Virtual Poster Session: **Spine/OA/Basic Science** | **Friday June 12, 2020** 1:30 - 2:50pm EDT
https://vimeo.com/showcase/7214866

29| Josh K Briar- Wilfrid Laurier University; Masters (In progress or completed)

"Effects of Delamination Rate on the Adhesive Properties of the Interlamellar Matrix"

Introduction: Low back pain is the leading cause of disability worldwide, and degeneration of the intervertebral discs (IVD) interspacing vertebrae in the spine, is one of the most prevalent causes of this issue. The intervertebral disc is a complex structure, but recent evidence suggests that separations between fibrous layers of the outer annulus may contribute to degeneration's development in the spine. The purpose of the present experiment was to examine annular separation and quantify the rate-dependent mechanical response of the layer-adjoining interlamellar matrix (ILM).
Methods: Twelve IVDs were dissected from four bovine tails (three IVD per tail). Two multi-layered (approximately 10 layers) AF samples were collected from each IVD (total=24, mean bond width=3.82±0.96mm) and randomly assigned to three rate-controlled delamination groups; 0.05mm/sec, 0.5mm/sec, 5mm/sec. Samples were initially manually delaminated between the two most middle layers and then subjected to a 180˚ peel test to mechanically propagate delamination. Lamellar adhesion force was determined by finding the mean value of the plateau region of the force-displacement curve of each sample and normalized by dividing by the sample bond width.
Results: Tissues were found to have similar mean adhesion strengths ($p=0.385$) and stiffness ($p=0.97$) across all conditions. A significant difference in lamellar adhesion strength variability was observed between the 5mm/sec condition when compared to both the 0.5mm/sec and 0.05mm/sec conditions ($p<0.05$).
Conclusion: The results indicated that the viscoelasticity of the annulus did not have a significant effect on the strength or stiffness of the ILM under the current rates. However, there was increased adhesion strength variability at increased delamination rates, which may suggest that the distribution of force between adjacent layers may be non-uniform at different rates of stress.

34| Mitch C Whittal- Wilfrid Laurier University; Masters (In progress or completed)

"Mechanics of Induced Intervertebral Disc Degeneration in SPARC-Null Mice"

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Background. Intervertebral disc (IVD) degeneration is accompanied by mechanical changes to the spine. Secreted protein acidic and rich in cysteine (SPARC) is a protein that regulates homeostasis of the extracellular matrix. SPARC-null mice display accelerated IVD degeneration and behavioural signs of pain; serving as a model for painful human lumbar IVD degeneration [1,2].
Purpose. Examine SPARC-null mouse axial spine mechanics as compared to wild-type (WT) mice.
Methodology. SPARC-null (n=36) and WT (n=18) mice aged 14-25 months were dissected and subjected to mechanical testing. Excised lumbar spines were tested in cyclic axial tension and compression to determine neutral zone (NZ) length and stiffness for both SPARC-null and WT mice. Three mechanical tests were completed for each spine to further determine if there was an effect of the number of IVDs being tested in series (one vs two vs three IVDs).
Results. SPARC-null spine NZs were 26.5% stiffer ($p < .001$) and 10.7% smaller in length ($p < .001$) than WT spines. Collapsed across condition (SPARC-null and WT), NZ length increased as the number of IVDs tested in series increased. Correlation analysis revealed a weak negative correlation ($r = -.24$, $p = .02$) between age and NZ length in SPARC-null mice and a weak positive correlation ($r = .30$, $p = .03$) between age and NZ stiffness in WT mice.
Significance. Increased NZ stiffness above the physiological norm indicated that the SPARC-null model exhibited typical mechanics of late-stage degeneration [3].

References

[1] Elliott, D. M., et al. (2004). Spine, 29(7), 713–722.
[2] Krock, E., et al. (2018). Osteoarthritis and Cartilage, 26(9), 1236–1246.
[3] Panjabi, M.M. (2003). J Electromyogr Kinesiol, 13, 371–9.

37| Grant Dickey- Western University; Masters (In progress or completed)

"Understanding Neck Neuromuscular Response during mild Traumatic Brain Injury"

Background: Mild Traumatic Brain Injuries (mTBI) are reported to affect neuromuscular response besides direct neuronal cell damage. How the mTBI-affected muscles influence future mTBI risk remains unclear. Moreover, recent literature indicates that muscular studies have focused specifically on the superficial cervical muscles, rather on all components of the cervical region. The novelty of our study is that we used a numerical approach for comprehensively analyzing the responses of the entire cervical region (spinal column [C1-C7], ligaments/tendons/muscles), and brain response during relaxed-state concussive impacts. We hypothesized that not only the muscles but the whole cervical region could have an influence on the risk of TBI.
Methods: Using the validated GHBMC head and neck model alongside a pneumatic impactor with an initial velocity of 3 m/s, we simulated 7 mTBI impact conditions. We analyzed internal energy absorption for 26 cervical muscles, cervical spinal column (C1-C7), 13 ligaments, and tendons.
Results: Our results demonstrated that brain response varied significantly depending on impact location. On average, we found that the cervical spinal column, including ligaments/tendons, contributed to 66.0% (standard deviation 6.9%) energy absorption, which was greater than muscle contribution. In the lateral direction of impact, cervical muscles contributed to the minimum energy absorption compared to other directions.
Significance: Our results highlighted that the cervical spine is equally as important as the cervical muscles in regard to brain response. When developing muscle-related mitigations to reduce mTBI risk, the consideration of ligament/bone response is needed in addition to the traditional focus on muscles. Moreover, direction-specific muscle contribution is recommended and more comprehensive studies on this aspect are needed.

52| Jeffrey L Hutchinson- Western University; Masters (In progress or completed)

"Effect of growth hormone and anabolic steroids on the intervertebral disc"

The most recent Global Burden of Disease study reported that low back pain is the leading cause of years lived with disability worldwide, with a lifetime prevalence of up to 84%. Back pain affects ~632 million people globally, causing socioeconomical impact estimated at $100 billion annually. IVD degeneration (IVDD), defined as changes in cellularity and matrix composition resulting in structural failure, is believed to be the major contributor to low back pain in ~40% of cases. Currently the only treatments are physiotherapy and pain management. A recent case study demonstrated that anabolic steroid (AS) and growth hormone (GH) injections decreased incidence and severity of patient self-reported back pain. Moreover, clinical observations suggest that suspected AS use in athletes is associated with increased IVD height. To date however, there are no investigations exploring how AS or GH affect the IVD biology. GH and AS doping is common in competitive athletes, with anecdotal evidence suggesting decreased pain and fat mass, promotion of muscle growth and recovery times, further suggesting potential benefits. The current study was designed to directly assess the effects of GH and AS on the IVD both in-vitro and in-vivo. We first adopted an ex-vivo murine IVD-explant organ model system to assess direct effects of GH and AS on IVD cell biology. Organ cultures were incubated with increasing concentrations of GH, AS, or both for up to 7 days. Preliminary histological analysis suggest that AS and GH exposure induces hypertrophy and cell proliferation respectively. Subsequent studies will further quantify effects using molecular, histological, and biochemical analysis of markers of IVD health. This will be complimented by an in-vivo murine model assessing changes in IVD height, hydration, and biomechanical properties upon GH and AS exposure. Together these studies will explore effects of GH and AS on IVD biology and its potential as a therapeutic intervention for IVDD.

63| Yiming Lin- Western University; Masters (In progress or completed)

"Characterization of Microvasculature in Duchenne Muscular Dystrophy"

Introduction: Duchenne muscular dystrophy (DMD) is an x-linked neuromuscular disease characterized by progressive muscular degeneration. In DMD, angiogenesis appears to be attenuated, leading to ischemia and diminished endogenous muscle repair. Two angiogenic factors are angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2). Ang-1 is an agonist for the endothelial cell receptor Tie-2, leading to downstream pro-survival PI3K/Akt pathway activation. Ang-2 is known as a Tie-2 antagonist in the presence of Ang-1. The ratio between the two may be irregular within dystrophic tissue, resulting in an aberrant microvasculature.
Hypothesis: We hypothesize that Ang/Tie2 signaling is abnormal in DMD and that vessel maturation/stability is abnormal in DMD.
Materials and Methods: Muscle tissues (gastrocnemius and diaphragm) from our DMD mouse model will be collected at several time points representing the development of fibrosis. Immunoblots will be used to assess the expression of Angiopoietin/Tie-2 factors and downstream signaling markers. Intravital multiphoton microscopy to assess vasculature density within the tissue.
Results: Our preliminary results show no significant decreases in the concentration of Ang-1 in diaphragm tissue of the disease model. Additionally, no significant increases in Ang-2 were observed. Interestingly, when observing individual ratios of Ang-1/Ang-2, 8 week-old DMD mice models had significantly lower Ang-1/Ang-2 ratio compared to healthy wildtypes.
Discussion and Conclusions: The decrease in Ang-1/Ang-2 may account for many of the vascular deformities noted in DMD. Indeed, we hope to visualize the vascular density and observe changes in vessel morphology. Characterization of the microvasculature is vital and may lead to new therapeutic interventions.

66| Joseph U. Umoh- Robarts Research Institute; Mid Career Researcher (reviewed for acceptance but not eligible for an award)

"The Determination of the Relationship Between Visceral Adipose Tissue and Whole-Body Adipose Tissue in Rats and Mice Using Micro-CT "

Background: Adipose tissue stores energy and provides insulation for some organs. However, the accumulation of excess visceral adipose tissue is linked to different risks of obesity or diabetes. Research interest in adipose tissue is growing not only because investigators are seeking solutions to the problem of obesity but also because of the realization that adipose tissue in human is a potential reservoir of adult stem cells, which could be used in tissue repair and engineering.
Rationale: Clinical research in adipose tissue often begins in rat and mouse models. A study showed that male mice of the same age and on the same diet as females, tend to weigh more than female. In this study, the mass of visceral adipose tissue (VAT) in the abdominal cavity and whole-body adipose tissue (WAT) were measured from micro-CT scans in rats and mice. While WAT measurement is easily automated, VAT quantification requires manual segmentation and is time consuming.
Objectives: Our objectives are to determine an empirical relationship between VAT and WAT in both rat and mouse models, and to investigate whether the relationship is different in the male and female rats and mice. The relationships will enable VAT to be easily computed from WAT.
Methods: The mass of WAT and VAT were computed in 91 rats and 80 mice (male and female). The data were grouped into male and female rats as well as male and female mice. A regression analysis was applied on the data to determine their relation.
Results and Conclusions: This study has determined a linear relationship between the visceral and whole-body adipose tissue, for both rats and mice, but the model parameters are different for male and female rats, as well as for male and female mice. This relationship could be used to predict the mass of visceral adipose tissue using whole-body adipose tissue, in both male and female rats and mice. The formulae could be applied in obesity studies involving rat or mouse model.

0| Ghazaleh Tavallaee- Krembil Research Institute; PhD (In progress or completed)

"MiR-27b-3p regulates key synovial extracellular matrix components during osteoarthritis"

Background: Synovial fibrosis, characterized by excessive deposition of extracellular matrix (ECM), is an important contributor to osteoarthritis (OA)-related joint destruction. We have previously shown microRNA(miR)-27b-3p is increased in the synovial fluid of advanced radiographic knee OA patients.
Purpose: To determine the expression pattern, function and signalling of miR-27b-3p in the OA synovium.
Methodology: In situ hybridization was used to visualize miR-27b-3p expression in human synovium and surgically induced-OA mouse knee joints. miR-27b-3p mimic-transfected OA fibroblast like synoviocytes (FLS) were subjected to ECM qPCR array and RT-qPCR validation. Immunohistochemistry was used to localize ECM proteins in the mouse knee joints. RNA sequencing and integrative computational analysis were performed to elucidate miR-27b-3p signalling pathway. The role of miR-27b-3p-modulated genes was investigated by siRNA transfection.
Results: miR-27b-3p expression is increased in OA FLS and synovial lining of advanced knee OA patients concomitant with enhanced ECM deposition, and in the synovial lining and fibrocartilage of mouse OA knee joints. miR-27b-3p mimic increased OA FLS migration, and transcript and protein levels of type I collagen (COL1A1), the major ECM component of the synovium. Array screening and validation identified marked elevation of COL1A1, COL5A1, COL14A1, fibronectin (FN1), and a disintegrin and metalloproteinase with thrombospondin motifs-8 (ADAMTS8) in OA FLS. COL5A1, COL14A1, and ADAMTS8 showed similar localization patterns to miR-27b-3p in the mouse knee joints. RNA sequencing analysis revealed ECM-related putative targets of miR-27b-3p in OA FLS including ADAMTS8. ADAMTS8 siRNA attenuated miR-27b-3p-induced upregulation of COL14A1, COL5A1 and FN1, indicating a contribution of ADAMTS8 to regulation of miR-27b-3p-modulated ECM genes.
Impact: miR-27b-3p may play an essential role in the regulation of key synovial ECM components during OA.

93 | Helal Endisha- Krembil Research Institute; Post-Doctoral Fellow

"MicroRNA-34a-5p Promotes Joint Destruction during Osteoarthritis"

BACKGROUND: MicroRNA-34a-5p (miR-34a-5p) expression is increased in the synovial fluid of late compared to early-stage radiographic knee OA patients; however, its exact role, signaling, and therapeutic potential in OA remains to be fully elucidated. We hypothesize that miR-34a-5p contributes to knee OA pathophysiology and could serve as a therapeutic target.
METHODS: Experiments were conducted with miR-34a-5p mimic and antisense-oligonucleotide (ASO) in human OA chondrocytes and fibroblast-like synoviocytes (FLS) (in vitro) and mouse OA models (in vivo), including a severe high-fat diet (HFD)-induced OA mouse model. miR-34a-5p-knock-out (KO) mice were subjected to destabilization of the medial meniscus (DMM) surgery to induce OA. Wild-type (WT) and KO mouse chondrocytes were subjected to RNA-sequencing and computational analysis.
RESULTS: MiR-34a-5p expression was increased in plasma, articular cartilage and synovium of late-stage knee OA patients compared to healthy controls and early-stage knee OA patients, respectively, as well as articular cartilage and synovium of DMM-surgery mice. Plasma miR-34a-5p expression was elevated in obese versus non-obese late-stage knee OA patients, and in plasma and knee joints of HFD-induced obese mice. In human OA chondrocytes and FLS, miR-34a-5p mimic increased OA pathology markers, while miR-34a-5p ASO improved cellular gene expression. Intra-articular injection of miR-34a-5p mimic in mouse knees induced an OA-like phenotype. Conversely, intra-articular miR-34a-5p ASO injections imparted cartilage-protective effects in moderately severe (DMM) and severe (HFD+DMM) surgical mouse models of OA. miR-34a-KO mice exhibited substantial protection from surgery-induced cartilage damage. Finally, RNA-sequencing of WT and KO chondrocytes revealed a putative miR-34a-5p signaling network.
CONCLUSIONS: This study, for the first time, provides comprehensive evidence on the role and therapeutic potential of targeting miR-34a-5p in knee OA.