and localize around the chondrocytes. Recently, we have also shown BPG diffusion following the same results in normal bovine knee cartilage ex vivo and normal rabbit knee cartilage in vivo. This is the first demonstration of BPG cartilage molecular engineering in human arthritic cartilage. The localization of these large anionic molecules around chondrocytes may be due to preferential binding to collagen I present in the pericellular matrix and collagen II in the rest of the ECM. This localization may also be due to an interaction with chondrocytes, which helps explain why there is less BPG10 and CS aggregation in more degenerative cartilage samples which have fewer cells. Biomimetic proteoglycans have the potential to replace lost aggrecan in the cartilage ECM in a minimally invasive manner through intra-articular injections. Infiltration through the cartilage surface may serve as a method to introduce BPGs into arthritic cartilage to molecularly engineer the tissue and restore hydration and mechanical properties.

SIGNIFICANCE: Pain reduction and joint mobility for patients with facet joint degeneration and osteoarthritis in other joints is a major challenge. Restoration of hydration and mechanical properties of early degenerative cartilage may be achieved by intra-articular injections of BPG solutions, allowing for passive diffusion of BPGs into the cartilage ECM. This study shows the first BPG cartilage molecular engineering in arthritic human cartilage.


ACKNOWLEDGEMENTS: We would like to thank the state of Pennsylvania CURE for funding this project.

Figure 1: Left) Confocal images of cartilage samples (scale bar 100 µm). Contrast and fluorescent images for controls, CS, and BPG10 at Collins’ grades 0-1 and 2-3. Right Top) Contrast and fluorescent images for control and BPG10 at Collins’ grade 4. Right Bottom) Patient data table.
Comparison of Quantitative Imaging Techniques for Detecting Degenerative Changes in a Mouse Caudal Intervertebral Disc Injury Model.


Departments of Neurosurgery and Orthopaedic Surgery, University of Pennsylvania

Introduction: Intervertebral disc (IVD) degeneration has been strongly implicated in the development of low back pain, a major cause of morbidity, health care expenditures, and lost productivity in the United States. As conservative management of low back pain secondary to disc degeneration is the mainstay of therapy and outcomes from surgical fusions are unimpressive, there is a strong need to develop novel therapies that restore disc structure and function. Developing animal models that recapitulate the pathological changes within the degenerating IVD is instrumental to studying novel cell based regenerative therapies. The objective of this study was to build on a previously reported mouse model of disc degeneration[1] and, specifically, to compare the sensitivity of two quantitative, high-resolution imaging techniques – magnetic resonance imaging (MRI) and microcomputed tomography (µCT) – for detecting disc degeneration induced by 3 different needle sizes.

Methods: Following approval by the institutional IACUC, six C57BL/6J retired breeder mice were anesthetized and in standard aseptic fashion underwent percutaneous, fluoroscopic guided needle puncture at approximately the C6/7 and C8/9 disc levels randomized to either 27G, 29G, or 31G needles (n=4 for each group). The intervening C7/8 level served as a non-injury control (n=6). At 4 weeks post injury, mice were euthanized for post-mortem analyses. Quantitative MRI was performed on mouse tails on a 9.4T scanner. T2 relaxation constant maps were constructed and average T2 relaxation times were calculated as a surrogate for disc composition [2]. One level (mouse 6, 27G injury) was excluded from this analysis due to substantial soft tissue inflammation/destruction that precluded delineating IVD from bone. Whole mouse tails were scanned on a µCT scanner (µCT35; Scanco, Switzerland) at 6 µM resolution (up-scaled to 12 µM for analysis). Volumetric measurements of mean disc height and mean vertebral body height were performed in MatLab. DHI was calculated as the mean disc height/mean vertebral body height [1]. One-way ANOVA was utilized to compare T2 relaxation constants and pairwise height measurements across injury groups and pairwise comparisons were examined using Tukey’s post hoc tests. Significance and trend were defined as p<0.05 and p<0.1, respectively. All statistical analyses were performed using SPSS.

Results: All six mice underwent the disc injury procedure without issue, survived the full four weeks post-operatively, and were subsequently euthanized. Post-mortem MRI revealed a significantly different T2 relaxation times within the nucleus pulposus overall (p=0.01). Specifically, lower average T2 relaxation times (Figure 1) were observed within the nucleus pulposus (NP) in the 27G injury levels (19.7±8.7ms) compared with the intervening control (45.0±10.1ms, p=0.02), 31G injury level (49.2±13.3ms, p=0.01), and 29G injury level (43.1±9.1ms, p=0.05). Figure 2 depicts T2 relaxation population average color maps for each experimental level. Differences in disc height index (Figure 3) trended towards significance across experimental groups (p=0.08). The DHI for 27G injured discs (0.05±0.02) was less than the intervening control, but this only approached significance (0.1±0.004, p=0.08). A similar trend was not observed when the 27G injured disc was compared with the 29G (1±0.02, p=0.16) or 31G injured discs (0.08±0.02, p=0.54). Neither the 29G or 31G injured discs differed from control with regards to DHI. Figure 4 depicts example contour plots for disc height for 29G (A) and 27G (B) injured discs.

Conclusions: The results of this study demonstrate that both quantitative MRI and µCT are able to detect degenerative changes in the mouse caudal disc injury model, but that MRI may be more sensitive than µCT. Both these imaging techniques detect clinically relevant changes (i.e. decreased T2 for MRI and loss of disc height for µCT) present in human disc degeneration. Results also suggest that 27G needle injury represents the threshold for detecting degenerative changes for both of these techniques, which is consistent with previous findings [1]. Needle sizes smaller than 27G may therefore be appropriate for cell delivery to the mouse disc in future therapeutic studies, without inducing significant damage. Ongoing work will correlate imaging findings with disc histopathology, and future studies will apply these imaging techniques to assess the response of the mouse disc to therapeutic intervention.


Acknowledgements: Funding received from the Penn Institute on Aging, the Penn Center for Musculoskeletal Disorders, and the Department of Neurosurgery at the University of Pennsylvania. Technical support received from Dr Stephen Pickup of the Penn Small Animal Imaging Facility.
Biologic Response of Human Nucleus Pulposus Cells to Treatment with Soluble Collagen VI

Gregory D. Schroeder MD, Dessislava Z. Markova PhD, Panya Luksanapruksa MD, Paul W. Millhouse MD, Mayan Lendner BS, Jeffrey A. Rihn MD, Alan S. Hilibrand MD, Alexander R. Vaccaro MD, MBA, PhD, D. Greg Anderson MD, Christopher K. Kepler MD, MBA

1,3Department of Orthopaedic Surgery, Thomas Jefferson University, Philadelphia, PA
2Department of Orthopaedic Surgery, Rothman Institute, Philadelphia, PA
4Department of Orthopedic Surgery, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand


Introduction: Age-related intervertebral disc degeneration is associated with a variety of human ailments, including chronic back pain and disability. It has been shown that disc degeneration is associated with increased cell proliferation and clustering, possibly due to an over-expression of growth factors such as PDGF, IGF-I, bFGF and/or TGF-β stimulating cell proliferation via the ERK and Akt pathways. The IVD pericellular matrix is generally similar to that of other regions, although differentiated by the relative abundance of Collagen type VI, particular in the nucleus pulposus (NP). The presence of Collagen VI has previously been shown to increase cartilage tissue generation as well as cardiac myofibroblast differentiation. The aims of these studies were to determine the role of soluble Collagen VI (COLVI) on human NP cells.

Methods: Real-time q-PCR was used to investigate the expression of collagen, type VI (COL6A1) in NP tissues from degenerate human IVDs. To elucidate COLVI-induced signaling events, we exposed NP cells to soluble COLVI. NP cells were plated in 96-well plates at 1.10⁴ cells per well in 100μl DMEM with 10% FBS culture medium. After 24 h the cells were washed twice with opti-MEM with no FBS (starving medium) for 24h to synchronize cell cycles. After 24 h medium was changed to 1.0% FBS containing 5 or 10µg/ml human soluble ColVI (Corning, Discovery Labware, Inc. USA) and cells was treated for 48h. Proliferation assays were performed using the WST-1 kit (Takara.Bio.INC, Japan). After treatment with 5 μg/ml soluble COLVI for 48h cells were collected, RNA was isolated and gene expression analysis was performed using Human Transcriptome 2.0 Affymetrix chip arrays to identify differential gene expression between untreated and treated with COLVI cells. Total cellular extracts were analyzed by Western blot for phosphorylation and expression of ERK1/2 and Akt.

Results: COL6A1 expression increased with degeneration in human IVD tissue samples. Level of mRNA increased significantly with Pfirrmann grade IV (4.4 fold, p = 0.024) and grade V (5.3 fold, p = 0.014) disc degeneration versus grade III. Soluble COLVI showed a stimulating effect on cell proliferation, the absorbance increased by 1.204 (p < 0.001) at 5ug and 1.355 (p = 0.001) at 10μg versus untreated control cells. Treatment with soluble COLVI induced phosphorylation of ERK½ and Akt. In total, 1,279 expressed genes were upregulated or downregulated by more than 1.5-fold in treated cells. The top ten upregulated genes included: IGF1(7.9 fold), TSPAN2(5.3), GABBR2(5.2), HOMER2(4.9) LGR4(4.9), ANX8(4.7), TNFAIP6(4.5), ADAMTS16(3.7), DOCK10(3.6), GXYLT2(3.6) and highly downregulated genes included: LGR5(-6.7), ISM1(-6.6), DPP4(-5.6), TOX(-4.5), ARHGAP20(4.5), SPTLC3(-4.3), AOX1(-4.1), CAMK2N1(-4.1), GREM1(-3.9), PDGF(-3.9).

Discussion: Results of this study showed that COL6A1 expression levels increase with degeneration of human IVD cells. Treatment with soluble COLVI showed a stimulating effect on cell proliferation which may occur via the ERK1/2 and Akt signaling pathways. Fibrosis is a process characterized mainly by excessive accumulation of collagen and other components of the ECM. Type VI collagen is a key regulator of extracellular matrix composition and fibroblast behavior and plays a role in wound repair and tissue regeneration, and inhibiting the expression of COLVI may be desirable for reducing fibrosis and scar formation. Intervertebral disk degeneration appears to involve a process similar to that of fibrosis in other tissues.

Significance: Atraumatic intervertebral disc degeneration is one of the most common pathologies of the musculoskeletal system, but to date the causes and mechanisms are poorly defined. This study identifies COLVI as a key regulator of fibrosis in the intervertebral disc. Further studies are need to verify this finding and to determine if COLVI may be a target for future therapies.
Correlation between Interlukin-6 Serum Levels and Pre-Operative Health Related Quality of Life Outcome Metrics in Patients Undergoing an Anterior Cervical Discectomy and Fusion for Cervical Radiculopathy.

Gregory D. Schroeder MD1,2, Dessislava Z. Markova PhD1, Paul W. Millhouse MD1, John D. Koerner MD1, Mayan Lendner BS1 Jeffery A. Rihn MD1,2, D. Greg Anderson MD1,2, Alan S. Hilibrand MD1,2, Alexander R. Vaccaro MD, PhD, MBA1,2, Christopher K. Kepler MD, MBA.1,2
1Department of Orthopaedic Surgery, Thomas Jefferson University, Philadelphia, PA, 2Department of Orthopaedic Surgery, Rothman Institute, Philadelphia, PA, 3New Jersey Spinal Medicine and Surgery, Hackensack, NJ

Disclosures: G.D. Schroeder; 5; Medtronic. 6; Medtronic, AOSpine. D.Z. Markova: None. P.W. Millhouse: 4; Globus Medical. J.D. Koerner; 5; Biomet, Medtronic, Pfizer. M. Lendner: None. J.A. Rihn: 3B; Pfizer. 5; DePuy. G. Anderson: 1; DePuy. 3B; DePuy. 4; ISD, PST. 6; Thieme. 7; Thieme. A.S. Hilibrand: 1; Aesculap/B.Braun, Amedica, Biomet. 4; Amedica, Benyeme Medical, Lifespine, Nexgen, Paradigm Spine, PSD, Spinal Ventures, Vertiflex. A.R. Vaccaro: 1; Aesculap/B.Braun, Globus Medical, Medtronic, Stryker. 3B; DePuy, Ellipse, Expert Testimony, Gerson Lehrman Group, Globus Medical, Guidepoint Global, Innovative Surgical Design, Medacorp, Medtronic, Orthobullets, Stout Medical, Stryker. 4; Advanced Spinal Intellectual Properties, Avaz Surgical, Bonovo Orthopaedics, Computational Biodynamics, Cytonics, Dimension Orthotics, Electrocure, Flagship Surgical, FlowPharma, Gamma Spine, Globus Medical, In Vivo, Innovative Surgical Design, Location Based Intelligence, Paradigm Spine, Prime Surgeons, Spine Medica, Spinology, Stout Medical, Vertiflex. 6; Elsevier, Jaypee, Medtronic, Taylor Francis/Hodder and Stoughton, Thieme. 7; Elsevier, Jaypee, Taylor Francis/Hodder and Stoughton, Thieme. C.K. Kepler; 5; Biomet, Medtronic, Pfizer.

Introduction: An anterior cervical discectomy and fusion (ACDF) for persistent cervical radiculopathy leads to significant improvements in Health Related Quality of Life (HRQOL) outcome metrics in up to 90% of patients; however, at the time it is unclear why a small minority of patients do not have relief of their symptoms. Interlukin-6 (IL-6) is an inflammatory cytokine that is increased both systemically and locally in patients with lumbar radiculopathy, and serum levels of IL-6 have been shown to lead to persistent radicular symptoms with surgical or non-surgical care. To date, the role of IL-6 in patients with cervical radiculopathy has not been evaluated. The goal of this study is to determine if either the baseline serum IL-6 levels or the change in IL-6 levels after an ACDF correlates to severity of cervical radiculopathy symptoms.

Methods: Preoperative and postoperative day one serum samples from 11 patients undergoing a one- or two-level ACDF for cervical radiculopathy were collected. The concentrations of IL6 in serum were measured by using commercially available Enzyme linked immunosorbet assay’s (ELISA). Spearman rank correlation coefficient was determined for the preoperative IL-6 concentration and the Short-Form 12 Physical Component Score (SF-12 PCS), Short-Form 12 Mental Component Score (SF-12 MCS), Neck Disability Index, (NDI), Visual Analog Score (VAS) neck pain and VAS arm pain. Similarly the correlation between Interlukin-6 Serum Levels and Pre-Operative Health Related Quality of Life Outcome Metrics

Results: The average HRQOL outcomes and the preoperative and postoperative IL-6 serum concentrations were calculated (Table 1). No significant correlation was found between pre-operative IL-6 serum concentration and SF-12 PCS (-0.436, p = 0.17), SF-12 MCS (0.491, p = 0.12), NDI (0.100, p = 0.76), VAS arm (-0.182, p = 0.58) or VAS neck (-0.40, p = 0.21). A significant negative correlation was found between an increase in the IL-6 serum concentration postoperatively and the baseline SF-12 PCS (-0.624, p = 0.048), but no correlation was found between an increase in IL-6 serum concentration postoperatively and SF-12 MCS (0.152, p = 0.66), NDI (0.394, p = 0.24), VAS arm (0.188, p = 0.58) or VAS neck (0.273, p = 0.43).

Discussion: No correlation between baseline HRQOL outcome metrics and pre-operative IL-6 serum levels were identified, however, a negative correlation between an increase in IL-6 serum concentration postoperatively and SF-12 PCS indicates that people with worse baseline physical function have a significantly increased production of IL-6 in the postoperative phase. Further studies are needed to determine if this significant increase of IL-6 levels correlates with HRQOL outcomes.

Significance: Persistent cervical radiculopathy is one of the common reasons an ACDF is performed, but the role of inflammatory cytokines in this disease process has yet to be evaluated. It is possible that the inflammatory cytokine pathway may play a critical role in the expected results from surgery; further research is needed to determine if the changes in IL-6 levels are predictive of long-term HRQOL outcomes, and furthermore if early intervention may affect outcomes.

Table 1: Average HRQOL outcome metrics and average IL-6 serum concentration

<table>
<thead>
<tr>
<th>SF-12 PCS</th>
<th>SF-12 MCS</th>
<th>NDI</th>
<th>VAS Arm</th>
<th>VAS Neck</th>
</tr>
</thead>
<tbody>
<tr>
<td>33.68 +/- 7.54</td>
<td>48.84 +/- 10.93</td>
<td>42.54 +/- 22/58</td>
<td>6.67 +/- 2.26</td>
<td>6.75 +/- 1.81</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>IL-6 Pre-operative serum concentration</th>
<th>IL-6 Post Operative Day 1 Serum Concentration</th>
<th>Change in IL-6 serum concentration after surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.65 +/- 1.96 ng/ml</td>
<td>23.98 +/- 29.02 ng/ml</td>
<td>22.45 +/-27.45 ng/ml</td>
</tr>
</tbody>
</table>
Serum MMP-9 levels Correlate with the Severity of Intervertebral Disc Herniation in Patients

Timothy Walden1,2, Christopher Filippi2, Aysya N. Khan1, Mitchell Levine2, Beth A. Winkelstein1, Nadeen O. Chahine1,2
1Feinstein Institute for Medical Research, Manhasset, NY, 2Hofstra Northwell School of Medicine, Hempstead, NY, Department of Bioengineering, University of Pennsylvania, Philadelphia, PA. nchahine@northwell.edu

DISCLOSURES: The authors have nothing to disclose

INTRODUCTION: Low back pain (LBP) is a symptom of many diseases, including intervertebral disc (IVD) herniation (DH), spinal stenosis (SS), and degenerative disc disease (DDD). LBP is one of the most common reasons for physician visits in the USA, with direct healthcare expenditures exceeding $100 billion/year [1]. Despite the multiple etiologies for LBP, treatment options overlap and response to treatment varies widely with the patient population. Systemic biomarkers, including matrix metalloproteinases, are a potential way to differentiate between pathologies as well as the severity of disease associated with pain and disability. We have previously shown that serum levels of MMPs vary by diagnosis of LBP, with different levels in DH patients compared to control subjects with no LBP [2]. The aim of this study is to investigate the relationship between DH severity and systemic biomarkers in patients. We hypothesize that increased indicators of tissue remodeling exists systemically in the blood in patients and the levels increase with the severity of disc herniation.

METHODS: Subjects were consented and enrolled into this IRB approved study. Adult subjects were recruited from those undergoing surgery for intervertebral disc herniation (n=41) in the lumbar spine. Blood serum was collected prior to surgical intervention. Severity of herniation was accessed on pre-operative MRI based on degree of herniation. Measurements of herniation (cm) and central canal size (cm) were made by two observers, one of which was a neuroradiologist. The degree of spinal canal intrusion (%) was computed from the ratio of mean herniation relative to the mean canal size, consistent with literature recommendations [5]. Canal compromise of less than one third of the canal at the location of the herniation was considered ‘mild’, between one and two-thirds as ‘moderate’, and greater than two-thirds as ‘severe’ [5]. The MSD 3-Plex Ultra Sensitive assay was performed to measure levels of matrix metalloproteinases MMP-1, MMP-3, and MMP-9 in the serum of each subject. MMP levels were natural log (Ln) transformed, and results from subjects within each severity group were compared using a one-way ANOVA and Bonferroni post hoc test (p<0.05 considered significantly, p<0.1 considered a trend, STATISTICA). In addition, the percent-protrusion of herniation was analyzed relative to MMP levels using Spearman correlations (STATISTICA).

RESULTS: The mean age of subjects was 49 ± 12 years, with 66% of the subjects female and 34% male. The average BMI was 28 ± 6. Twenty four of the subjects (58%) reported chronic pain, with a duration of pain symptoms for 6 months or longer. Of the 41 subjects, 16 (39%) were characterized as having ‘mild’ herniation, 19 (46%) as having ‘moderate’ herniation and 6 (14%) as having ‘severe’ herniation. When analyzing serum levels of MMPs, levels of MMP-1 did not significantly change with DH severity. However, MMP-3 levels were found to be significantly greater in ‘moderate’ vs. ‘mild’ herniation (p=0.04). In comparing DH severity groups, a trend was observed for the effect of severity on MMP-9 levels (p=0.07) (Figure 1). Interestingly, a significant correlation was observed for MMP-9 levels and percent intrusion into the spinal canal (Spearman rho =0.42, p=0.006) (Figure 2). However, no significant correlations were detected between percent intrusion into the spinal canal and either MMP-1 or MMP-3 levels.

DISCUSSION: The goal of this study was to examine systemic levels of degradative biomarkers associated with increasing severity of IVD herniation. These findings reveal higher serum MMP-3 in moderate herniations than in mild herniations. In addition, MMP-9 levels were significantly correlated with increasing severity of IVD herniation. Recent reports have shown MMP-3 to affect the resorption process of herniated IVD tissue, directly through the proteolysis of extracellular matrix components, and indirectly by inducing neoangiogenesis in the periphery of IVDs and by promoting macrophage infiltration [4]. The severity-dependent response observed here is consistent with the systemic level findings of elevated MMP-3 in ‘moderate’ but not ‘severe’ DH groups relative to ‘mild’. Additionally, since the distribution of subjects in the different groups is not homogenous, additional subject recruitment is needed in the ‘severe’ group to improve statistical power. MMP-9 levels have been previously described to be elevated in herniated disc tissue and have been implicated to participate in disc remodeling [3]. Both duration of symptoms and high pain intensity have also been shown to correlate with elevated MMP-9 mRNA levels in disc tissue, with such changes within the tissue being attributed to the extent of extended neovascularization around herniated IVD fragments [4]. Interestingly, while there is increasing work suggesting MMPs have a role in neuronal activity and synaptic remodeling [6], their relationship, and that of MMP-9 in particular, to nerve root compression severity is not fully understood. With continued enrollment and expansion of the patient cohorts, future studies will examine relationship between systemic biomarker levels with DH severity, pain duration, and pain intensity in patients. Using these profiles to clarify pathogenesis and to monitor outcomes may lead to predictive models for targeted management and treatment of LBP caused by DH.

SIGNIFICANCE: This study shows that disc herniation severity is associated with changes in the blood levels of matrix degradation enzymes in patients. These findings extend our previous studies linking local degenerative changes associated with disease to systemic effects that can be measured in the blood. This approach supports the potential for identifying disease biomarkers of DH as a prognostic indicator and for therapeutic interventions.


ACKNOWLEDGEMENTS: NSF CAREER 1151605, NIH R41AG050021, R01AR069668, New York State Department of Health (ECRIP).

Figure 1: Levels of MMP-9 in serum of subjects with DH severity in three categories. A trend effect of severity on MM9 levels was observed (p=0.07).

Figure 2: Serum levels of MMP-9 are significantly correlated with herniation intrusion into the spinal canal (Spearman rho =0.42, p=0.006).
Grow and Differentiate Stem Cells in Macroporous Scaffolds and Potential Application to Intervertebral Disc Repair

Mingkun Wang¹, Chunxiao Chui¹, Mazen Ibrahim², Rebekah Decker², John Lawrence³, Maurizio Pacifici², Li-Hsin Han¹
¹Drexel University, Philadelphia, PA; ²Children’s Hospital of Philadelphia, Philadelphia, PA

ABSTRACT
INTRODUCTION: Tissue regeneration by stem cells provides a tremendous hope for people who suffer from joint injuries including intervertebral disc herniation. The success of stem cell disc regeneration relies on an ideal integration of mechanical and biochemical properties in the surrounding matrix. A proper range of elasticity is needed to promote chondrogenesis via the stem cells' mechanosensing cascades. Recently, growing evidence reveals that the porosity in the matrix is another key element for controlling stem cell fate. Macropores, which are pore spaces with the size no less than the typical diameter of cells (>10 μm), regulate the shape of stem cell by forming contact guidance, which regulates the cells' sensitivity to matrix elasticity by tailoring cytoskeleton morphology, and ultimately regulates the RhoA, Ras, and myosin-mediated mechanosensing signals that control multiple downstream pathways including ROCK, MAPK and PI3K, which in turn regulate stress fiber formation and the activation of nucleic transcriptional factors, and ultimately control cell differentiation. For chondrogenesis, inhibited cell spreading was found to hinder the cytoskeleton reorganization in mesenchymal stem cells (MSC), regulate transcription cofactors activity and promote the formation of hyaline cartilages. On the other hand, our recent study showed larger pores (>100 μm) promotes connective tissue formation via enhanced cell production of collagen and proteoglycan-rich matrix. According to these results, we hypothesize that there is an optimal pore size for chondrogenesis and disc forming. A suitable platform for verifying such hypothesis was lacking, however. Existing 3D platforms including hydrogels, woven meshes, and pre-fabricated scaffolds are limited due to the lack of mechanism to control matrix macroporosity or the difficulty of delivering cells in 3D. To overcome such limitation, we create crosslinkable micro/nanofibers as a novel platform that promotes macropores of customizable sizes ranging from hundreds of microns down to subcellular scale (<1 microns). We verified the existence of an optimal pore size for stem cell-based-like tissue regeneration using such micro/nanofibers.

METHODS: Stretch-and-Fold Method for Fiber Formation. We invented a method to produce micro/nanoscale, crosslinkable fibers (C-fibers) with a widely tunable diameter. We repeatedly folded and stretched a precursor ring that contained a core and a sheath compartments (Fig. 1A-F). The core contained hydrated gelatin, and the sheath was made of a solvent-soluble polymer that kept the cores separated. The stretch-and-fold cycles (n) increased the cores number (N) exponentially (N = D0/2^n) while decreasing the core diameter exponentially (D = D0/2^n) (Fig. 1G-J). 26 stretch-and-folding cycles, for example, turns a 2-mm gelatin core into 67,108,864 parallel fibers and 250nm average diameter (Fig. 1H). C-fibers were retrieved by acetone leaching, aldehyde-fixed, methacrylated, dialyzed and freeze-dried for storage. Experiment-I: We first examined the effect of large macropores. Cellularized scaffolds containing 100 to 200 μm-sized pores were prepared by encapsulating human mesenchymal stem cells (MSC) (20M/cm³ density) by 50μm-wide C-fibers (7.5 wt%) (Fig. 2E). To examine the effects of porosity, a control group was prepared by encapsulating MSC in hydrogels made of methacrylated gelatin, which formed 1–2μm-sized pores that were much smaller than MSC (~15 μm) (Fig 2F). Scaffolds were cultured with chondrogenic medium under 37°C and 5% CO2. Samples were collected on day 1 and 21 for analysis. Experiment-II: Next, we examined the effect of intermediate pore size by repeating Experiment-I using 4 μm and 25 μm C-fibers (prepared by 14 vs. 20 stretch-and-fold cycles), which formed 10–20μm and 100–200μm pores, respectively. In experiment II, we fixed the cell density from 20 to 10 million/cm³, in order to exclude or minimize the effect of cell-cell contacts, which is known to promote chondrogenesis via the activation of HIPPO signaling. All groups have repeated N > 3.

RESULTS
SECTION: (1) Macropores facilitate ECM formation. The 100–200 μm pore scaffolds exhibited dramatically increased compressive moduli (Δ ≈ 260 kPa) on day 21 vs. day 1, while the 1–2μm pore samples had only slightly increased moduli (Δ ≈ 15 kPa) (Fig 2A). DNA and biochemical analyses showed twice as many cells per gram and 2.5-times as much glycosaminoglycan (GAG) per gram in the C-fiber scaffolds in comparison with in the hydrogels (Fig 2B). Examination of the spatial distribution of GAG, aggrecan (Agg) and type-II collagen (Col-II) on the day-21 scaffolds showed that these ECM components formed interconnected networks through the 100–200 μm pore samples (Fig 2GJ), but formed only pericellular precipitates in the 1–2μm pore samples (Fig 2HJ). Material mechanics theory reveals that the observed morphology of ECM was responsible for the dramatic difference in bulk stiffening, as Collagen and GAG may dominate the bulk stiffness by extending across the bulk volume. (2) Cell morphology determines cell phenotype. The 100–200 μm pores led to spreading cell morphology resembling fibroblasts (Fig 2K). In contrast, the 1–2μm pores resulted in round cell morphology resembling mesenchymal stem cells (MSC) (Fig. 2G). In Fig. 2K, we show examples of stem cells (MSC) forming interconnected networks through the 100–200 μm pore samples (Fig. 2GJ), but formed only pericellular precipitates in the 1–2μm pore samples (Fig 2HJ). Material mechanics theory reveals that the observed morphology of ECM was responsible for the dramatic difference in bulk stiffening, as Collagen and GAG may dominate the bulk stiffness by extending across the bulk volume. The success of stem cell disc regeneration relies on an ideal integration of mechanical and biochemical properties in the surrounding matrix. A proper range of elasticity is needed to promote chondrogenesis via the stem cells' mechanosensing cascades. Recently, growing evidence reveals that the porosity in the matrix is another key element for controlling stem cell fate. Macropores, which are pore spaces with the size no less than the typical diameter of cells (>10 μm), regulate the shape of stem cell by forming contact guidance, which regulates the cells’ sensitivity to matrix elasticity by tailoring cytoskeleton morphology, and ultimately regulates the RhoA, Ras, and myosin-mediated mechanosensing signals that control multiple downstream pathways including ROCK, MAPK and PI3K, which in turn regulate stress fiber formation and the activation of nucleic transcriptional factors, and ultimately control cell differentiation. For chondrogenesis, inhibited cell spreading was found to hinder the cytoskeleton reorganization in mesenchymal stem cells (MSC), regulate transcription cofactors activity and promote the formation of hyaline cartilages. On the other hand, our recent study showed larger pores (>100 μm) promotes connective tissue formation via enhanced cell production of collagen and proteoglycan-rich matrix. According to these results, we hypothesize that there is an optimal pore size for chondrogenesis and disc forming. A suitable platform for verifying such hypothesis was lacking, however. Existing 3D platforms including hydrogels, woven meshes, and pre-fabricated scaffolds are limited due to the lack of mechanism to control matrix macroporosity or the difficulty of delivering cells in 3D. To overcome such limitation, we create crosslinkable micro/nanofibers as a novel platform that promotes macropores of customizable sizes ranging from hundreds of microns down to subcellular scale (<1 microns). We verified the existence of an optimal pore size for stem cell-based-like tissue regeneration using such micro/nanofibers.

DISCUSSION: Our results verified the existence of optimal matrix pore size for stem cell-based chondrogenesis. This optimal pore size may simultaneously promote stem cell chondrogenic differentiation and facilitate intervertebral disc matrix production. We enabled the above study by creating a novel biomaterials, the micro/nanoscale C-fibers using a Stretch-and-Fold method.

SIGNIFICANCE: This research aims to provide a novel tissue-engineering platform that may one day realize the ultimate goal of complete intervertebral disc repair. If successful, numerous patients with injured and degenerative discs can benefit from the 3D macroporous scaffolds.

![Fig. 1 Stretch-and Fold Method for Micro/nanoscale C-fiber preparation. Scale bars for G and H: 100 μm.](Image)

![Fig. 2 Verifying the effects of matrix porosity on Chondrogenesis. Scale bar in G,H,M,N: 100μm. Staining in K,L: blue, cell nuclei; green, microtubules; red, actin filament.](Image)
How Does Rod Diameter and Material Affect Motion and Stress Concentrations in Lumbopelvic Reconstructions?
-A Finite Element Analysis

Wenhai Wang, PhD; Mark Moldavsky, MS; Brandon S. Bucklen, PhD; Bryan W. Cunningham, PhD; Sigurd H. Berven, MD

1Musculoskeletal Education and Research Center, Globus Medical Inc., Audubon, Pennsylvania
2 Department of Orthopedic Surgery, University of Maryland Medical System; Baltimore, Maryland
3 Department of Orthopedic Surgery, University of California San Francisco Medical Center; San Francisco, California

INTRODUCTION: Implant material selection has been reported to influence instrumentation complications in adult spinal deformity surgery. Clinically, iliac screws with smaller diameter rods have been identified as potential risk factors for rod fractures. Clinical concerns still remain regarding the failure mode and potential stress risers in posterior lumbopelvic reconstructions. The current investigation uses a computational model to compare segmental motion and rod stress/strain of three different rod materials, and two diameters, in lumbopelvic reconstructions.

METHODS: A lumbopelvic finite element model (L1-Pelvis) was developed and validated with cadaveric range of motion (ROM) data. Vertebral segments were modeled as three-dimensional solid elements. Intervertebral discs, including nucleus and annulus fibrosis, were structured as hyperelastic materials. The sacroiliac joint was modeled as articular cartilage contacts surrounded by six types of strong ligaments, which were depicted as spring elements. Bilateral L1-S1 rigid (Sacral), right L1-S1 and left L1-pelvis rigid (Unilateral Iliac), and bilateral L1-pelvis rigid (Bilateral Iliac) constructs were compared using three rod materials of differing modulus of elasticity (E): titanium (E=113.8 GPa), cobalt chrome (E= 241 GPa) and stainless steel (E=196 GPa). Both 5.5mm and 6.35mm diameter rods were simulated. A ±12.5 N-m moment was applied at the L1 superior endplate to model flexion-extension. ROM and stress/strain at the lumbosacral and sacroiliac junctions were compared.

RESULTS: The effect of rod diameter on simulated strain was minimal at the L5-S1 level. However, the sacroiliac junction experienced less stress when a 6.35mm rod was used. This effect was consistent across all materials and constructs. The construct itself appeared to have a greater effect than rod diameter on stresses within rod, at both measured points. Titanium exhibited the highest stresses, on average, while CC spinal rods reduced stress across analogous constructs/diameters (Ti > SS > CC). The unilateral iliac group with titanium had the highest rod strain, which was reduced by adding a secondary rod (bilateral group), or by not extending across the SI-junction. However, the role on screw loosening was not investigated.

DISCUSSION: Rod stress and strain under flexion and extension was shown to depend on material, rod diameter, and construct choice, but most importantly on construct choice. Cobalt chrome rods achieved the highest level of rigidity with the least rod deformation. When extending across the sacroiliac joint, a unilateral connector rod resulted in higher rod stresses than using bilateral connectors. Clinical failure could be mitigated by increasing the rod diameter or using a material with a higher modulus.

SIGNIFICANCE: This study provides insight into which factors are important that affect the motion and stress concentrations in lumbopelvic reconstructions.
Overexpression of Human Interleukin (IL)-8 in Mouse Intervertebral Disc Tissue to Model Patients with Back Pain


Introduction: The etiology of axial back pain has been intensively investigated. We have previously shown that inflammatory mediators found in annulus fibrosus (AF) tissues from patients with discogenic back pain are likely produced by intervertebral disc (IVD) cells, and may play a key role in back pain. Among the chemokines identified, IL-8 is the most inducible in vitro: following IL-1β stimulation, IL-8 mRNA expression increased over 20,000 fold in NP and AF cells, while protein released increased by over 1,000 fold. To investigate the molecular mechanism of IL-8 signaling in the development of disc degeneration in vivo, we have generated a conditional IL-8 transgenic (Tg) mouse model. Our aim was to demonstrate that IVD cells produce interleukins that may, at least in part, be responsible for pain generation.

Methods. Tissue culture studies: IVDs from human spine segments (donor age range 21-75y), procured by the Gift of Hope Human Donor and Tissue Network of Illinois, were used with an approved IRB protocol. IVDs were dissected and AF and nucleus pulposus (NP) were separated. Cells were isolated by sequential enzymatic digestion of disc tissue and plated in 12- or 24-well plates. NP and AF cells were cultured in monolayer, with DMEM/F12 medium with 10% or 20% FBS, until they reached confluency. Cells were then serum-starved for 24 hours and subsequently stimulated with IL-1β (10 ng/mL) for 24 hours. IL-8 gene expression was analyzed using real-time PCR. IL-8 protein in the conditioned media was analyzed using enzyme-linked immunosorbent assay (ELISA). Cytokine array analysis: AF tissues were collected from patients undergoing spinal surgeries at Thomas Jefferson University, with an approved IRB protocol. Detailed discography scores and MRI grades were also recorded. Protein was extracted from the tissue and used to probe human cytokine array membranes. Cytokine profiles of painful and non-painful IVDs of the same MRI grades were compared. Transgenic mouse generation: pCALL2 plasmid was used to construct human IL-8 transgene. pCALL2-IL-8 mice were then bred with GDF5- Cre mouse (generously provided by David Kinsley, Stanford University) to conditionally express the transgene in cartilage and intervertebral disc tissues. Transgene expression was confirmed with PCR and IL-8 ELISA. Mouse behavioral testing was performed with Laboras (laboratory animal behavior observation, registration and analysis system, Metris®), a fully automatic and non-invasive system, to record more than 18 spontaneous behaviors.

Results. Following IL-1β stimulation, IL-8 gene expression increased 26,541 fold in NP cells (n=4, p=0.0083) and 22,429 fold in AF cells (n=4, p=0.0105). IL-8 protein released by the NP cells in response to IL-1β treatment also increased, from 31pg/ml to 74,056pg/ml, a 2,388 fold increase (n=4, p=0.0004). Similarly, IL-8 protein released by the AF cells increased, from 53pg/ml to 94,540pg/ml, a 1,784 fold increase (n=4, p=0.0055). IL-8 protein concentration in the AF tissues from patients with axial back pain is 1.8 fold of that in patients undergoing surgery for reasons other than back pain (e.g., scoliosis). But, due to the large individual variation, the difference is not statistically significant (p= 0.19). At 12 weeks of age, male Tg mice with IL-8 over-expression induced by breeding with GDF5-Cre mice showed a trend of decrease in ambulation and grooming (n=7, P=0.08), while female mouse behavior did not differ significantly.

Conclusion. We have shown that cultured IVD cells produce a massive amount of IL-8 in response to IL-1β stimulation, and generated a Tg mouse line to overexpress IL-8. We have preliminary evidence that male mice ambulate and groom less than negative controls. We will continue to characterize mouse behavior with a larger sample number, and will examine inflammatory cell infiltration and joint and spine tissue morphology.
Sponsor Information

Globus Medical, Inc. is a leading musculoskeletal implant manufacturer and is driving significant technological advancements across a complete suite of spinal products. Founded in 2003, Globus’ single-minded focus on advancing spinal surgery has made it the fastest growing company in the history of orthopedics. Globus is driven to utilize superior engineering and technology to achieve pain-free, active lives for all patients with spinal disorders.

More information at globusmedical.com

NuVasive, Inc. is a world leader in minimally invasive, procedurally integrated spine solutions. From complex spinal deformity to degenerative spinal conditions, NuVasive is transforming spine surgery with innovative technologies designed to deliver reproducible and clinically proven surgical outcomes. NuVasive’s highly differentiated, procedurally integrated solutions include access instruments, implantable hardware and software systems for surgical planning, and reconciliation technology that centers on achieving the global alignment of the spine.

More information at nuvasive.com