
# Abstract book


# 24th Canadian Connective Tissue Conference

May 23 – 25, 2018 | Toronto, ON

## The Canadian Connective Tissue Conference 2018

It is with great pleasure that we welcome you to the 24th Annual Canadian Connective Tissue Conference (CCTC) 2018. The 2018 CCTC is hosted at the Ontario Institute for Studies in Education of the University of Toronto (OISE/UT).

This year's conference includes national and international researchers as invited speakers. Highlights of this year's program include scientific sessions on: Skin and Matrix Biology, Bone and Developmental Biology, Cartilage and Intervertebral Disc Biology**,** Novel Therapeutic Strategies in Connective Tissue Disease, Bioengineering, Artificial Intelligence and Computational Biology, and Tissue Fibrosis with the visionary talk on bridging Translational Research in Canada.

The program also features oral presentations and 3-minute elevator pitch from selected submitted abstracts, as well as two poster sessions. Various prizes will be awarded for the best trainee oral, 3-minute elevator pitch and poster presentations.

The CCTC 2018 organizing committee members look forward to seeing you all in Toronto in May 2018.

Prof. Saeid Amini Nik
University of Toronto

Prof. Mohit Kapoor
University of Toronto

CCTC co-chair

# The Canadian Connective Tissue Society



Dear Colleague's and Friends,

Welcome to Toronto and the 24th Canadian Connective Tissue Conference. Yearly conferences have been held since 1994 and are now the flagship of the Canadian Connective Tissue Society, which was founded in 2012. This annual conference brings together leading researchers in Canada working in the field of connective tissues including bone, cartilage, cardiovascular tissues and skin.

The Canadian Connective Tissue Conference was established primarily to bridge the gaps in our scientific and clinical knowledge of connective tissues. It has encouraged and motivated the next generation young Canadian scientists by providing them with an opportunity to present their research as well as to interact with leading Canadian and international researchers in this field.

This year's organizing committee, chaired by Professor's Saeid Amini Nik and Mohit Kapoor, has put together an outstanding program that promises once again to encompass basic research, translation and clinical application. Without a doubt, I believe this program will interest both trainees and experienced researchers alike.

On behalf of the board it is my pleasure to welcome you to Toronto

President, Canadian Connective Tissue Society

## Organizing Committee

### Co-Chairs

Dr. Mohit Kapoor
Dr. Saeid Amini-Nik

### Event Coordinators

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Dr. Poulami Datta
Helal Endisha
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Dr. Irina Voronov (Chair, Abstract Review Committee)

***CCTC-2018 would like to thank following experts for reviewing the abstracts:***

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## Venue: Earth Sciences Centre - ES

5 Bancroft Avenue, TORONTO, ON, Canada, M5S 3B3

# Contents

# SCIENTIFIC PROGRAM

## Day 1: Wednesday May 23, 2018

Earth Sciences Centre, University of Toronto
5 Bancroft Avenue, Toronto, ON M5S 3J1

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|---|---|
| **5:15 – 6:15pm** | CCTS Board Meeting |
| **6:00 – 9:00pm** | **Registration & Opening Reception** |
| **6:00 – 6:30pm** | Registration<br>*Location: Auditorium Lobby* |
| **6:30 – 6:45pm** | Opening Remarks: **Lisbet Haglund** |
| **6:45 – 7:15pm** | Invited Speaker: **Sandeep (Sonny) Kohli**<br>*The future of health care delivery: the convergence of exponential technologies* |
| **7:15 – 9:00pm** | **Reception: Food and Drinks** |
| **End of Day 1** | |

# SCIENTIFIC PROGRAM

## Day 2: Thursday May 24, 2018

Location: Auditorium & lobby

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| **7:45 – 8:30am** | **Breakfast**<br>*Location: Auditorium Lobby* |
| **8:30 – 8:45am** | Welcome Remarks |
| **Session I: Skin and Matrix Biology - Health and Disease**<br>*Co-chairs: Anie Philip and Afsaneh Alavi* | |
| **8:45 – 9:15am** | Invited Speaker: **Vincent Piguet**<br>*Dendritic Cells in Skin Diseases* |
| **9:15 – 10:10am** | Selected abstracts for oral presentation |
| **9:15am** | Invited Abstract: **Aziz Ghahary**<br>*Trans-differentiation of Immune Cells into Skin Cells in Large Skin Injuries* |
| **9:30am** | **Heena Kumra:** *Molecular interaction between elastogenic proteins impacting matrix assembly and function* |
| **9:40am** | **Maria Zena Miranda:** *Regulation of TAZ expression by chemical and mechanical fibrogenic stimuli* |
| **9:50am** | **Shruthi Venugopal:** *Pro-fibrotic cytokines induce the RhoA exchange factor GEF-H1 through a cytoskeleton-dependent self-regulatory cycle: possible role in epithelial-mesenchymal transition* |
| **10:00am** | **Nazihah Bakhtyar:** *Exosomes Enriched In The Wharton's Jelly Of The Human Umbilical Cord Enhances Skin Wound Healing* |
| **10:10 – 10:25am** | **Coffee Break**<br>*Location: Auditorium Lobby* |
| **Session II: Bone and Developmental Biology - Health and Disease**<br>*Co-chairs: Morris Manolson and Marc Grynpas* | |
| **10:25 – 10:55am** | Invited Speaker: **Jeffrey Dixon**<br>*Unraveling the complex network of purinergic signaling in bone* |
| **10:55 – 11:35am** | Selected abstracts for oral presentation |
| **10:55am** | **Lina Saad:** *Osteoclastogenesis is modulated by the phosphatase Inpp4b through the PKCβ/GSK3β/Nfatc1 pathway* |
| **11:05am** | **Elie Akoury:** *Zoledronic acid-loaded silica nanoparticles to treat cancer-induced bone metastasis* |
| **11:15am** | **Boraschi-Diaz:** *Mechanisms underlying increased osteoclastogenesis in the mouse model of osteogenesis imperfecta due to mutation in collagen type I* |

# SCIENTIFIC PROGRAM

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| **11:25am** | **Garthiga Manickam:** *The role of SMPD3 in skeletal tissues during fracture healing* |
| **Session III: Bridging Translational Research in Canada**<br>*Chair: Svetlana Komarova* | |
| **11:35am - 12:05pm** | Invited Speaker: **James Jaquith**<br>*Zucara Therapeutics:* Centre For Drug Research and Development (CDRD)*/MaRS Innovation* |
| **12:05 – 1:20pm** | **Lunch and Poster Presentation Session 1**<br>*Lunch Location: Auditorium Lobby*<br>*Poster Presentations will take place from 12:45-1:15 in lobby* |
| **12:20 – 1:20pm** | CCTS Board Meeting |
| **Session IV: Cartilage and Intervertebral Disc Biology - Health and Disease**<br>*Co-chairs: Frank Beier and Stephane Roy* | |
| **1:20 – 1:50pm** | Invited Speaker: **Frank Barry**<br>*Stromal Cell Therapy for Arthritic Diseases: Progress and Obstacles* |
| **1:50 – 2:30pm** | Selected abstracts for oral presentation |
| **1:50pm** | **Fackson Mwale:** *Link N retards disease progression in a rabbit model of osteoarthritis* |
| **2:00pm** | **Daniel G. Bisson:** *Role of Toll-like receptor 2 in degenerating articular cartilage* |
| **2:10pm** | **Mable Chan:** *Modulation of inflammation and cartilage degradation using ex vivo polarized monocytes/macrophages within a human osteoarthritic joint explant model* |
| **2:20pm** | **Michael P. Grant:** *Tungsten accumulation in the intervertebral disc regulates degeneration and markers of discogenic pain* |
| **Session V: Novel Therapeutic Strategies in Connective Tissue Disease**<br>*Chair: Peter Kannu* | |
| **2:30 – 3:00pm** | Invited Speaker: **Rita Kandel**<br>*Biological intervertebral disc replacement: advancing towards a new treatment option* |
| **3:00 – 4:00pm** | **Coffee + Poster Presentations Session 2**<br>*Coffee Location: Auditorium Lobby*<br>*Poster Presentation Location: Lobby* |
| **3 Minute Elevator Pitch - Session A**<br>*Chair: Jason Rockel* | |
| **4:00 – 4:45pm** | Selected abstracts for 3 minute elevator pitch |

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| | **Boaz Wong:** *Myofibroblasts attract macrophages in fibrillar collagen*<br><br>**Nuno M. Coelho:** *Major vault protein holds the key for DDR1-β1 integrin interaction*<br><br>**Nina Noskovičová:** *CUB Domain Containing Protein 1 (CDCP1) is a novel negative regulator of TGFβ signaling and myofibroblast differentiation*<br><br>**Pardis Pakshir:** *Mechanisms Of Macrophage Mechanosensation In Collagen Matrix*<br><br>**Mohammadali Sheikholeslam:** *Engineering And Basic Science Meet Clinic: Dermal Skin Substitute For Burn Patients*<br><br>**Jose Ramirez-GarciaLuna:** *Impact of non-steroidal anti-inflammatory drugs on mast cells during bone repair*<br><br>**Xueting Mei:** *Microfluidic Platform for Investigation of Mechanoregulation of Breast Cancer Bone Metastasis*<br><br>**Sotcheadt Sim:** *BMP-2 Revealed Enhanced Healing in Fractured Mouse Tibia using Micro-CT and Torsion Test*<br><br>**Zeeshan Sheikh:** *A new approach for reversing osteoporotic bone loss with a conjugated drug (C3) in an ovariectomized (OVX) rat model*<br><br>**Rayan Fairag:** *Low-cost 3D printed biodegradable scaffolds, a potential graft substitute materials* |
| **End of Day 2** | |
| **6:00 – 9:00pm** | **Gala Dinner**<br>*Location: BMO Education & Conference Centre, 60 Leonard Avenue, Toronto* |

# SCIENTIFIC PROGRAM

## Day 3: Friday May 25, 2018
Location: Auditorium

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| **8:00 – 8:30am** | **Breakfast**<br>*Location: Auditorium Lobby* |
| | **Session VI: Bioengineering**<br>*Chair: Craig Simon* |
| **8:30 – 9:00am** | Invited Speaker: **Cari Whyne**<br>*The impact of metastatic disease on vertebral bone quality* |
| | **Session VII: Artificial Intelligence and Computational Biology**<br>*Co-Chairs: Dieter Reinhardt and Christopher McCulloch* |
| **9:00 – 9:30am** | Invited Guest Speaker: **Igor Jurisica**<br>*Precision medicine - from data to models to insights and treatments* |
| **9:30 – 9:50am** | Selected abstracts for oral presentation |
| **9:30am** | **Christie Costello:** *Metabolomics signature for non-responders to total joint replacement therapy in primary osteoarthritis patients* |
| **9:40am** | **Anne-Christin Hauschild:** *Influence of subtype and gender on disease progression and treatment response in osteoarthritis* |
| | **Session VIII: High-throughput Screening**<br>*Chair: Guangju Zhai and Aziz Ghahary* |
| **9:50 – 10:20am** | Invited Guest Speaker: **David Andrews**<br>*High Content Analysis to redefine subcellular organization* |
| **10:20 – 10:40am** | Selected abstracts for oral presentation |
| **10:20am** | **Supinder Kour Bali:** *RNA-SEQ Transcriptome Profiling To Investigate The Role of Glycogen Synthase Kinase 3 Signaling in Cartilage Biology* |
| **10:30am** | **Shabana Amanda Ali:** *Next generation sequencing as a tool for microRNA discovery in knee osteoarthritis* |
| **10:40 – 10:50am** | **Coffee Break**<br>*Location: Auditorium Lobby* |
| | **3 Minute Elevator Pitch - Session B**<br>*Chair: Casimiro Gerarduzzi* |

# SCIENTIFIC PROGRAM

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| **10:50 - 11:20am** | Selected abstracts for 3 minute elevator pitch<br><br>**Helal Endisha**: *microRNA-34a: Role In The Development Of Osteoarthritis During Obesity*<br><br>**Alejandro Gómez-Aristizábal:** *Monocyte/macrophages in osteoarthritic synovial fluid are correlated with patient-reported outcomes*<br><br>**Margaret Man-Ger Sun:** *Investigating The Role Of Retinoid X Receptor In Cartilage Development And Homeostasis*<br><br>**Akihiro Nakamura:** *Intra-articular injection of microRNA-181a-5p inhibitor attenuates cartilage degeneration in both facet and knee osteoarthritis animal models*<br><br>**Abhinav Parashar:** *A mechanistic study to understand vascular calcification caused by MGP deficiency*<br><br>**Rongmo Zhang:** *Fibrillin-1-mediated regulation of microRNA signaling and cell function*<br><br>**Mathieu Hélène:** *Identification of candidate genes potentially involved in Adolescent Idiopathic Scoliosis*<br><br>**Gyu-Tae Kim:** *High-Throughput Drug-Screening Using Human Cardiomyocytes In Mechanically Controlled Environments* |
| **11:20-11:50** | **CCTS General Assembly** |
| **Session IX: Tissue Fibrosis: Mechanisms and Therapeutic Targets**<br>*Chair: Carl Richards* | |
| **11:50 – 12:20am** | Invited Guest Speaker: **David Lagares**<br>*New Therapeutics for Fibrotic Diseases: from Biology to Targeted Therapy* |
| **12:20am - 12:50pm** | Invited Guest Speaker: **Andras Kapus**<br>*Mechanosensitive transcription factors: regulation and prominent roles in organ fibrosis* |
| **12:50 – 1:15pm** | Awards Presentation & Concluding Remarks: **Robin Poole** |
| **End of Conference** | |

## Opening Ceremony Speaker

### Dr. Sandeep (Sonny) Kohli

Dr. Sonny Kohli is a Physician in Internal Medicine & Critical Care in Canada, where he also serves as faculty at nearby McMaster University. His desire to improve access to care, deepened by his work as a global medical volunteer, inspired him to co-found Cloud DX, a company focused on utilizing unique sensors, acquisition of raw data, and cloud-based intelligence to transform health care. Cloud DX ultimately made it to the finals of the Qualcomm Tricorder XPRIZE competition, where they were bestowed with the 'Bold, Epic Innovator' award. Sonny is formerly a CSA Astronaut Candidate, trained Flight Surgeon and alumnus of the International Space University, and new faculty chair of health at Singularity University, Canada.

# Abstracts for Oral Presentations

# Session I: Skin and Matrix Biology – Health and Disease

## Invited Speaker: Dr. Vincent Piguet

*Dermatology & Immunology*

Dr. V. Piguet, MD, PhD, FRCP, trained at the School of Medicine, University of Geneva and graduated in 1995 before spending two years doing research in virology and immunology at the Salk Institute in San Diego, USA. He obtained his specialist certification in Dermatology & Venereology in 2004 from the Swiss Medical Association. In 2010 he was appointed Professor at Cardiff University, Wales, and in 2014 became a Fellow of the Royal College of Physicians. Professor Piguet was formerly the Chair of the Department of Dermatology and Wound Healing and Director of the Institute of Infection and Immunity at Cardiff University, prior to being appointed as Professor and Department Division Director, Division of Dermatology, Department of Medicine, University of Toronto and Division Head, Division of Dermatology, Women's College Hospital, Toronto. He is the Past-President of the European Society for Dermatological Research and past President-Elect of the European Dermatology Forum, and in 2017 was honored with membership of the Academia Europaea.

## Trans-differentiation of immune cells into skin cells in large skin injuries

Y Li[1], RT Kilani[1], A Ghahary[1]

[1]University of British Columbia, BC, Canada

**Introduction and Hypothesis**: Upon any kind of large dermal injury, keratinocytes and fibroblasts migrate from the edge of injury site to the wound site where they proliferate and promote wound healing. However, it is unlikely that these cells from the edges of large injury site to be able to migrate to a very long distance to cover the wound site. Here, we hypothesize that skin injury initiates a signal through which a subset of circulating immune cells become de-differentiated into stem like cells and these cells then become the major source of skin cells during the healing process.

**Methods**: The potential role of releasable factors from the proliferating fibroblasts on trans-differentiation of immune cells to multi-potent stem like cells was evaluated by culturing immune cells in fibroblast conditioned medium for 6 days. Cells were then examined for their morphology and the expression of a set of stem cell markers and their capacity to further differentiation into other cell types.

**Results**: The finding showed that culturing a subset of blood derived immune cells have the capacity to be de-differentiated into fibroblast like cells when co-cultured with proliferating fibroblasts. These cells were then identified to be fibroblast like cells with capacity to express a panel of stem cell markers such as alkaline phosphatase, formation of embryonic bodies, and expression of other pluripotent stem cells markers. Further, these cells showed a capacity to further differentiate into fibroblasts, osteocytes, adipocytes, smooth muscle cells, endothelial cells, neural cells. This finding was further confirmed in a mouse model by showing an easy detection of SSEA-1, a main marker for PSCs in wounded but not in normal tissues.

**Conclusions**: These data confirm that a subset of circulating immune cells have the capacity to become de-differentiated into PSCs within the wound environment and that these cells become the main source of skin cells in large wounds including burn.

**Applicability of Research to Practice**: Identifying the factors responsible for conversion of immune cells to skin cells would make it possible to topically apply these factors to promote the healing and reduce skin related inflammation diseases. Canadian Institute of Heath Research (CIHR) and WorkSafe BC supported this study

# Molecular interaction between elastogenic proteins impacting matrix assembly and function

Heena Kumra*[1], Valentin Nelea*[2], Dieter P. Reinhardt[1,2] *Co-first authors

[1]Faculty of Medicine and [2]Faculty of Dentistry, McGill University, Montreal, Canada

**Introduction:** Elastogenesis is an intricate and well-orchestrated multi-step process by which cells form functional elastic fibers. This hierarchical process begins with self-aggregation of tropoelastin, the soluble precursor of mature elastin, on the cell surface. The aggregates are then deposited to fibrillin-1 (FBN-1) enriched microfibrils, which require fibronectin (FN) for their assembly. The deposition of tropoelastin onto microfibrils requires various accessory proteins including fibulin-4 and 5 (FBLN-4 and -5) and latent transforming growth factor binding protein (LTBP)-4. The assembled tropoelastin is then crosslinked by enzymes of the lysyl oxidase family (LOX, LOXL) to form the mature and functional elastic fibers, which provide elasticity to various soft tissues including blood vessels and skin.

Phenotypic evidences from mouse models suggest the existence of a crosstalk between various ECM proteins involved in elastogenesis. Despite the known functional relationship between various elastogenic proteins, it is not clear how these proteins physically interact and affect each other at the molecular level, which then affects the organization of the matrix.

**Results:** Surface Plasmon resonance spectroscopy (SPR) showed that FBLN4 can interact with FN *in vitro*. Deletion of FN using fibroblasts from *Fn* knockout mice disrupted the assembly of FBLN4. To analyze if the dependency of FBLN4 on FN is direct or is dependent on other FN dependent ECM proteins, fibroblasts from *Fbn1* knockout mice were utilized. Knocking out *Fbn1* (FN assembly is still intact) did not affect FBLN4 assembly. This data shows di

rect dependency of FBLN4 on FN for its assembly. FBLN4 assembly is also known to be dependent on LTBP4. Mouse studies showed the existence of a functional relationship between the two. To obtain molecular details of this crosstalk, atomic force microscopy (AFM) of samples of LTBP4 mixed with FBLN4 was performed. The analysis revealed that the interaction of FBLN4 with LTBP4 induces an unexpected conformational change in the structure of LTBP4, switching the conformation from a compact to an elongated structure. Furthermore, this conformational change triggered an increased binding of LTBP4 to FBN1, but a decreased binding to FN, as shown by SPR and solid phase binding assays. Immunofluorescence analyses showed that the conformational change in LTBP4 induced by FBLN4 also affected LTBP4 assembly/deposition in cell culture.

Next, we examined if FBLN4 acts as a chaperone to induce this conformational and functional change in LTBP4 or if the two proteins form a complex. To test this, we passed LTBP4 over a FBLN4 column. This facilitated a transient interaction between the two proteins. It was observed that a transient exposure to FBLN4 is sufficient to induce a conformational change in LTBP4 as observed by AFM and dynamic light scattering. The interaction also imposes a functional change in LTBP4 as observed SPR.

**Conclusion:** The results show that FBLN4, which requires FN for its assembly, acts as a molecular chaperone to induce conformation and functional change in LTBP4.

## Regulation of TAZ expression by chemical and mechanical fibrogenic stimuli

Maria Zena Miranda[1,2], Pam Speight[2], Katalin Szaszi[2], Andras Kapus[1,2]

[1]Department of Biochemistry University of Toronto, [2]Li Ka Shing Knowledge Institute St. Michael's Hospital

It is increasingly recognized that fibrosis-related phenotype transitions (e.g. EMT and fibroblast-myofibroblast transition) are brought about by the interplay between chemical (e.g. TGFβ) and mechanical (e.g. stiffness, stretch) inputs, which in part act through cytoskeleton-regulated transcription factors. Among these, the Hippo effector TAZ and the Rho/actinregulated myocardin-related transcription factor (MRTF) are TGFβ- and mechano-sensitive transcriptional co-activators, which play key roles in the pathogenesis of organ fibrosis. Previously we have shown that TAZ and MRTF are both necessary for myofibroblast transition but the potential relationship between these factors was unknown. Intriguingly, we observed that besides its nuclear localization, TAZ is also controlled at the level of its expression. Thus, we first investigated the impact of TGFβ on TAZ expression. TGFβ caused a substantial rise in TAZ mRNA in mesenchymal (10T1/2) cells, a progenitor of the myofibroblast. Since the TAZ gene promoter contains a CArG box (the cis-element for MRTF) we wondered whether MRTF is a critical regulator of TAZ expression. TGFβ increased MRTF activity as verified by an MRTF-dependent luciferase reporter. Importantly, downregulation of MRTF by siMRTF or addition of CCG-1423, a pharmacological MRTF inhibitor strongly suppressed TGFβ-triggered TAZ mRNA and protein expression. TGFβ also activated a TAZ promoter luciferase reporter (TAZLuc), an effect which was abolished by MRTF inhibition or the mutation of the CArG box (mtTAZ-Luc). Mechanistically, TGFβ induced TAZ mRNA expression in a Smad3-independent and p38-mediated manner, leading to the phosphorylation and activation of MRTF without a substantial increase in nuclear MRTF levels. Although TGFβ triggered TAZ expression predominantly via non-canonical (Smad3-indpendent) pathways, downregulation of Smad2 significantly reduced TAZ expression. Interestingly, transfection of Smad2 increased the activity of the co-transfected MRTF on TAZ-Luc, suggesting a synergy between these factors. Next we asked if mechanical stimuli can also drive TAZ expression. Cyclic stretch (10% 1 Hz) increased TAZ mRNA levels and caused a ~2-fold rise in TAZ-Luc but not in mtTAZ-Luc activity, with a concomitant increase in the activity of the MRTF reporter. While, at least in epithelial cells, stretch could induce TGFβ synthesis in an MRTFdependent manner, the timing of the stretch effect as well as its insensitivity to a TGFβ receptor inhibitor argued against the possibility that stretch acts via such autocrine fashion. Instead, stretch directly stimulates MRTF, which in turn is a significant contributor to the ensuing TAZ expression.

Taken together, our studies show that critical chemical and mechanical fibrogenic stimuli increase TAZ expression via MRTFmediated mechanisms. We propose that the resulting rise in TAZ expression, combined with the concomitant nuclear translocation of TAZ and MRTF accelerate and exacerbate fibrogenic phenotype transitions.

## Pro-fibrotic cytokines induce the RhoA exchange factor GEF-H1 through a cytoskeleton-dependent self-regulatory cycle: possible role in epithelial-mesenchymal transition

Shruthi Venugopal[1], Qinghong Dan[1], Faiza Waheed[1], Razieh Rabani[1], Pam Speight[1], Andras Kapus[1], Katalin Szaszi[1]

[1]Keenan Research Center for Biomedical Science, St Michael's Hospital and Department of Surgery University of Toronto

Upon injury- and pro-fibrotic cytokine exposure epithelial cells undergo phenotypic shifts such as partial or full EpithelialMesenchymal Transition (EMT), an important step enhancing tissue regeneration. However, ongoing injury and EMT also promote organ fibrosis. Mesenchymal reprogramming involves junction disruption, loss of apico-basal polarity and major cytoskeleton remodelling. Many of these effects are mediated by Rho family small GTPases. Downstream from RhoA, central effectors of EMT-associated reprogramming are the transcriptional co-activators Myocardin Related Transcription Factor (MRTF) and its partner Serum Response Factor (SRF). We have previously shown that pro-fibrotic and inflammatory cytokines activate the Rho-Rho kinase (ROK) pathway through the Guanine Nucleotide Exchange Factor GEF-H1. However, the regulation of GEF-H1 during EMT has not been elucidated. Here we show that prolonged stimulation of LLC-PK1 tubular cells with various EMT-promoting stimuli including TGFβ1, mechanical stress (cell stretch) or the proinflammatory cytokine Tumor Necrosis Factor-α (TNFα) elevates expression of GEF-H1. Tubular GEF-H1 expression was also increased in unilateral ureteral obstruction (UUO), a mouse model of kidney fibrosis, as shown using laser capture microdissection and micro-PCR. Silencing or inhibiting RhoA, ROK or MRTF reduced basal GEF-H1 protein expression in LLC-PK1 cells and prevented the TGFβ1- and TNFα-induced elevation. Conversely, Jasplakinolide that induces actin polymerization and activation of MRTF elevated GEF-H1 expression. Further, we also show that the effect involves changes in GEF-H1 gene expression, as TNFα and TGFβ activated a luciferase coupled GEF-H1 promoter construct and increased mRNA expression through ROK and MRTF. Interestingly, silencing endogenous GEF-H1 also reduced the activity of the GEF-H1 promoter construct, suggesting a feedback regulation. Finally, TNFα augmented TGFβ-induced expression of the EMT marker smooth muscle actin (SMA) in a GEF-H1-dependent manner. In summary, our study shows that GEF-H1 expression is elevated by pro-fibrotic stimuli through cytoskeleton remodeling and the RhoA/Rho kinase/MRTF pathway, and this can further augment EMT. Such a self-

regulatory positive feed-back cycle might play a key role in the progression of kidney
fibrosis.

# Exosomes enriched in the Wharton's jelly of the human umbilical cord enhances skin wound healing

Nazihah Bakhtyar[1], Marc Jeschke*[1,2,3], Mohammadali Sheikholeslam[1], Elaine Herer[1], Arthur Zaltz[1], Saeid Amini-Nik*[1,3,4] *Co-corresponding authors

[1]Sunnybrook Research Institute, [2]Institute of Medical Science, University of Toronto, [3]Department of Surgery, [4]Department of Laboratory Medicine and Pathobiology

We recently reported that acellular gelatinous Wharton's jelly (AGWJ) from the human umbilical cord enhances wound healing *in-vitro* and *in-vivo*. However, the active ingredient(s) of AGWJ is not known. **Hypothesis:** There are extracellular factors in the AGWJ which are beneficial for wound healing. **Purpose:** Investigate the active ingredient(s) in the jelly and identify any abundant proteins. **Methods:** Isolated and fractionated acellular WJ. Mass spectrometry on AGWJ to identify proteins, then isolated exosomes from AGWJ. *In-vivo,* 6mm punch biopsies on the backs of BALb/c male mice were performed; wounds were treated with control matrigel and matrigel containing exosomes derived from AGWJ. Mice were sacrificed on day 7; histology was performed on wounds. Mass spectrometry also conducted on exosomes from AGWJ. **Results:** Mass spectrometry on AGWJ revealed enrichment of proteins characteristic of exosomes. After isolating exosomes, i*n-vitro*, exosomes enhanced cellular behavior by interacting with cells and by becoming engulfed in the cell over time. These exosomes treated onto murine wounds lead to significantly enhanced wound healing. Mass spectrometry revealed that exosomes from the AGWJ contain a high amount of alpha-2-macroglobulin (a2M). Subsequent *in-vitro* experiments using a2M displayed similar results to exosomes on cellular behavior, including cell proliferation and migration, *in-vitro*. **Conclusion:** Data suggests that exosomes in AGWJ enhance wound healing by becoming engulfed into cells over time, these exosomes have an abundance of a2M within their secretome which aids in wound healing properties.

# Session II: Bone and Developmental Biology – Health and Disease

## Invited Speaker: Dr. Jeff Dixon

*Mineralized Tissues*

Dr. Jeff Dixon, DDS, PhD, is a Distinguished University Professor in the Department of Physiology and Pharmacology, and School of Dentistry at Western University in London, Canada. He studies the cellular and molecular mechanisms underlying the resorption and formation of mineralized tissues. Collaboratively, he is investigating mechanotransduction in skeletal cells and developing advanced materials for bone regeneration and dental applications. From 2001-2009, Dixon directed the CIHR Group in Skeletal Development and Remodeling, an interdisciplinary team focusing on musculoskeletal and dental health research. Dixon currently serves on the Advisory Board of the Institute of Musculoskeletal Health and Arthritis (one of the Canadian Institutes of Health Research) and on the leadership team of the Bone and Joint Institute at Western University (http://boneandjoint.uwo.ca).

## Osteoclastogenesis is modulated by the phosphatase Inpp4b through the PKCβ/GSK3β/Nfatc1 pathway

Lina Saad[1], Monica Pata[1], Jean Vacher[1,2,3]

[1]Institut de Recherches Cliniques de Montréal (IRCM), [2]Département de Médecine, Université de Montréal, Montréal, Québec, Canada, [3]Department of Medicine, Division of Experimental Medicine, McGill University, Montréal, Québec, Canada

Osteoclast bone remodeling activity is critical for skeletal development and bone tissue homeostasis. The disruption of osteoclast differentiation or maturation leads to pathologic conditions such as osteopetrosis and osteoporosis. We isolated and characterized the mouse homologue of the human and rat inositol polyphosphate 4-phosphatase b (Inpp4b) as a negative regulator of osteoclastogenesis. Systemic Inpp4b ablation induced a nuclear accumulation of Nfatc1, an increase in osteoclasts number, size and activity, resulting in an osteopenic phenotype. However, the molecular mechanism through which Inpp4b regulates osteoclast differentiation and Nfatc1 signaling pathway remains elusive. Here, we show that among several osteoclast-specific signaling cascades analyzed upon RANKL stimulation and upstream of Nfatc1, only the PKCβ pathway was modulated in absence of Inpp4b. We found that Inpp4b depletion induces the activation of the kinase PKCβ, concomitant with a significant increase in the phosphorylation level of the protein with time. Interestingly, we also demonstrated that the GSK-3β protein, a downstream effector of PKCβ and a key regulator of Nfatc1 localization, was inactivated by phosphorylation in the same cells. Pharmacological inhibition of PKCβ results in a decrease in osteoclast formation which was associated with a significant reduction of NFATc1 gene expression. As expected, the phosphorylation of GSK-3β was also decreased following PKCβ inhibition. In addition, co-IP experiments suggest that Inpp4b can interact directly with the phosphorylated forms of PKCβ and GSK-3β. Together our results indicate that Inpp4b can regulate osteoclast Nfatc1 activity and osteoclast differentiation through the modulation of PKCβ and GSK-3β phosphorylation levels and activities.

## Zoledronic acid-loaded silica nanoparticles to treat cancer-induced bone metastasis

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Objective: Zoledronate (Zol) is a Bisphosphonate (BP) class drug used in treatment of patients suffering from bone metastases, including the spine. Systemic BP treatment is the current standard for delivering BPs to patients. However, BP can cause multiple adverse effects. Interestingly, our group has shown that local Zol delivery blocks tumor-induced osteolysis in mice while preventing the occurrence of side effects associated with systemic administration. Over the past decades, nanoparticles have been explored as an exciting method for delivering anti-cancer drugs directly to tumor sites. These nanoparticles allow for high and local administration of drug while avoiding the complications of systemic delivery. Our aim is to develop a valuable tool that delivers Zol locally for the treatment of bone metastasis.

Methods: Testing Zoledronate release from nanobeads: fluorescent Zol was incubated overnight with mesoporous silica nanoparticles that were either uncoated or coated with a thin chitosan shell. The nanoparticles were dispersed in aqueous media. Aliquots from the dispersed solution were taken daily for up to 14 days and fluorescent Zol was measured using a plate reader. Testing the effect of Zol on a prostate cancer cell line and prostate-induced bone metastasis cells: cells were seeded, incubated with non-fluorescent Zol for 7 days and assessed for proliferation, metabolic activity, migration and invasion using the vybrant® MTT cell proliferation, alamarBlue®, transwell migration and 3D culture spheroid cell invasion assays respectively.

Results: Chitosan-coated nanoparticles hold and release more Zol over time in aqueous media compared to uncoated nanoparticles. The prostate cancer cell line or patient-derived cells treated directly with Zol shows significantly reduced cell proliferation, metabolic activity, migration and invasion. In ongoing experiments, the zoledronate bead release is being tested on the prostate cancer line and patient-derived cells.

Conclusions: Nanoparticle-releasing Zol could constitute a therapeutic promise to combat metastatic spine tumors. These nanoparticles can be integrated into commercial bone putty to develop a bioactive bone graft following bone tumor resection to deliver localized BP drug facilitating bone stability and healing while preventing tumor recurrence.

# Mechanisms underlying increased osteoclastogenesis in the mouse model of osteogenesis imperfecta due to mutation in collagen type I

I. Boraschi-Diaz[1,2], E.C. Niemi[3], F. Rauch[2,4], M.C. Nakamura[3], S.V. Komarova[1,2]

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**Introduction**: Osteogenesis imperfecta (OI) is the most common heritable bone fragility disorder, usually caused by dominant mutations in genes coding for collagen type I alpha chains, *COL1A1* or *COL1A2.* Although, osteoclasts do not express or produce collagen, stimulated osteoclastogenesis and bone destruction were reported in OI.

**Objective**: The aim of this study was to examine the osteoclast phenotype in a mouse model of dominant severe OI caused by a *Col1a1* mutation, Col1a1$^{Jrt/+}$ mice.

**Methods**: Bone marrow and spleen osteoclast precursors were extracted, characterized and differentiated into mature osteoclasts by culturing with macrophage colony-stimulating factor and RANKL in vitro.

**Results**: We found that more and larger osteoclasts were formed in vivo from bone marrow and spleen precursors of *Col1a1*$^{Jrt/+}$ (OI) mice compared to wild-type littermates (WT). However, expression of the osteoclast markers genes, or signaling by calcium or NFATc1 were not significantly different between WT and OI. Osteoclast precursor frequency was similar in *Col1a1*$^{Jrt/+}$ and WT mice, however OI precursors were more sensitive to RANKL compared to WT. No significant difference was found in the protein expression of RANKL, OPG or RANK between OI and WT bone extracts or in vitro generated osteoblasts or osteoclasts. We have previously established that osteoclast formation is inhibited by collagen type I degradation fragments. The molecular weight of isolated *Col1a1*$^{Jrt/+}$ collagen type I was approximately 50 kDa and was easily degradable by cathepsin K. Osteoclast-inhibitory effect of full length collagen type I from OI was significantly stronger compared to WT, while OI collagen degradation fragments had no osteoclast-inhibitory activity.

**Conclusion**: We demonstrate that *Col1a1* mutation results in an osteoclast phenotype even though osteoclasts themselves do not express this protein. One of the potential mechanisms is rapid degradation of mutated collagen type I resulting in complete lack of fragments with osteoclast inhibitory activity, and thus an absence of a negative feedback for osteoclastogenesis in OI.

# The role of SMPD3 in skeletal tissues during fracture healing

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Traumatic bone fractures can be a serious and frequent problem for patients suffering from osteoporosis, metastatic bone cancer and congenital bone disorders. The promotion of new bone formation and mineralization during the healing process of fractures can facilitate and shorten the time of healing, as well as yield stronger union of the fractured bones. Our laboratory has identified that sphingomyelin phosphodiesterase 3 (SMPD3), a lipid-metabolizing enzyme present in bone and cartilage, has important roles in the developing skeleton including the promotion of apoptosis of hypertrophic chondrocytes and mineralization of both cartilage and bone extracellular matrix (ECM). Using a conditional knockout mouse model, *Smpd3*$^{flox/flox}$*;Osx-Cre*, where *Smpd3* is deleted in both chondrocytes and osteoblasts, we showed that SMPD3 activity in both these cell types is required for skeletal ECM mineralization and normal skeletal development. Conversely, using an inducible system, ablation of SMPD3 only in adult skeletal cells showed that SMPD3 has less critical functions in the adult skeleton as we did not see any gross phenotypic differences between 3-month old *Smpd3*$^{flox/flox}$*;Osx-Cre* and the control *Smpd3*$^{flox/flox}$ mice. Although we did not observe a major role for SMPD3 in the adult skeleton, considering that bone fracture healing involves a recapitulation of the steps seen during bone development, we hypothesized that mice lacking SMPD3 in chondrocytes and osteoblasts would adversely affect the process of fracture healing.

**Objective:** To investigate the role of SMPD3 during fracture healing

**Methods:** To investigate the role of SMPD3 in fracture healing, we generated a conditional knockout mouse, *Smpd3*$^{flox/flox}$*;Osx-Cre*, which lacks *Smpd3* in both chondrocytes and osteoblasts. Rodded immobilized fracture surgeries were performed in the tibia of these mice. The bones were then analyzed at 2 and 4 weeks post-surgery by micro-CT, histology and histomorphometry.

**Results:** Micro-CT analysis did not show any differences in callus size and the amount of mineralized tissue at the fracture site between *Smpd3*$^{flox/flox}$*;Osx-Cre* mice and the control mice at both 2 and 4 weeks post-surgery. Histomorphometric analysis showed a significant increase in osteoid volume at the fractured site in *Smpd3*$^{flox/flox}$*;Osx-Cre* mice compared to its control at both time points.

**Conclusion:** Our data provides compelling evidence that SMPD3 activity plays a role during bone fracture healing. The novel insight generated through this study will add to the current understanding of SMPD3 regulation in skeletal tissues and has potential to pave the way for the development of therapeutic approaches to expedite healing of fractured bones.

## Zucara Therapeutics: A CDRD/MaRS Innovation

James Jaquith, Ph.D.[1], Head of Medicinal Chemistry

[1]The Centre for Drug Research and Development (CDRD)

CDRD, Canada's national Centre for Drug Research and Development, is a global bridge that translates discoveries into innovative therapeutic products and improved health outcomes. In collaboration with partners we advance exciting therapeutic discoveries toward novel, world class therapeutics for the treatment of human diseases.

In collaboration with Professor Michael Riddell of York University and MaRS Innovation, and with support from the Juvenile Diabetes Research Foundation (JDRF), CDRD has validated and developed a novel therapeutic approach for the treatment of hypoglycemia, a significant health problem for people living with Type 1 diabetes. The technology was first discovered by Mladen Vranic, Professor Emeritus at the University of Toronto's Banting & Best Diabetes Centre. Together, these efforts have resulted in the creation of Zucara Therapeutics Inc. to advance the technology into clinical development. Recently Zucara has announced initial financing aimed at completing preclinical development of a drug candidate discovered at CDRD.

An overview of CDRD, its mission, and Zucara's translation from bench to bedside will be reviewed.

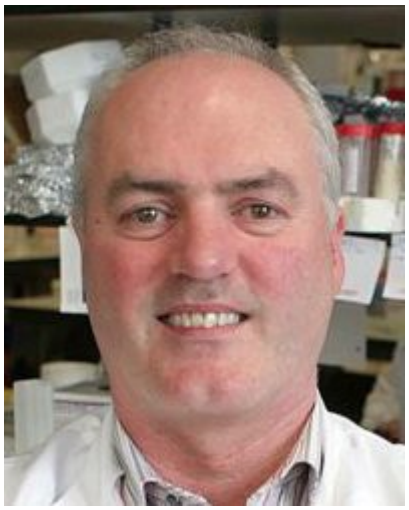
# Session III: Bridging Translational Research in Canada

## Invited Speaker: Dr. James Jaquite

*Medicinal cheimstry*

Dr. James Jaquith is the Head of Medicinal Chemistry at The Centre for Drug Research and Development (CDRD), Canada's national translational and commercialization centre. CDRD plays a key role in the evaluation, funding, de-risking, and strategic development of academic research programs from Canadian investigators. Current in-house programs focus on novel approaches for the treatment of pathogenic infections, peripheral neuropathies, diabetes, Parkinson's disease, and cancer, with a special focus on novel ADC platforms. Dr. Jaquith also leads CDRD's Regenerative Medicine, Inflammation and Fibrosis Task Force which specifically seeks to identify research projects that are at the cutting edge of the Regenerative Medicine field. Our primary focus in on the development of small molecule, antibody and antibody drug conjugate therapies for inflammatory and fibrotic diseases. Prior to joining CDRD, Dr. Jaquith was the Director of Chemistry at Aegera Therapeutics (Pharmascience Inc.). Dr. Jaquith received his B.Sc. and M.Sc. in Chemistry from the University of Waterloo, and a Ph.D. from the University of Ottawa, before joining Apoptogen Inc. as an NSERC Industrial Research Fellow.

# Session IV: Cartilage and Intervertebral Disc Biology – Health and Disease



## Invited Speaker: Dr. Frank Barry

*Cell Therapy, Tissue Repair and Joint Injury*

Frank Barry is Professor of Cellular Therapy at the Regenerative Medicine Institute (REMEDI), National University of Ireland Galway. Here he directs a large group of researchers who focus on the development of new repair strategies for musculoskeletal conditions, especially osteoarthritis. Previously he was Director of Arthritis Research at Osiris Therapeutics in Baltimore, MD Assistant Professor at the University of South Florida College of Medicine and a Research Fellow at Shriners Hospital for Children in Tampa, FL. He has contributed to the fields of tissue engineering and regenerative medicine by developing innovative and successful cellular therapies for tissue repair, joint injury and arthritic disease. In a career that has spanned both industry and academic research, he has been a driver in the development of cellular therapy as a biological repair strategy. It is his belief that the application of new technologies in regenerative medicine, including cellular therapy, gene therapy, growth factor augmentation, implantable scaffolds and nanomaterials, will have a profound impact in medicine in years to come. Frank Barry has been the recipient of the Marshall Urist Award for excellence in tissue regeneration research from the Orthopaedic Research Society.

# Link N retards disease progression in a rabbit model of osteoarthritis

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**Introduction:** Osteoarthritis (OA) is a degenerative joint disease characterized by a slowly progressive degradation of articular cartilage, thickening of the subchondral bone, formation of osteophytes, inflammation of the synovium, degeneration of ligaments and the menisci, and hypertrophy of the joint capsule. Currently, there is no medical treatment to reverse or even retard OA. The purpose of the present study was to determine if short Link N (sLN), a recently discovered fragment of the Link N peptide, could retard disease progression in a rabbit model of OA.

**Methods:** Skeletally mature New Zealand white rabbits underwent unilateral anterior cruciate ligament transection (ACLT) of their left femorotibial joints to induce joint degeneration. Beginning 3 weeks post-operatively, and every three weeks thereafter for 12 weeks, either saline (1 mL) or sLN (100 µg in 1 mL saline) was injected intraarticularly in the operated knee. Additional rabbits underwent Sham surgery but without ACTL. The effects on gross morphology and histologic changes were evaluated.

**Results:** In all joint compartments of the Sham group, the articular cartilage macroscopic grades were 1 (normal: intact surface with no sign of lesion). In the Saline group, fibrillation of articular cartilage occurred in all joint surfaces, and prominent erosion occurred in both femoral condyle compartments and the lateral compartment of the tibial plateau. sLN treatment reduced the severity of the cartilage damage in three compartments of the knee showing erosion. In the lateral tibial plateau (LTP) and medial femoral condyle (MFC) sLN prevents the erosion, and reduces erosion in the lateral femoral condyle (LFC) compartment. In the medial tibial plateau (MTP) compartment only minor fibrillation is observed with saline or sLN. Overall there is a trend in reducing the severity of joint damage, reaching statistical significance in the MFC compartment. Thus, sLN attenuates the severity of articular cartilage damage.

**Discussion & Conclusions:** When all histologic parameters were combined from each site to obtain a total joint score, statistically significant differences were detected between the Saline treated joints and the sLN group ($p = .04$), showing that sLN reduces the joint lesion severity in a statistically significant manner. Thus, pharmacologically, sLN supplementation could be a novel therapeutic approach for treating osteoarthritis by retarding disease progression.

**Acknowledgements:** The authors would like to thank our funding sources Canadian Institute of Health Sciences (CIHR) and Trepso Therapeutics.

# Role of Toll-like receptor 2 in degenerating articular cartilage

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Adolescent Idiopathic Scoliosis (AIS) is a progressive 3-dimmensional bending of the spine which affects the intervertebral disc (IVD) and the facet joints. Our facet joint characterization study has revealed the presence of degeneration in scoliotic cartilage through decreased proteoglycan content and elevated secretion of inflammatory cytokines and matrix degrading proteases. Recently, a novel degenerative pathway in IVDs involving Toll-like receptor 2 (TLR2) was found. When activated by fragmented extracellular matrix components (alarmins), TLR2 receptors in disc cells initiate the production of inflammatory cytokines, proteases and neurotrophins leading to tissue degradation and pain. In this study, we investigate the potential role of TLR2 receptor activation in facet joints from AIS patients as a cause of early degeneration. TLR presence was assessed by rt-qPCR and immunocytochemistry on cells isolated from AIS and cadaveric non-scoliotic cartilage samples with consent. TLR2 receptors were activated in monolayer AIS chondrocytes using a TLR2 agonist (Pam2CSK4, Invivogen). Cartilage explants were isolated from the subchondral bone and cultured in the presence and absence of Pam2CSK4 in chondrocyte media, which was analyzed by ELISA for degenerative marker secretion. After the culture period, the cartilage was cryosectionned and stained with SafraninO – Fast green dyes to reveal proteoglycan content. Gene expression analysis revealed a significant higher TLR2 expression in AIS chondrocytes compared to controls. Interestingly, TLR2 mRNA in AIS chondrocytes correlated positively and significantly ($p<0.05$) with degenerative factor (MMP3, MMP13, IL-1b, IL-6 and IL-8) mRNA levels using Pearson's correlation. These correlations were not as strong (MMP13, IL-6) or not present at all (MMP3, IL-1b and IL-8) in non-scoliotic healthy chondrocytes. In the presence of the TLR2 agonist, gene and protein expression analysis showed significantly elevated levels of TLR2 receptor, proteases, inflammatory cytokines and pain-related factors such as MMP3, MMP13, IL-6 and NGF. Furthermore, histological staining of cultured cartilage explants revealed a significant decrease in proteoglycan content after treatment with Pam2CSK4 in AIS cartilage, but not in the non-scoliotic controls. In conclusion, our data show that TLR2 activation lead to a degenerative cycle with the upregulation of the receptor itself and secretion of proteases, inflammatory cytokines and pain-related factors that ultimately trigger proteoglycan loss in the affected cartilage. Our study reveals TLR2 as a potential therapeutic target to treat degenerating articular cartilage and subsequent pain.

# Modulation of inflammation and cartilage degradation using ex vivo polarized monocytes/macrophages within a human osteoarthritic joint explant model

Mable Wing Yan Chan[1,2], Alejandro Gómez-Aristizábal[1], Rajiv Gandhi[1], Wayne Marshall[1], Nizar Mahomed[1], Sowmya Viswanathan[1,2,3]

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**Background**: Inflammatory monocyte infiltration and synovitis are implicated in the progression of osteoarthritis (OA). Monocytes/macrophages (Mϕs, as a heterogeneously differentiated population) are innate immune cells that mediate both inflammation and homeostatic tissue repair; dysregulation of their function and polarization is implicated in many inflammatory and degradative diseases. We are investigating the role of polarized inflammatory (M1) versus homeostatic (M2) Mϕs within OA and propose that Mϕ population modulation via ex vivo polarized Mϕ-based cell therapy will serve as a multimodal treatment that reduces both inflammation and matrix degradation.

**Hypothesis**: To generate proof-of-concept pre-clinical evidence, we propose that addition of *ex vivo* polarized homeostatic Mϕs into a cartilage and synovium human OA explant model will reduce synovial inflammation and cartilage degradation.

**Methods and Results**: We optimized an in-house *ex vivo* human OA joint explant model using culture of cartilage and synovial tissue obtained from end-stage OA total knee replacements. CD14+ Mϕs were isolated from peripheral blood and ex vivo polarized using standard 48h cytokine protocols into M1/M(IFN-γ+LPS) and M2/M(IL-10+TGF-β). After polarization, Mϕs, which are stable even when challenged, were added to OA explants for up to 7 days.

The model was validated through negative and positive inflammatory controls for the explant tissue that demonstrated reproducible changes in gene expression and extracellular matrix (ECM) loss that mimicked OA inflammation and degradation. 5 ng/mL of OSM + IL-1β upregulated inflammatory and catabolic gene expression (qRT-PCR; N=3) and proteoglycan loss in cartilage explants (Safranin-O histology and DMMB assay), while 100 ng/mL IFN-γ upregulated inflammatory, downregulated homeostatic genes in synovium (N=4).

Relative to negative baseline co-culture of cartilage with synovium, M1 Mϕs upregulated cartilage catabolic (MMP1), inflammatory (INOS,IL6), and chemotactic (CCL2, CXCL8) genes while downregulating anabolic ECM genes (COL2A1, ACAN, PRG4, TIMP1), whereas M2 Mϕs upregulate anabolic ECM genes (PRG4, TIMP1, COL1A1, COLX), all at greater than 2-foldchange of expression (N=3). Synovium gene expression is more variable than cartilage, but M1 Mϕ treatment trends to upregulate inflammatory and chemotactic genes (N=3). Levels of protease inhibitors TIMP-1 and TIMP-2 in the explant culture conditioned medium are higher in the M2 Mϕ treatment group and lower with M1 Mϕ group (N=3).

**Conclusion:** Our data shows that polarized inflammatory versus homeostatic Mϕs play differential roles in modulation of the OA joint inflammatory and degradative environment; inflammatory Mϕs promote expression of inflammatory, chemotactic, and catabolic genes, whereas homeostatic Mϕs upregulate ECM anabolism. Our human OA joint explant is a useful, clinically relevant tool for reproducible demonstration of differential Mϕ effects, contributing to proof-of-concept data that supports further investigation of *ex vivo* polarized Mϕ treatment for OA.

# Tungsten accumulation in the intervertebral disc regulates degeneration and markers of discogenic pain

Michael P. Grant[1], Alicia Bolt[1], Laura M. Epure[1], Hsiang Chou[1,2], John Antoniou[1,2], Koren K. Mann[1,2], Fackson Mwale[1,2]

[1]SMBD-Jewish General Hospital, Lady Davis Institute for Medical Research, Montreal, QC, Canada; [2]McGill University, Montreal, QC, Canada

**Introduction:** Tungsten has been increasing in demand for use in manufacturing and recently, medical devices, as it imparts flexibility, strength, and conductance of metal alloys. Given the surge in tungsten use, our population may be subjected to elevated exposures. For instance, embolism coils made of tungsten have been shown to degrade in some patients. In a cohort of breast cancer patients who received tungsten-based shielding for intraoperative radiotherapy, urinary tungsten levels remained over tenfold higher 20 months post-surgery. In an in vivo mouse model, tungsten exposure increased tumor metastasis by altering the tumor microenvironment. In a recent report, tungsten was shown to rapidly accumulate in bone and enhance the adipogenesis of bone marrow-derived mesenchymal stem cells while inhibiting osteogenesis. Whether tungsten accumulates in other tissues and affects viability and/or function remains unknown. We recently determined that when mice are exposed to tungsten [15 ug/mL] in their drinking water, it bioaccumulates in the intervertebral disc [~3 ppm], equivalent to what is observed in bone. This study was performed to determine the toxicity of tungsten on intervertebral disc cells.

**Methods:** Bovine nucleus pulposus (bNP) and annulus fibrosus (bAF) cells were isolated from bovine caudal tails. Cells were expanded in flasks then prepared for 3D culturing in alginate beads at a density of 1*10^6 cells/mL. Beads were cultured in medium supplemented with increasing tungsten concentrations in the form of sodium tungstate [0, 0.5, 5, 15 ug/mL] for 12 days. A modified GAG assay was performed on the beads to determine proteoglycan content and Western blotting for type II collagen (Col II) synthesis. Cell viability was determined by counting live and dead cells in the beads following incubation with the Live/Dead Viability Assay kit (Thermo Fisher Scientific). Cell numbers in beads at the end of the incubation period was determined using Quant-iT dsDNA Assay Kit (Thermo Fisher Scientific).

**Results:** Tungsten dose-dependently decreased the synthesis of proteoglycan in IVD cells, however, the effect was significant at the highest dose of 15 ug/mL. (n=3). Furthermore, although tungsten decreased the synthesis of Col II in IVD cells, it significantly increased the synthesis of Col I. Upregulation of catabolic enzymes ADAMTS4 and -5 were also observed in IVD cells treated with tungsten (n=3). Upon histological examination of spines from mice treated with tungsten [15 ug/mL] in their drinking water for 30 days, disc heights were diminished and Col I upregulation was observed (n=4). Cell viability was not markedly affected by tungsten in both bNP and bAF cells, but proliferation of bNP cells decreased at higher concentration. Surprisingly, histological examination of IVDs and gene expression analysis demonstrated upregulation of NGF expression in both NP and AF cells. In addition, endplate capillaries showed increases in CGRP and PGP9.5 expression as determined on histological sections of mouse IVDs, suggesting the development of sensory neuron invasion of the disc.

**Conclusions:** We provide evidence that tungsten affects matrix protein synthesis in IVD cells, possibly enhancing disc fibrosis. Tungsten toxicity may play a role in disc degeneration disease.

**Acknowledgements:** This research was supported by CIHR.

# Session V: Novel Therapeutic Strategies in Connective Tissue Disease

## Invited Speaker: Dr. Rita Kandel

*Regenerative Medicine*

Dr. Rita Kandel MD FRCP(C) obtained her medical degree and did her Pathology residency at the University of Toronto. She did postdoctoral fellowships at Tufts University and Harvard University in Boston. She is currently the Chair of the Department of Laboratory Medicine and Pathobiology at the University of Toronto and Chief of Department of Pathology and Laboratory Medicine at Sinai Health System. Her research has focused on utilizing regenerative medicine approaches to develop biological treatments for back pain and arthritis. She has published over 240 papers and multiple book chapters. Among other honours and awards, Dr. Kandel is a Fellow of the Canadian Academy of Health Sciences.

# Session VI: Bioengineering

## Invited Speaker: Dr. Cari Whyne

*Orthopaedic Biomechanics / Bioengineering*

C. Whyne is a Senior Scientist and the Director of the Holland Musculoskeletal Research Program at Sunnybrook Research Institute. She is a Professor in the Department of Surgery, Institute of Biomaterials and Biomedical Engineering and Institute of Medical Sciences at the University of Toronto. The focus of her work within the Orthopaedic Biomechanics Laboratory is clinically translational bioengineering research. Dr. Whyne's research integrates biomechanical analyses with basic science and preclinical investigations, including extensive work in computational image analysis, micro-imaging and finite element modeling techniques. Her work also incorporates design, simulation, evaluation and clinical translation of novel less/minimally invasive surgical techniques and devices. The primary foci of Dr. Whyne's research are cancer in bone, spinal/lower extremity/thin bone biomechanics and fracture fixation/healing.

# Session VII: Artificial Intelligence and Computational Biology

## Invited Speaker: Dr. Igor Jurisica

*Computational Biology / Informatics*

I. Jurisica is Tier I Canada Research Chair in Integrative Cancer Informatics, Senior Scientist at Krembil Research Institute, Professor at U Toronto and Visiting Scientist at IBM CAS. He is also an Adjunct Prof. at the School of Computing, Pathology and Molecular Medicine at Queen's U, Computer Science at York U, scientist at the Institute of Neuroimmunology, Slovak Academy of Sciences and an Honorary Professor at Shanghai Jiao Tong University. Since 2015, he has also served as Chief Scientist at the Creative Destruction Lab, Rotman School of Management. He has published extensively on data mining, visualization and cancer informatics, including multiple papers in Science, Nature, Nature Medicine, Nature Methods, J Clinical Oncology, and has over 12,163 over the last 5 years. He has been included in Thomson Reuters 2016, 2015 & 2014 list of Highly Cited Researchers, and The World's Most Influential Scientific Minds: 2015 & 2014 Reports.

# Metabolomics signature for non-responders to total joint replacement therapy in primary osteoarthritis patients

Christie Costello[1], Ming Liu[1], Weidong Zhang[1], Andrew Furey[2], Edward Randell[3], Proton Rahman[4], Guangju Zhai[1]

[1]Discipline of Genetics, Faculty of Medicine, Memorial University of Newfoundland, St. John's, NL, Canada, [2]Department of Surgery, Faculty of Medicine, Memorial University of Newfoundland, St. John's, NL, Canada, [3]Department of Laboratory Medicine, Faculty of Medicine, Memorial University of Newfoundland, St. John's, NL, Canada, [4]Discipline of Medicine, Faculty of Medicine, Memorial University of Newfoundland, St. John's, NL, Canada

**Background:** While total joint replacement therapy (TJR) is by far the most effective treatment for advanced osteoarthritis (OA), up to one third of patients fail to reach the minimal clinically important difference (MCID) in pain relief or function improvement. **Objectives:** To identify metabolic markers for predicting non-responders to TJR in primary OA by a metabolomics approach.

**Methods:** 704 TJR patients were recruited and their plasma samples were collected prior to surgery. The MCID was assessed based on the patient-reported Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain and joint function subscales. Patients were classified as non-responders to pain if the change score was less than 7 points (of 20 points total) for WOMAC pain subscale from pre-surgery to post-surgery. For physical function, patients were non-responders if the change score was less than 22 points (of 68 points total) from pre-surgery to post-surgery. Metabolic profiling (186 targeted metabolites) was performed on plasma samples collected at baseline. 130 metabolite concentrations passed the quality control procedure. Pair-wise metabolite ratios as proxies for enzymatic reactions were then calculated and used in the analysis. The significance level was defined at $a<0.0004$ with Bonferroni correction for multiple testing, assuming these metabolites represent 130 independent metabolic pathways. **Results:** After excluding patients with secondary OA and other joint diseases, a total of 449 TJR patients (124 hips and 325 knees) due to primary OA were included in the analysis. The average age was 65±7.8 years; 57% were females. The average post-surgery follow-up time was 3.9±1.4 years. Of these patients, 17% were classified as pain non-responders, while 16% were function non-responders. No significant association was found between non-responders and age, sex, BMI, or joints. A total of 15 metabolite ratios were significantly associated with pain non-responders (all $p<0.0004$). Collectively, these ratios had an area under the curve (AUC) of 73.0% in the ROC analysis to predict pain non-responders. In the multivariable regression analysis including all 15 ratios, one ratio - lysophosphatidylcholine (lysoPC) acyl (a) C18:1 to phosphatidylcholine (PC) acyl-alkyl (ae) C36:4 - was independently and significantly associated with pain non-responders. LysoPC to PC ratio was also previously found to be associated with OA risk and progression. A total of 6 metabolite ratios were significantly associated with function non-responders (all $p<0.0004$). Collectively, these ratios had an AUC of 66.4% in the ROC analysis to predict function non-responders. In the multivariable regression, four ratios - citrulline to PC ae C38:5, hydroxysphingomyelin C22:1 to PC ae C38:5, PC ae C36:0 to PC ae C38:3, and threonine to lysoPC a C17:0 - were independently and significantly associated with function non-responders. **Conclusion:** We found that several novel metabolic ratios were associated with non-responders to TJR. These ratios suggest the alteration of metabolic pathways in energy, phosphatidylcholine, and amino acid metabolism including citrulline, methionine, and threonine in non-responders to TJR. While confirmation is needed, our results demonstrated a great potential of a metabolomics signature in the clinical decision-making process when contemplating TJR for primary OA patients.

# Influence of subtype and gender on disease progression and treatment response in osteoarthritis

Anne-Christin Hauschild[1], Chiara Pastrello[1], Igor Jurisica[1,2]

[1]Krembil Research Institute, [2]University of Toronto

**Background**: Osteoarthritis (OA) is a progressive joint disease that features the continuous loss of articular cartilage, and is considered the most common form of arthritis. Given the increasing prevalence and incidence of OA, estimated lifetime risk for knee OA is 40% in men and 47% in women, it is now considered a major public health problem worldwide. Being a disabling disorder, it significantly reduces the quality of life of nearly 4.6 million Canadians.

**Aim**: The aim of this study is to identify temporal clinical and biomedical attributes indicative of arthritis severity or treatment response, controlling for sex-specific differences.

Samples: The Osteoarthritis Initiative (OAI) is a multi-center, longitudinal, prospective observational study of knee OA. It includes clinical data, radiological images and biospecimen data from 4,796 patients (1,992 men and 2,804 women), of age 45-79, undergoing regular checkups every 6 to 12 months. We extracted 1,071 temporal variables, which cover life style related attributes, such as scores for physical activity (e.g., PASE), or OA severity (e.g., WOMAC/KOOS), as well as response variables, including information about the potential occurrence and time period of joint replacements.

**Method**: Our analysis focuses on clinical variables and their associations and predictive potential for OA. At first, each of the compared variables are standardized in order to evaluate their co-linearity over time. The subsequent analysis requires the modelling of the groups in order to estimate their influence on the clinical attributes. Therefore, we utilize mixed-effect models (MEM), which is a particularly well-suited technique to model longitudinal data. MEM are an extension of the more general linear model, which only contains fixed effects representing explanatory variables, such as sex or treatment while modeling the disease progression. Additionally, the MEM consider random variance components that assume a hierarchy of different populations within the data set. We assume that each patient has a specific measurement profile, following a certain distribution around the average of the general population. This variation is modeled by the so called random effect of the MEM.

**Results**: Using MEM, we confirmed known and expected correlations as well as identified interesting new relations. For instance, BMI correlated with body weight and abdominal circumference. We also found an anti-correlation between physical activity and pain score, suggesting a clear advantage of exercise on OA management. It has been previously shown that physical activity has a positive influence in WOMAC but also affects quality of life and depression scores (Mesci et al., 2015). We then focused on sex-specific differences, and identified unhealthy eating habits in men or supplements (vitamins, medication) in females. For example, studies indicated that females with OA hugely benefit from bisphosphonates treatment compared to male OA patients. Further, a possible role for vitamin D as OA treatment has been proposed, but a consensus is still lacking (Garfinkel et al., 2017). We will present how these behavioral and environmental factors affect the disease progression and treatment, controlling for sex-specific differences.

# Session VIII: High-throughput Screening

## Invited Speaker: Dr. David Andrews

*Molecular/Cellular Biology & High Throughput Screening*

Director of and Senior Scientist in Biological Sciences at Sunnybrook Research Institute (SRI), Professor of Biochemistry and Medical Biophysics at University of Toronto and a Tier 1 Canada Research Chair.His research comprises, the molecular mechanisms by which Bcl-2 family proteins regulate apoptosis, the cellular mechanisms of resistance and sensitivity to cancer chemotherapy, the assembly of proteins into membranes, high-content screening and development of new microscopes for fluorescence lifetime imaging microscopy (FLIM) and the application of high content screening to precision cancer treatment. Dr. Andrews uses fluorescence spectroscopy and fluorescence spectroscopic microscopy to study interactions between proteins in membranes using purified proteins and in live cells. By quantifying interactions in live cells his group identifies and validates new drug targets. Using high throughput screening they identify tool compounds used in early drug discovery of novel chemotherapy agents. He has established a facility for image-based high-content cellular analysis at SRI that includes the most sophisticated FLIM and automated confocal microscope based screening instruments in the world. Using these tools his laboratory has discovered and characterized small molecules that have application to cancer and regenerative medicine. Dr. Andrews has collaborated with a number of pharmaceutical companies including ABBVIE, Eli Lilly, Genentech and others on the development of new anti-cancer drugs and biosensors. He is a member of scientific advisory boards including at the Max Plank Institute. He holds licensed patents in areas such as translational regulation, in vitro evolution, peptide display technologies and optical microscopy, and has participated in start-up companies. He is developing a new generation automated high-speed hyperspectral FLIM confocal that is being commercialized.

# RNA-Seq based transcriptome profiling to investigate the role of glycogen synthase kinase 3 signaling in cartilage biology

Supinder Kour Bali[1], Lauren Solomon[2], Dawn Bryce[1], Frank Beier[1]

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Osteoarthritis (OA), the most common form of arthritis that affects diarthrodial joints, is classically characterized by the breakdown of articular cartilage but also involving other tissues in the affected joint in disease pathophysiology and progression. Therefore, prevention or reversal of OA-associated degenerative changes in cartilage are the prime targets in order to maintain and restore joint health. It is, thus, crucial to identify molecular pathways that regulate regenerative potential of cartilage, with the long-term goal to design strategies to regenerate damaged articular cartilage and to prevent or reverse OA progression. Studies have shown the involvement of glycogen synthase kinase (GSK) 3 alpha and beta as key regulators of chondrocyte biology, but their roles in cartilage health and disease are not well understood. In this study, we aim to decipher key GSK-3 dependent processes and functions by RNA-Seq based comparative differential transcriptome profiling of normal mouse articular chondrocytes and those treated with a pharmacological inhibitor of GSK-3. mRNA from these chondrocytes was enriched using rRNA depletion approach, followed by RNA sequencing and bioinformatics analyses using HISAT2 to map alignment of query sequence reads on mouse genome GTF file 'mm10' (publically available at UCSC and Ensembl); HTSeq2 for characteristic count; and DESeq2 for differential gene expression analysis. Enrichment analysis was done using both DAVID and g:Profiler. We used two publically available databases – UCSC and Ensembl - to analyze our RNA-Seq sequence reads; and only included the transcripts represented and annotated in both the databases for enrichment analyses. Results from bioinformatics analysis were further validated by gene expression studies using real-time PCR and other supporting experiments.

Bioinformatics analyses of RNA-Seq data for comparative differential gene expression between the two experimental groups, using a significantly changed gene-list at a corrected-P value cut-off of 0.05, highlighted 324 genes involved in various biological, cellular and molecular processes, as well as skeletal abnormalities associated with the reported human phenotypes. For further validation of results from RNA-Seq data, we performed gene expression analysis by real-time PCR using a subset of these 324 genes, involved in important biological processes as well as in musculoskeletal health and disease. Gene expression of molecules important in cartilage such as *Acan*, *Timp3, Adamts5, Col10a1* and *Frzb* were significantly downregulated upon GSK-3 inhibition in iMACs, supporting our RNA-Seq results. In addition, we also observed significant downregulation of *Ihh, Pthrp*, *Nos2* and *Tnfsf11*, which were not highlighted in comparative differential gene expression from RNA-Seq. Together, these results suggest significant involvement of GSK-3 signaling in cartilage health and disease, and highlighted potential molecular targets involved in various developmental skeletal defects and maintenance of cartilage health.

# Next generation sequencing as a tool for microRNA discovery in knee osteoarthritis

Shabana Amanda Ali[1], Konstantin Shestopaloff[1], Rajiv Gandhi[1], Mohit Kapoor[1]

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**Objectives**: While there are currently no biomarkers that can be used in a therapeutic, prognostic, or diagnostic manner for osteoarthritis (OA), circulating microRNAs have emerged as promising biomarkers for many diseases. As small non-coding RNAs, microRNAs repress expression of target genes. Our lab was the first to perform global array screening of microRNAs in OA. This approach was useful for identifying known microRNAs, but there may be previously unidentified microRNAs that contribute to OA. Next generation sequencing is a high-throughput technology that can identify all known and novel microRNAs in a biological sample. The objective of this study is to use next generation sequencing to identify microRNAs in plasma samples from patients with OA. This technology has never been applied to identifying circulating microRNAs in OA, and has the sensitivity and specificity to detect microRNAs that are unique to various cohorts, including early OA versus late OA.

**Methods**: Plasma samples from early OA and late OA patients were obtained from the Knee Osteoarthritis BioBank at the University Health Network in Toronto, Canada. Cohorts were defined based on Kellgren-Lawrence radiographic grading for OA severity where early OA is grade 1 and late OA is grades 3 & 4. Plasma samples from 10 normal donors, 5 early OA patients, and 10 late OA patients were subjected to next generation sequencing of microRNAs. Differentially expressed microRNAs were identified in an initial sample of 5 normal and 5 late OA patients using negative binomial count modelling with the edgeR (v3.18.1) package in R (v.3.2.2). To determine whether these differentially expressed microRNAs persist in independent samples, analysis of sequencing data from an additional 5 normal donors and 5 late OA patients is underway. To determine whether this expression pattern changes according to disease severity, analysis of sequencing data from 5 early OA patients is underway.

**Results**: Sequencing analysis of 5 normal donors and 5 late OA patients revealed a total of 2579 known and 59 novel microRNAs. After filtering for microRNAs with at least 10 counts per million of classified sequences in at least 2 of 10 samples, 314 known and 15 novel microRNAs were identified. From this, a list of top 20 differentially expressed microRNAs was generated based on defined criteria, including false discovery rate 2, log fold change > 1.5, and p-value < 0.0003. Hierarchical clustering of these microRNAs was visualized and revealed a distinct expression pattern between normal and late OA samples. Among these microRNAs is a known candidate previously shown to be dysregulated in OA synovial fluid, showing a 3.22-fold increase in OA plasma, and a novel putative microRNA that has not previously been characterized, showing a 3.95-fold decrease in OA plasma.

**Conclusions and Future Directions**: Our results suggest that next generation sequencing is a useful technology for identifying known and novel microRNAs in OA. Ongoing sequencing experiments that include early OA samples will be followed with validation by real-time PCR in larger cohorts [early OA (N=100), late OA (N=100), and normal donors (N=100)]. Statistical analysis will allow identification of microRNA signatures that can be used to distinguish patient cohorts. We anticipate identifying specific circulating microRNAs as valid and reliable biomarkers with potential applications for improving OA detection and treatment.

# Session IX: Tissue Fibrosis: Mechanisms and Therapeutic Targets

## Invited Speakers: Dr. David Lagares

### *Matrix and Mechanobiology*

Dr. David Lagares is the Director of the Matrix and Mechanobiology Program at the Fibrosis Research Center in the Massachusetts General Hospital (MGH) and a faculty member at Harvard Medical School (HMS). Dr. Lagares holds B.Sc. and M.Sc. degrees in Biochemistry and Genetics, respectively. He earned his Ph.D. degree in Biomedical Research in 2012 from the Autonomous University of Madrid, Spain, and completed his postdoctoral training in the laboratory of Dr. Andrew Tager at MGH/HMS, Boston in 2014. Dr. Lagares recently established his own laboratory in the Division of Pulmonary and Critical Care Medicine at MGH. His laboratory investigates the fundamental mechanisms that balance tissue regeneration and fibrosis following injury, with an emphasis on the biochemical and biomechanical drivers of scar-forming myofibroblast activation. Specifically his current research focuses on reversing organ fibrosis via targeted apoptosis of myofibroblasts, mechanobiology of fibrotic disease, and the ADAM10-sEphrin-B2 pathway in tissue fibrogenesis. His laboratory utilizes cutting edge molecular biology techniques, new bioengineering assays, genetic manipulation of mice, animal modeling of fibrotic disease, and translational studies in humans to understand these disease processes. Ultimately, the goal of the Lagares Laboratory is to develop innovative anti-fibrotic therapies for the treatment of human diseases such as systemic sclerosis (scleroderma) and idiopathic pulmonary fibrosis (IPF).

## Invited Speaker: Dr. Andras Kapus

*Cell (patho)physiology and Cell Biology*

Dr. Kapus obtained his MD (1986) and PhD (1990) from cell physiology at the Semmelweis University, Budapest Hungary. He was postdoctoral fellow at the Division of Cell Biology in the Hospital for Sick Children (1992-1995) in Toronto, and was then recruited as a basic scientist to the Toronto General Hospital Research Institute and Dept. Surgery in 1997. He was an MRC Scholar (1999-2004). Since 2005 he works at the St. Michael's Hospital/Keenan Research Centre (KRC) for Biomedical Research. He is a full professor (Dept. Surgery and Dept. Biochemistry), the Associate Vice Chair of Research at the Dept. Surgery and the Director of the Critical Care/Trauma/Inflammation Research Platform at KRC. His research area is basic cell (patho)physiology and cell biology, specifically cellular stress signaling, volume and pH regulation, cytoskeleton remodeling and the role of the cytoskeleton in gene expression, epithelial-mesenchymal transition, cell plasticity. He explores molecular mechanisms whereby the cytoskeleton regulates nuclear traffic of transcription factors and thereby cell fate and phenotype. This has strong relevance to his current major focus: the pathobiology of organ (kidney) fibrosis. He has published >147 peer-reviewed papers (H-index: 58), and have been continuously supported by CIHR, the Kidney Foundation of Canada and NSERC. He has been involved in the graduate training of >50 students. He was the recipient of the Elsie Winifred Crann Memorial Trust Award for Medical Research, Scholar of the Medical Research, the Premier's Research Excellence Award, Mel Silverman Mentorship Award, the James Waddell Mentorship Award and the Keenan Scientist Legacy Award.

# Abstracts for 3-minute elevator pitch

**Myofibroblasts attract macrophages in fibrillar collagen**

Boaz Wong[1], Pardis Pakshir[1], Boris Hinz[1]

[1]University of Toronto

**Background:** Successful tissue repair requires the coordinated appearance and clearance of inflammatory macrophages (Mφ) and collagen-remodelling myofibroblasts (MFs). Dysregulated communication between both cell types results in either fibrosis or chronic healing. We found that contractile MFs mechanically attract migratory Mφ in collagen extracellular matrix (ECM). The mechanism how fibroblast remodeling activities guide Mφ migration in ECM and which Mφ receptors mediate mechanosensing are yet unknown.

**Hypothesis:** MF contraction of collagen produces mechanical signals in the ECM that attract Mφ.

**Objective:** To elucidate the molecular mechanism and receptors of Mφ mechanosensation in response to mechanical cues produced by contracting MF in collagen ECM.

**Methods:** To study whether Mφ migration is guided by dynamic or structural cues in collagen ECM, primary mouse MFs and human MRC-5 fibroblasts pre-remodelled collagen gels for 0-18 h. Collagen alignment was assessed using confocal reflection microscopy. Mouse and lineage THP-1 human Mφ were then added and their migration was tracked relative to MF positions for another 6 h using live video microscopy. The density of Mφ around MFs after 6 h was quantified from immunostaining. To identify Mφ mechanoreceptors, THP-1 were seeded onto collagen or control fibronectin substrates and processed for Western blotting for known receptors of fibrillar collagen.

**Results:** The degree and range of collagen fibril alignment increased over time of MF remodelling. In contrast, the displacement rate (velocity) of ECM fibres decreased with increasing remodelling time and distance from the MF. Mouse and human Mφ migrated towards contracting MFs only on fibrillar collagen that underwent active displacement. Concomitantly, the percentage of attracted Mφ decreased with increasing collagen remodelling from 65-68% (1-3 h), over 29% (6 h), to 27% (18 h), when collagen alignment was highest but displacement rate lowest. Human Mφ expressed DDR-1 and β1 integrin. DDR-1 phosphorylation was upregulated in Mφ on fibrillar collagen versus fibronectin.

**Conclusions:** Mφ-to-MF attraction requires an active collagen displacement component potentially sensed through DDR-1 receptors in fibrillar collagen ECM.

**Major vault protein holds the key for DDR1-β1 integrin interaction**

Nuno M. Coelho[1], Max Kotlyar[2], Igor Jurisica[2,3], Christopher A. McCulloch[1]

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The discoidin domain receptor 1 (DDR1) is a collagen adhesion mechanoreceptor associated with fibrotic conditions of kidney, liver, lung and perivascular tissues. We showed earlier that DDR1 is centrally involved in mechanical realignment of collagen by traction forces, which is consistent with the increased expression of DDR1 in several different types of cancer and fibrotic conditions. We considered here that DDR1 contributes to cancer and tissue fibrosis by regulating β1 integrindependent, MAPK signaling. DDR1 over-expression reduced ERK phosphorylation 5-fold in cells expressing β1 integrin when plated on collagen, while plating on fibronectin or inhibition of DDR1 phosphorylation with nilotinib rescued ERK phosphorylation. A phospho-site screen indicated that DDR1 activation inhibit β1 integrin-dependent ERK signaling through regulation of the focal adhesion kinase (FAK) autophosphorylation. In FAK null mouse embryonic fibroblasts, and in cells treated with a FAK inhibitor, collagen-induced ERK activation was reduced, indicating that collagen-induced ERK activation is dependent on FAK. Immunoprecipitation and mass spectrometry showed that while DDR1 and FAK do not interact directly, the major vault protein (Mvp) binds to DDR1 and FAK. In cells expressing both DDR1 and β1 integrins and then cultured on collagen, Mvp associated with DDR1 while in cells cultured on fibronectin, Mvp associated with FAK. Mvp knockdown rescued ERK activation in DDR1 over-expressing cells cultured on collagen. Our results indicate that collagen-induced DDR1 activation downregulates β1 integrin-dependent ERK signaling by sequestering Mvp. Thus DDR1 is a strong regulator of β1 integrin-dependent pro-fibrotic signaling pathways.

**CUB Domain Containing Protein 1 (CDCP1) is a novel negative regulator of TGFβ signaling and myofibroblast differentiation**

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**Rationale:** Fibroblasts are essential producers of extracellular matrix (ECM) in normal lung to maintain tissue homeostasis. During tissue fibrosis, transforming growth factor-beta1 (TGF-β1) activates fibroblasts and transforms them into myofibroblasts, which secrete increased amounts of ECM and exhibit enhanced contractile activity. Apart from well-studied TGF-β1 receptors, little is known about other growth factor receptors regulating myofibroblast activation. We recently identified Cub domain containing protein 1 (CDCP1) in cell-surface proteome analysis to be strongly downregulated by TGFβ1. CDCP1 is a transmembrane glycoprotein with yet unknown function in human lung fibroblasts.

**Hypothesis**: CDCP1 modulates TGF-β1-induced lung fibroblast-to-myofibroblast activation.

**Objective**: To investigate the impact of TGF-β1 on CDCP1 expression and function in lung fibroblasts.

**Methods:** To analyze CDCP1 expression in human lung fibroblasts, we immunostained lung tissue slices obtained from healthy donors and patients with idiopathic pulmonary fibrosis. To study the regulation of CDCP1 during myofibroblast activation, we performed qPCR, Western blotting, immunoprecipitation, FACS analysis, and cell adhesion assays with primary human lung fibroblast cultures ±TGF-β1. To investigate CDCP1 function, we knocked-down CDCP1 in cultured lung fibroblasts using specific siRNA.

**Results:** CDCP1 is expressed in fibroblasts, but not in myofibroblasts in fibrotic lung tissue. *In vitro,* TGF-β1 treatment resulted in downregulation of CDCP1 expression on mRNA (2.9-fold) and protein (2.8-fold). CDCP1 downregulation upon TGF-β1 treatment was independent from ubiquitin-mediated proteasomal degradation. TGF-β1-treatment reduced the percentage of surface CDCP1-positive lung fibroblasts (73.5±14.8%) compared to non-treated fibroblasts (85.7±10.0%). CDCP1-depleted lung fibroblasts exhibited an increased expression of profibrotic markers collagen V and α-smooth muscle actin, independently from TGF-β1. Knock-down of CDCP1 in the presence of TGF-β1 resulted in increased fibroblast adhesion (2.0-fold) and significantly enhanced Smad3 phosphorylation (2.2-fold).

**Conclusion:** CDCP1 is a novel negative regulator of TGF-β1 signaling in fibroblast-to-myofibroblast transdifferentiation.

**Mechansims of macrophage mechanosensation in collagen matrix**

Pardis Pakshir[1,2,3], Moien Alizadehgiashi[4], Boaz Wong[5], Nuno Miranda Coelho[2], Christopher McCulloch[2], Boris Hinz[1,2,3]

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**Background:** Intimate communication between macrophages (Mφ) and fibroblasts is important for tissue repair after injury and miscommunication can lead to pathological healing and fibrosis. Our previous results show that single contracting fibroblasts generate deformation fields in fibrillar collagen extracellular matrix (ECM) that provide far-reaching physical cues for Mφ. We found that Mφ detect and follow local strain rate changes in their substrate but the mechanisms of Mφ mechanosensing remain elusive.

**Hypothesis:** Mφ use collagen binding receptors and stress-activated membrane channels to sense and respond to local deformations in their ECM.

**Objective:** To elucidate the molecular mechanism and receptors of Mφ mechanosensation in response to dynamic mechanical cues transmitted through collagen ECM.

**Methods:** Mouse bone marrow-derived Mφ were seeded onto fibrillar collagen ECM. To identify potential mechanosensors, we assessed fibrillar collagen receptors: discoidin domain receptors (DDRs), integrins α1β1 and α21β1, using Western blotting and flow cytometry. To study the involvement of these receptors in mechanosensing, Mφ were incorporated into 3D collagen ECM and subjected to cyclic uniaxial strain. Mφ were then fixed and stained for cytoskeletal markers of cell migration polarization to identify Mφ mechano-responses to ECM strain. To mimic force fields produced by single contractile fibroblasts, Mφ migration was tracked on collagen ECM that was gradually deformed using micromanipulator-controlled microneedles. Both experimental series were performed in presence and absence of collagen receptor and ion channel inhibitors.

**Results:** Recruitment of α2β1 integrin was upregulated in Mφ exposed to fibrillar collagen versus non-coated substrates. Upon periodic stretching, Mφ oriented within collagen gels, indicating mechanosensing. Collagen receptor function-blocking antibodies, most prominently β1 integrin inhibitors, altered Mφ alignment indicating their functional involvement in Mφ mechanosensation. Mφ migrated towards actuated microneedles, which was impaired in the presence of selective ion channel blockers indicating a functional role of specific membrane channels in Mφ mechano-responses to ECM strain.

**Conclusions:** Mφ mechanosensing of strain in collagen ECM is dependent on membrane ion channels and β1 integrins.

**Engineering and basic science meet clinic: dermal skin substitute for burn patients**

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**Hypothesis:** There is an immediate need for affordable skin substitutes despite significant developments in the management of severe skin loss. Gelatin is an economical, natural biomaterial which is frequently used for tissue engineering applications. However, it suffers from a lack of sufficient mechanical strength and associated difficulties with handling. Poly-carbonate urethanes (PU) are biodegradable elastomeric biomaterials that can be spun into fibrous scaffolds, with excellent cell compatibility and non-toxic degradation products. We hypothesized that the addition of 20% PU to gelatin (Gel80-PU20) would improve the mechanical strength and degradation rate of electrospun gelatin without alteration in the cell behavior.

**Methods**: A new gelatin-based electrospun scaffold was fabricated for skin regeneration via the addition of 20% PU. It was compared with gelatin (Gel100) and Integra® as a commercial dermal regenerative matrix (DRM). Physical and mechanical characterization as well as *in vitro* viability assay were performed to evaluate the effect of adding PU. Also, the scaffolds were transplanted on mice and pigs for 20 and 30 days respectively for *in vivo* assessments.

**Results & Discussions**: Scaffold morphology is a determining factor for cell compatibility and successful regeneration. Adding PU to gelatin did not change the fiber and pore size of the scaffold while tensile strength and elongation were significantly improved. This shows the role of PU in the polymer. In relation to the same effect, collagenase degradation assay showed that adding 20% PU to Gel100 improves degradation resistance from few hours to 14 days. The composite polymer can also hold liquid 10 times of its dry weight which is important in wound healing in terms of absorbing wound exudates and also preventing the wound from drying. Also more than 90% of the cells were viable on all scaffolds at 7 days post seeding *in vitro*, showing no adverse effect due to the presence of PU. More α-SMA expression, characteristics of the myofibroblastic phenotype, was seen on the Gel100 scaffold that can be due to faster degradation of the Gel100 scaffold. Trichrome staining revealed that the transplanted electrospun scaffold on the mice was more populated with host cells compared with DRM. It was also seen that as the cells infiltrate from wound bed, they degrade the scaffold. F4/80 immunostaining was performed to unravel the cellular subtype of infiltrated cells and to verify the extent of inflammation. Significantly fewer macrophages were observed on the Gel80-PU20 scaffold compared with the DRM. This indicates that the composite scaffold does not elicit a foreign body response or chronic inflammation after transplantation and probably the granulation tissue matured, shifting to the remodeling phase. Ideal skin substitute should prevent excessive wound contraction which can further give rise to scarring. α-SMA immunostaining showed that significantly more $\alpha SMA^{+}$-myofibroblasts are present on the DRM compared to Gel80-PU20. This might be beneficial for preventing wound contraction and scarring in favor of skin regeneration.

**Conclusions:** Gel80-PU20 scaffold is a promising composite polymer in preclinical studies and might serve as an ideal scaffold for skin substitute using various skin progenitor cells.

**Impact of non-steroidal anti-inflammatory drugs on mast cells during bone repair**

Jose Ramirez-GarciaLuna[1,2], Karla Rangel-Berridi[1,2], Timothy H. Wong[1,3], Janet Henderson[1,2], Paul Martineau[1,2]

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**Introduction**: Bone repair starts with an inflammatory response during which immune and vascular precursor cells are attracted to the defect and stem cells differentiate into osteoblasts to repair the tissue. This process can potentially be modified by disturbances in the inflammatory environment, such as the chronic use of non-steroidal anti-inflammatory (NSAIDs) drugs, which are clinically administered for pain relief. Our previous work has demonstrated that mast cells, a subgroup of inflammatory cells, regulate angiogenesis, osteoclast activity, and the inflammatory environment during bone healing. The goal of this project is to identify the mechanism whereby chronic administration of NSAIDs modifies bone repair. Our objectives are to determine the impact of NSAID treatment on mast cell, macrophage, osteoblast and osteoclast activity in mice with and without systemic inflammation secondary to LPS administration, and untreated controls.

**Methods**: Bone repair in skeletally mature Bl6 mice was quantified in 2mm cortical window defects drilled in the femur. Mice were randomly assigned into treatment groups: 1) NSAID (5 mg/kg diclofenac); 2) NSAID + LPS (diclofenac + 25µg LPS); or 3) placebo. The mice were allowed free ambulation for 14 days before euthanasia and femur harvest. The quantity and quality of bone and re-vascularization of the defect were quantified using micro CT. Osteoblast, osteoclast, vascular endothelial cell, mast cell and macrophage distribution pattern and activity were assessed by histology. Comparisons were made by ANOVA and Tukey post-hoc tests.

**Results**: NSAID and NSAID + LPS treated mice had significantly less bone than control mice, with fewer trabeculae that were less well connected and more porous. These morphological features were reflected in the histological sections, where significantly less osteoblast and osteoclast activity were identified. The pattern of re-vascularization differed significantly amongst the groups. Control animals treated with placebo showed an extensive network of capillaries in the repair tissue and adjacent bone marrow. NSAID treated mice showed an enlarged capillary network around the newly formed bone, but few blood vessels in the marrow, which was heavily infiltrated by fat. NSAID + LPS treated animals showed disorganized revascularization with numerous, smaller vessels and multiple isolated CD34 positive cells. Mast cell number and distribution varied across groups. While in control mice mast cells were predominantly at the proximal end of the defect, those in NSAID treated mice were grouped at the distal side and in significantly higher numbers. In NSAID + LPS treated animals, we found significantly less mast cells than in the placebo treated mice with no difference in the distribution pattern. Macrophage number and distribution closely matched that of mast cells, strongly suggesting a functional axis between these two types of cells. Build-up of fibrous tissue could be observed near where macrophages clustered.

**Conclusions**: Systemic treatment with NSAIDs during the inflammatory phase of bone repair impairs healing by inhibiting bone formation and turnover. These drugs affect the repair tissue probably by modifying mast cell and macrophage activity and distribution, which in turn affect blood vessels and modify the extracellular matrix. The widespread use of NSAIDs for pain management in patients who are at risk of complications in bone healing should be re-considered.

**Microfluidic Platform for Investigation of Mechanoregulation of Breast Cancer Bone Metastasis**

Xueting Mei[1,2], Kevin Middleton[2], Yu-Heng Ma[2], Liangcheng Xu[2], Noosheen Walji[1], Edmond Young[1,2], Lidan You[1,2]

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**INTRODUCTION:** Approximately 70% of advanced breast cancer patients experience bone metastasis (1). Breast cancer cells (BCC) that invaded across the endothelium to the bone have devastating impacts on bone quality by interacting with osteoclasts to alter the bone remodeling process. Exercise, an often-used cancer intervention strategy, regulates bone remodeling process and could affect BCC metastasis to bone. As the major mechanosensory cell in bone, osteocytes can be a key regulator. Although recent *in vitro* studies showed that mechanically stimulated osteocytes increased BCC migration and modified endothelial cell (2), there lacks a physiological relevant *in vitro* model that addresses trans-endothelial invasion of BCCs in response to mechanically stimulated osteocytes. Therefore, we present the design and validation of an *in vitro* microfluidic tri-culture model for studying mechanical regulation of breast cancer metastasis.

**METHODS:** A previously established microfluidic endothelial lumen model (3) will be modified to study the effect of mechanically stimulated osteocytes on BCC invasion. More specifically, highly metastatic MDA-MB-231 human BCCs will be cultured inside an endothelialized lumen (human umbilical vein endothelial cells, HUVECs) that is adjacent to a population of either static or mechanically-stimulated osteocyte-like MLO-Y4 cells. Soluble factors will diffuse through hydrogel-filled side channels to instigate inter-cellular communication between MLO-Y4 cells and BCCs over a period of 3 days.

Microfluidic model validation experiments were based on BCC transendothelial invasion to the adjacent MLO-Y4 cells under static condition, with or without RAW264.7 differentiated osteoclast-conditioned media (CM). BCC transendothelial invasion distances were measured against the edge of lumen, normalized to the control with acellular osteocyte growth media, and compared between the MLO-Y4 cells with or without osteoclast CM. In addition, MLO-Y4 calcium signaling experiment will be used to validate the integration of on-chip mechanical loading, where MLO-Y4 cells stained with Fura 2AM dye will be exposed to oscillatory fluid flow (OFF) (1Pa; 1Hz; 4min) produced by a customized pump (4).

**PRELIMINARY RESULTS:** An endothelial lumen was successfully cultured in the microfluidic device, with DAPI (blue) and VEcadherin (green) stained. Invasion validation results show that BCCs invaded further toward static osteocytes in the presence of osteoclast CM, with 37% increase ($p<0.02$) compared to without osteoclast CM, likely due to osteoclast support of cancer cell growth (5).

**DISCUSSION AND CONCLUSION:** This microfluidic system is a significant improvement on the current *in vitro* models for studying bone metastasis. This platform allows the integration of physiological relevant OFF and real-time signaling between different cell populations. Future work with this platform will elucidate the effects of bone mechanical loading on BCC transendothelial invasion and determine the key mechanisms involved in osteocyte regulation of BCC metastasis.

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**BMP-2 revealed enhanced healing in fractured mouse tibia using micro-CT and torsion test**

Sotcheadt Sim[1], Theresa Farhat[2], Martin Pellicelli[2], Martin Garon[1], Eric Quenneville[1], Rene St-Arnaud[2]

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Bone morphogenic protein (BMP)-2 is known as a potent inducer of bone formation and fracture repair and is FDA approved for clinical use for open tibia fractures. Torsional testing offers a great advantage in studies of treated/fractured bones since they are not affected by the orientation of asymmetric calluses. Thus, in this study, we investigated the ability of torsion tests to monitor the mechanical properties of fractured mouse tibias after treatment with BMP-2.

An intramedullary rodded surgical fracture repair model was performed on three-month old wild type female C57BL/6 mice. An absorbable collagen sponge (ACS) was used as a carrier to deliver 10µg of recombinant human BMP-2 (rhBMP-2) diluted in 3µl sterile water, at the fracture site. Two treatment groups were defined as a vehicle group receiving water-loaded ACS and a treatment group receiving 10µg of recombinant human BMP-2 (rhBMP-2)-loaded ACS. The healing bones were collected and assessed through micro-CT and torsional testing at day 15 and day 21 post-fracture. Tibiae were scanned using a SkyScan 1272 high-resolution micro-CT (Bruker) and reconstruction was performed using the SkyScan NRecon Program (Bruker). Callus total volume ($TV_{Callus}$, $mm^3$), callus bone volume ($BV_{Callus}$, $mm^3$) and callus mineralized volume fraction ($BV/TV_{Callus}$, %) were determined for each specimen. Subsequently, torsional testing was performed using the mechanical tester Mach-1 model v500ct (Biomomentum) with a 70N multi-axial load cell (torque resolution of 25 µN·m). Each specimen was kept hydrated during sample preparation and mounting. Then, each sample was subjected to a torsion test with a loading rate of 5 deg/s. The ultimate torque ($T_{max}$, N·mm) and torsional stiffness (*GJ*, N·mm/deg) were extracted from the torque-rotational displacement curves.

At 15 days post-fracture, mice that received BMP-2 displayed significant higher $TV_{Callus}$ (50.4±1.4$mm^3$ *vs* 22.6±6.3$mm^3$; $p<0.0001$), $BV_{Callus}$ (8.8±0.5$mm^3$ *vs* 4.5±1.3$mm^3$; $p<0.0001$), ultimate torque (12.4±2.6N·mm *vs* 6.7±1.0N·mm; $p=0.01$) and torsional stiffness (0.47±0.09N·mm/deg *vs* 0.27±0.05N·mm/deg; $p=0.008$) than the vehicle group. However, the callus mineralized volume fraction was significantly lower in BMP-2 treated group (17.4±1.0% *vs* 19.7±1.1%; $p=0.01$). At 21 days post-fracture, significant higher $TV_{Callus}$ (44.3±6.6$mm^3$ *vs* 17.3±2.8$mm^3$; $p<0.0001$) and $BV_{Callus}$ (5.3±0.6$mm^3$ *vs* 2.9±1.2$mm^3$; $p=0.003$) were still observed in the treated group while the mineralized volume fraction (12.1±0.5% *vs* 16.2±4.1%; $p=0.06$), the ultimate torque (11.1±2.7N·mm *vs* 13.2±3.4N·mm; $p=0.9$) and torsional stiffness (0.38±0.11N·mm/deg *vs* 0.59±0.04N·mm/deg; $p=0.17$) were similar in both groups. This suggests that mechanical competence of the bone (forces required to disrupt the callus), mechanical strength and elasticity of bone of the callus has been reached faster in the BMP2 treated group.

The present study is a first step toward the establishment of torsion testing as a means of understanding the mechanical behavior of callus tissues and their role in reestablishment of stiffness and strength during the fracture healing of bone.

# A new approach for reversing osteoporotic bone loss with a conjugated drug (C3) in an ovariectomized (OVX) rat model

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**Introduction**: Pathological bone loss is a regular feature of osteoporosis owing to negative bone balance due to increased bone tissue turnover and resorptive activity which is much greater than bone formation. There is an unmet need to find new anabolic drugs to rebuild bone lost to osteoporosis. By linking a PGE2 receptor analog (EP4a) to a bisphosphonate (C1) we have shown that bone loss can be recovered. However this conjugated drug did not allow for bone remodeling and produced excessive bone tissue. In this research study we adopted a conjugate bone-targeting approach where a synthetic, stable EP4 agonist is covalently linked to an inactive alendronate (ALN) that still binds to bone and allows for physiological bone remodeling.

**Methods**: In this curative experiment, the following 7 groups of rats were treated for 8 weeks (after losing bone for 12 weeks). 1) Sham, 2) OVX, 3) OVX +C3 low and high doses (4), 5) OVX+C1, 6) inactive ALN alone and 7) mixture of unconjugated ALN and EP4a (to assess the effect of conjugation). We used microCT to determine bone architecture; static and dynamic histomorphometry to determine bone turnover and biomechanics to measure bone mechanical properties.

**Results**: This experiment demonstrated that C3 treatment significantly increased vertebral bone mineral density and trabecular bone volume versus OVX controls. Biomechanical testing showed that C3 treatment led to significant improvement in the load bearing abilities of the vertebrae compared to OVX controls; C3 stimulated bone formation and increased load bearing in femurs. We have shown that C3 led to significant anabolic effects on trabecular bone while anabolic effects associated with C1 were beyond physiological levels. Conjugation between the EP4a and ALN components was crucial to the anabolic efficacy.

**Conclusion**: The C3 conjugate demonstrate, for the first time, that a combined therapy using an anabolic agent linked to an inactive ALN has significant anabolic effects, yet allow bone remodelling.

**Low-cost 3D printed biodegradable scaffolds, a potential graft substitute materials.**

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**Introduction:** Disc degeneration is a leading cause of back pain. Once the disc reaches a very late stage of degeneration, Surgery to fuse the vertebral bodies become the only option and these defects often require grafting with either autografts, allografts or commercial materials such as cement. However, these standard methods of grafting bear multiple limitations such as donor site morbidity, insufficient quantity or infections. 3D printing provides an alternative attractive option to rapidly prototype numerous scaffold designs using biocompatible, biodegradable thermoplastics for tissue engineering applications. This study will demonstrate the ability of low-cost 3D printed poly-lactic-acid (PLA) scaffolds in conducting primary human osteoblast adhesion, growth and osteogenic matrix deposition. It will also test the hypothesis that human mesenchymal stem cells will adhere, proliferate, and adopt osteogenic phenotype when seeded within these scaffolds.

**Methods:** Orthogonal scaffolds were designed using Solidworks software in three different pore sizes (0.5, 0.75 and 1.0mm). They were printed using FDA approved PLA (MakerGeeks) on a Flashforge Creator Pro desktop printer with a 0.3 mm nozzle. Primary human osteoblasts were isolated from human vertebral bodies with institutional ethics approval. Scaffolds were seeded with $5x10^5$ cells/scaffold and allowed to equilibrate for 24 hours, after which osteogenic medium was applied for the whole 21 days culture period. Samples were either fixed in 4% PFA then stained with Alizarin red, or extracted in 4M gaunidine hydrochloride and subjected to western blot probing for osteopontin expression. Human MSCs were obtained commercially and seeded on the medium size (0.75mm) scaffolds. The same protocol was applied for culture and analysis.

**Results:** 3D-printed scaffolds were successfully fabricated using low-cost 3D printing. Mechanical tests showed increased stiffness with decreased pore size. Cell-seeded scaffolds showed cell growth on three pore sizes after 21 days culture. Mineralization and Calcium deposition was detected in all scaffolds (with the most on the 0.75 mm size) supporting osteogenic matrix deposition. Semi-quantitative data of osteopontin expression was highest on the 0.75mm scaffolds. Osteogenic differentiation markers will be followed at different time points of culture.

**Conclusion:** The low-cost 3D printer was able to reproducibly fabricate scaffolds of varying pore size with high-precision. Scaffolds showed high compatibility for cell growth and differentiation with appropriate mechanical properties in vitro. We conclude that the 0.75mm pores size will be suitable for bone-like matrix deposition. These results suggest that this technology is highly feasible for bone tissue engineering studies. Future studies aim to test the feasibility of bone repair using these scaffolds in vivo.

**MicroRNA-34a: Role in the development of osteoarthritis during obesity**

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**OBJECTIVE**: In humans, we have reported that microRNA-34a (miR-34a) is expressed at significantly higher levels in the synovial fluid of end-stage knee osteoarthritis (OA) patients compared to early OA. Studies have shown that miR-34a levels are also elevated in obesity. Previous studies in our lab show that mice fed a high-fat diet (HFD) exhibit an accelerated surgically-induced OA phenotype compared to lean diet (LD) mice. Despite the strong association between obesity and OA pathogenesis, no studies have examined the role of miR-34a in the development of OA during obesity. We hypothesize that during obesity expression of miR-34a is elevated and contributes to OA pathophysiology.

**METHODS**: Mouse blood was collected via saphenous vein at 9 weeks old and at the end of a HFD or LD course, which spans 18 weeks. Human plasma was taken at preadmission from end-stage OA patients undergoing total knee replacement. OA patients with no co-morbidities were segregated according to body mass index (BMI) into non-obese (BMI=18.5-29.9 kg/m2) and obese groups (BMI≥30kg/m2). Chondrocytes and synovial fibroblasts (SF) were transfected with 100nM miR-34a mimic or inhibitor for qRT-PCR or Western blot. In-vivo grade mir-34a mimic or inhibitor was injected intra-articularly in mouse knee joints.

**RESULTS**: Plasma miR-34a was significantly up-regulated in HFD mice compared to baseline mice and LD controls. Mice fed a LD showed no change in plasma miR-34a compared to baseline levels. Similarly, human plasma miR-34a was sig. upregulated in obese end-stage OA patients compared to non-obese end-stage OA patients. Notably, OA patients expressed sig. higher plasma miR-34a than healthy controls. In-situ hybridization showed HFD mouse knee joints expressed higher levels of miR-34a than LD mouse knees and localized to cartilage and synovial membrane. Chondrocytes treated with miR34a mimic had a sig. reduction of SIRT1 (a direct target of miR-34a), anabolic (type II collagen and aggrecan) and autophagy markers, as well as, elevated catabolic markers (MMP13), suggesting that miR-34a contributes to cartilage degeneration. Chondrocytes treated with mir-34a inhibitor reversed these effects. SFs treated with miR-34a mimic expressed sig. elevated inflammatory (TNF-α, IL-6), fibrotic (TGF-β, Type 1 Collagen), and autophagy markers, suggesting that miR-34a is involved in mediating synovial inflammation and fibrosis. SFs treated with miR-34a inhibitor reversed these effects. Interestingly, intraarticular injection of miR-34a mimic induced cartilage damage and loss of proteoglycan content; however, mir-34a inhibitor injections in surgically-induced OA mice was cartilage-protective.

**CONCLUSIONS**: This is the first report to show that mir-34a is up-regulated in the plasma of obese individuals with endstage OA compared to non-obese OA individuals, as well as, in the plasma, cartilage and synovium of obese mice compared to lean mice showing that miR-34a levels are highly responsive to a HFD. Next, our data shows that miR-34a is involved in cartilage degeneration by promoting chondrocyte loss and catabolic activity, as well as, promoting synovial inflammation and fibrosis. Inhibiting miR-34a in-vitro is not only chondroprotective, but also inhibits markers of synovitis. Notably, our preclinical studies complement these findings. Thus, targeting miR-34a may provide therapeutic benefit in delaying or preventing the progression of OA.

**Monocyte/macrophages in osteoarthritic synovial fluid are correlated with patient-reported outcomes**

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Osteoarthritis (OA) is a progressive and debilitating joint disease with high prevalence. OA involves chronic, low-grade inflammation of the synovium (synovitis). Inflammatory cells, particularly monocytes/macrophages (MΦs) are abundantly present in OA synovium and involved in OA progression. However, the types of MΦs present in the OA synovial fluid (SF) have not been investigated and the relationship between SF cells and patient symptoms have not been previously deciphered. We hypothesize that levels of SF-resident MΦ subsets are indicative of symptomatic OA.

**Methods**: Synovial fluid leukocytes (SFLs, N=81) and peripheral blood mononuclear cells (PBMCs, n=51) from OA patients were characterized. Linear models were used to determine the correlation between SF MΦs with patient-reported and radiographic outcomes; these models were adjusted for sex, age and body mass index.

**Results**: SF MΦs (36.5%) are the most abundant SFLs and are present in an activated state. Within these, inflammatory MΦs ($CD14^+CD16^+$, 39.2% vs 5.9% in circulating MΦs) are more abundant in the SF than circulation. Levels of $CD14^+CD16^+$ MΦs, which express high levels of PAR2 (61.5% PAR2+), a receptor involved in OA pain, correlates with worse patient-reported function, pain during locomotion and stiffness ($\beta>0.38$), but not with radiographic outcomes.

**Conclusion**: Prevalence of SF MΦ correlates with clinical outcomes, respectively and is indicative of symptomatic OA phenotype, and may serve as means to objectively measure OA therapy outcomes and targets of novel therapeutics.

**Intra-articular injection of microRNA-181a-5p inhibitor attenuates cartilage degeneration in both facet and knee osteoarthritis animal models**

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**Objectives**: Osteoarthritis (OA) is among the leading cause of severe pain and disability worldwide. The degeneration of cartilage is hallmark of OA. However, exact endogenous mechanisms associated with cartilage degeneration during OA are unknown. We recently discovered that miR-181a-5p plays an active role in destroying facet joint (FJ) cartilage by elevating the inflammatory, catabolic and apoptotic/cell death activities in vitro and in vivo (Filed in US patient; No 62/299,305). This led us to hypothesize that inhibition of miR-181a-5p may be a potential therapy not only in FJ but also other OA joints such as knee. Therefore, in this study we tested the effect of miR-181a-5p inhibition on degree of cartilage degeneration in both FJ and knee OA animal models. **Methods**: Injury (needle puncture)-induced FJ OA model in Sprague-Dawley rats and trauma (DMM)-induced knee OA model in C57BL/6J mice were used for our study as animal models. In-vivo grade miR-181a-5p inhibitor (anti-sense molecule; 5 µg/µl) or control inhibitor (negative scramble control; 5 µg/µl) were injected into the two levels of rat lumbar FJ joints (n=10/ group) at 3 and 6 weeks or mice knee joints at 2 and 4 weeks post-surgeries (n=10/ group). Histopathological analysis including safranin O, in situ hybridization (ISH) and immunohistochemistry (IHC) assessments (MMP13, COL10A1, PARP p85, cleaved caspase3 and cleaved COL2A1 markers) was performed at 12 weeks for rat FJ OA and 10 weeks for mouse knee OA models. Human knee OA cartilage/chondrocytes, rat FJ and mouse knee chondrocytes were cultured with either miR181a-5p in-vitro grade inhibitor or control inhibitor and then extracted RNA or cells were used for further studies including qPCR and flow cytometric analysis. **Results**: Using both ISH and qPCR, we first confirmed that expression of miR-181a-5p in FJ and knee OA cartilage is upregulated in both human and animal (rat and mouse) compared to control (non-degenerated) FJ and knee cartilage. We then discovered that silencing the expression of miR-181a-5p significantly down-regulates cartilage destruction markers and apoptosis/cell death activities, and up-regulates production of type II collagen (Col2a1) in vitro. We tested the effect of miR181a-5p in vivo inhibition on cartilage degeneration by intra-articular injection into rat FJ OA and mouse knee OA models. We observed a marked reduction in the severity of FJ OA and knee OA, including decreased cartilage degeneration, proteoglycan loss and chondrocyte cellularity compared to control. IHC assessment further revealed significant reduction in catabolic, hypertrophic, apoptotic/cell death and Col2a1 breakdown markers in both rat FJ and mouse knee cartilage injected with miR-181a-5p inhibitor compared to control inhibitor. Examining human knee chondrocytes (in vitro) and cartilage tissue (ex vivo), we determined that inhibition of miR-181a-5p also down-regulates the expression of cartilage catabolic and apoptotic/cell death activities, and up-regulates expression of COL2A1 assessed by qPCR and flow cytometric analysis. **Conclusion**: Our pre-clinical data strongly suggests that local injection of miR-181a-5p inhibitor may be a potential therapy to halt cartilage degeneration during both FJ and knee OA. We are currently focusing our studies on identifying the most

effective dose, frequency of injections as well as safety profile of this miRNA inhibitor in pre-clinical animal models for its future clinical use.

**Investigating the role of retinoid X receptor in cartilage development and homeostasis**

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**Purpose**: Osteoarthritis (OA) is a disabling joint disease characterized by cartilage degradation of the joint. A better understanding of the genetic regulations behind cartilage breakdown is needed to understand OA pathogenesis and develop novel therapeutic strategies. Previously, studies have shown activation of a nuclear receptor, the Liver X Receptor (LXR), to possess protective effects against cartilage degradation. However, LXR cannot regulate transcription without first forming obligate heterodimers with another nuclear receptor, the Retinoid X Receptor (RXR). RXR is also a heterodimeric partner of many other nuclear receptors, including the Peroxisome Proliferator Activated Receptors (PPARs). In order to elucidate the underlying mechanisms of LXR's protective effect in cartilage, we investigate the effects of RXR activation in cartilage development and homeostasis.

**Methods**: To examine RXR's genetic regulatory function in chondrocytes, immature murine articular chondrocytes (IMACs) were isolated from P5 mice and treated with vehicle control or a specific RXR agonist, SR11237, for 72 hours. RNA was isolated and Affymetrix microarray analysis was performed, followed by bioinformatics analyses, real-time PCR validation and functional validation through lipid mass quantification, and dimethylmethylene blue (DMMB) assay. To examine the role of RXR *in-vivo*, cartilage-specific deletion of RXRα was generated by crossing $RXR\alpha^{fl/fl}$ mice to mice carrying the Crerecombinase gene under the control of the type II collagen promoter. The overall skeletal phenotype and growth-plate morphology was characterized in wildtype and knockout littermates at three-weeks and six-weeks of age through skeletal preparations and microCT analyses, and long bone morphology was examined histologically using Safranin-O/Fast-Green staining.

**Results**: Microarray analysis revealed RXR activation to induce a strong lipocentric effect in chondrocytes, with differential expression observed primarily in genes implicated in lipid metabolic processes. Most highly enriched gene ontology terms and KEGG-pathways include lipid metabolic pathways, PPAR signaling pathway, and extracellular matrix components. Lipid mass quantification demonstrated RXR activation to increase cellular triglyceride levels, and DMMB assay revealed RXR activation to increase glycosaminoglycan release from femoral head explants. At three- and six-weeks of age, cartilagespecific RXRα deficiency in mice resulted in decreased body weight, decreased long bone length, delayed ossification, and histological alterations in the long bones and knee-joint.

**Conclusion**: Our findings demonstrate that RXR activation in chondrocytes causes differential expression of lipid metabolic genes, resulting in changes in cellular lipid profiles and extracellular matrix homeostasis. Loss of RXRα in cartilage appears to disrupt long bone histology resulting in decreased long bone length, suggesting RXRα to play an important role in endochondral ossification. Future studies will continue to examine the role of RXR in skeletal development and disease in order to explore its potential as a possible therapeutic target in different pathological contexts such as osteoarthritis.

**A mechanistic study to understand vascular calcification caused by MGP deficiency**

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**Background:** Mutations in the matrix Gla protein (MGP) gene in humans lead to Keutel syndrome, a rare autosomal recessive disorder hallmarked by cartilage and vascular calcification, midface hypoplasia and pulmonary stenosis. *MGP* is highly expressed in chondrocytes and vascular smooth muscle cells (VSMCs). *Mgp* 'knockout' (*Mgp-/-*) mice display most of the phenotypic traits of Keutel syndrome. Earlier, we demonstrated that the introduction of a mutation in *Phex* gene in *Mgp-/-* mice prevents vascular calcification. However, it was not clear whether fibroblast growth factor 23 (FGF23), a phosphate regulating hormone acting downstream of PHEX, plays a critical role in the process. MGP carries 4 **γ**-carboxylated glutamic acid (Gla) residues. Although these residues are thought to be critical for MGP's anti-mineralization function, so far no genetic experiment has been performed to examine their role in the vascular tissues.

**Aims:**

1) To investigate the effects of FGF23 on the vascular calcification phenotype in MGP-deficient mice.

2) To investigate the anti-mineralization property of mutated MGP that lacks the Gla residues.

**Methods:** We crossed *Mgp+/-* mice with the *ApoE-Fgf23* transgenic mice to generate *Mgp-/-;ApoE-Fgf23* mice. As is the case with the PHEX-deficient (*Hyp*) mice, *Mgp-/-;ApoE-Fgf23* mice show high levels of circulating FGF23 and hypophosphatemia. The vascular calcification phenotype in these mice was analyzed by microCT, histology and alizarin red staining of the thoracic aorta. For the second aim, we generated a new transgenic line (*SM22GlamutMgp*) expressing a mutant form of MGP in which the four conserved glutamic acid residues to alanine. We generated *Mgp-/-;SM22GlamutMgp* mice and examined the vascular calcification phenotype as above.

**Results:** Alizarin red staining and histological analyses showed that unlike *Mgp-/-* mice, at 4 weeks of age, *Mgp-/-;ApoEFgf23* mice did not show any sign of vascular calcification. A high phosphorus diet induces rapid vascular calcification in these mice. The *Mgp-/-;SM22Glamut* mice did not express the endogenous MGP, but express in the VSMCs a mutant form lacking the conserved glutamic acid residues. Surprisingly, these mice never developed vascular calcification. Under hyperphosphatemic conditions, these mice develop severe arterial calcification.

**Conclusions:** FGF23 and Inorganic phosphate axis acts as a major regulator of vascular calcification in *Mgp-/-* mice. Here we show that the known Gla residues are dispensable for MGP's anti-mineralization function under normophosphatemic condition This latter finding may explain why drugs that interfere with gamma carboxylation of Gla residues may result in high mortality in end stage renal disease.

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**Fibrillin-1-mediated regulation of microRNA signaling and cell function**

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**Introduction:** Fibrillin-1 is the major component of microfibrils in the connective tissue of many tissues, including bone and blood vessels. Fibrillin-1 mediates cell interactions via an evolutionarily conserved Arg-Gly-Asp (RGD) sequence. Abnormalities in the fibrillin-1 gene cause various disorders, including Marfan syndrome (MFS), dominant Weill-Marchesani syndrome, stiff skin syndrome, among others. microRNAs (miRNAs) are reported to post-transcriptionally control 30% of mammalian gene expressions, including ECM proteins. However, whether ECM proteins regulate miRNAs, and whether ECM-regulated miRNAs have therapeutic potential in connective tissue disorders is largely unknown. This study addresses how cell interaction of the fibrillin-1 RGD sequence regulates proliferation and differentiation-related mechanisms through miRNAs, and highlights the regulation of ERK1/2 by fibrillin-1-mediated miRNAs in the ascending aorta of a MFS mouse model.

**Results:** A miRNA global microarray analysis comparing fibroblast seeded on an RGD-containing fibrillin-1 fragment compared to a mutant RGA-containing control fragment presents a novel paradigm how fibrillin-1 controls miRNAs through integrin-ligation. Comparative mRNA studies by global microarray analysis identified genes involved in the transforming growth factor-beta pathway, MAPK pathway (includes ERK1/2), and focal adhesion formation as the most important functional groups regulated upon fibillin-1 ligation. Bioinformatic prediction and experimental validation of the targets of the selected miRNAs highlighted their roles in proliferation or differentiation-related mechanisms. Among these validated miRNAs, miR-503 was shown to participate in p-SMAD2 regulation by RGD ligation. Immunofluorescence indicated that miR1208 was involved in total ERK1/2 and cell proliferation regulation, and in fibroblast to myofibroblast differentiation. Furthermore, downregulation of miR-612 and miR-3185 by fibrillin-1 binding contributed to focal adhesion formation in fibroblasts.

To understand which of the fibrillin-1 regulated miRNAs identified in the microarray could play a role in MFS disease pathogenesis, we first compared them to miRNAs reported as dysregulated in MFS and aortic aneurysms, which identified 10 overlapping miRNAs. Three miRNAs (let-7a, let-7g and miR-27b) were downregulated in the ascending aortae of 10-week old female *Fbn1*$^{mgR/mgR}$ MFS mice, which are characterized by a ~80% reduced level of fibrillin-1. miRNA let-7g was also significantly reduced in male mice. Bioinformatic analysis (microT-CDS) predicted targets for let-7a, let-7g and miR-27b in the MAPK pathway (the critical pathway in MFS pathogenesis). Furthermore, functional analysis showed that let-7a and let7g mimics significantly downregulated total ERK1/2 in smooth muscle cells isolated from mouse ascending aortae.

**Conclusion:** Fibrillin-1 regulates a subset of miRNAs involved in TGF-beta signalling, proliferation, myofibroblast differentiation, and focal adhesion formation. Several fibrillin-1 regulated miRNAs involved in ERK1/2 signalling are dysregulated in MFS mice.

**Identification of candidate genes potentially involved in adolescent idiopathic scoliosis**

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**Introduction :** Scoliosis is a complex disease characterized by phenotypic heterogeneity with unknown etiology. Some forms of scoliosis, including adolescent idiopathic scoliosis (AIS), seem to have genetic contribution. Epidemiologic approach confirmed genetic involvement of AIS and suggested different type of inheritance. In this study, the aim is to identify the disease-causing gene by performing exome sequencing of affected candidates.

**Our hypothesis** is that AIS is a polygenic disorder and that AIS definitely involves several genes.

**Materials and methods:** The selected families were mostly French-Canadian and have at least two members with AIS with a Cobb Angle up to 15 degrees. We performed exome sequencing of at least two affected people in each family. Identified candidate variants were validated by Sanger sequencing, and we studied their segregation with the disease in each family. To prioritize genes for functional studies, we studied pathogenicity prediction using *in-silico* analysis.

**Results:** Here we studied 20 families were studied: 8 had recessive inheritance of AIS and 12 had dominant inheritance. Exome sequencing of recessive inheritance families lead to identify 9 genes with 2 rare variants as possible candidates. 25 genes with rare variants in at least 2 families and over-represented compared to control population were selected for dominant inheritance families. After confirmation by Sanger sequencing and segregation analysis, 13 genes were considered for future functional studies: FN1 and VLDLR for recessive inheritance families; CA4, CASP4, GLS2, LAMB3, OGFR, P3H1, PTGS1, SPATA31E1, SSPO, SIPA1L3 and TCEB3B for dominant inheritance families. Variants of 7 genes: FN1, VLDLR, CA4, GSL2, OGFR, PTGS1 and SIPA1L3, have been predicted pathogenic.

**Conclusion:** Selected genes are potentially involved in AIS and could play a role in spine development by affecting bones and cartilage morphogenesis. Molecular consequences of these selected genes will be validated *in vitro* using cellular model derived from AIS patients and *in vivo,* by introducing the mutations by CRISPR-Cas9 in zebrafish. Finding causative gene of AIS will help us understand the etiopathogenesis of AIS.

**High-throughput drug-screening using human cardiomyocytes in mechanically controlled environments**

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**Background:** Drug side-effects are a major risk for patients and one of the most common reasons for drug recall from the market. Nifedipine (procardia), can be used as drug intervention for mixed connective tissue disease, but has dangerous potential side-effects, such as acute myocardial infarction. Better preclinical test using human cardiomyocytes (CM) as sentinel cells in high throughput screening (HTS) are needed to detect drug side-effects earlier. However, no currently available HTS assay can directly assess CM contraction amplitude. We developed a HTS platform to quantify deformations (wrinkles), created by cell contractions on silicone polymer surfaces. The wrinkling silicone polymer substrates can be produced in a stiffness ranging from soft healthy to stiff diseased heart. Proof-of-principle tests with one substrate stiffness already demonstrated the suitability of the wrinkling assay to quantify contractions of induced pluripotent stem cell (iPSC)derived CM.

**Hypothesis:** Tuning culture surface stiffness to the mechanical conditions of the heart will enhance the validity of in vitro drug tests with human iPSC-derived CM.

**Objective:** To evaluate HTS with human iPSC-derived CM in wrinkling assays of different pathophysiological stiffness and in different drug treatment scenarios.

**Methods:** Wrinkling silicone polymer substrates with stiffness corresponding to healthy and diseased heart were cast onto the bottom of 96-well plates, covalently functionalized with matrix protein, and seeded with human iPSC-derived CM. CM beating frequency, amplitude, and regularity of contraction was quantified as a function of substrate stiffness and in response to a panel of established drugs. Wrinkling device performance was benchmarked against an industry gold-standard cardiotoxic test device (xCELLigence). In addition to modulating CM beating with drugs, we performed electrical stimulation (pacing) with different input frequencies and voltages. Further, CM contraction was simultaneously analyzed with calcium transients to assess cardiac excitation-contraction in proof-of-principle tests.

**Results:** Tests performed with 9 established drugs in 5 concentrations validated the wrinkling device for its capacity to detect dose-dependent drug effects on CM beating. Compared to xCELLigence, the wrinkling assay showed higher sensitivity and the unique ability to measure contraction amplitude changes. The wrinkling assay succeeded where xCELLigence failed to reveal expected drug effects, e.g., showing dose-dependent decrease in response to the cardiac myosin activator omecamtiv mecarbil. Culture on either soft or stiff wrinkling substrates modulated maturity of CM sarcomere structure and, consequently, responsiveness to drug treatment. The wrinkling contraction assay also reliably detected changes in rate and amplitude of CM contraction modulated by acute electrical pacing, and was compatible with simultaneous calcium analysis.

**Conclusion:** We validated the wrinkling HTS assay for its ability and high sensitivity to measure changes in rate and amplitude of CM contractions. The stiffness-tuneable culture material of the device allows to assess CM contraction under different mechanical pathophysiological conditions.

**Impact:** The wrinkling assay fills the gap of HTS test to measure CM contraction amplitude and provides additional features, such as pathophysiological mechanical conditions to improve *in vitro* drug tests.

# Poster Presentations Session 1

**Spring! Break! Design of elastic protein biomaterials**

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Elastin is an extracellular matrix protein of vertebrates that belongs to a class of proteins across biology whose main function is to confer elasticity to the tissue or material in which it is found. Although elastic proteins have predominantly structural roles, polymer chains within cross-linked materials must remain substantially disordered for elastic function, because returning to a state of higher entropy after extension is a main driving force for elastic recoil. Conformational disorder of elastic proteins is largely conferred by hydrophobic sequences that contain a high composition of structure-breaking proline and glycine residues. However, cross-linking domains differ substantially in structure between elastomeric proteins, and may be key to understanding the mechanical properties of natural biomaterials, which span orders of magnitude. A major emerging question is how structure, disorder and dynamics combine to modulate mechanical properties such as extensibility, tensile strength, and resilience. An improved understanding of the molecular basis of elastic mechanical properties will enable the design of protein scaffolds incorporating crucial physiological elasticity for tissue repair and engineering, mechanically tailored to direct stem cell fate and behaviour, and with tissue-specific mechanical profiles.

Here we describe three strategies for mechanically designing elastin-based materials based on modifying the balance of structure and disorder of the monomer “building block”: (i) disrupting local structural motifs, (ii) adding extended secondary structure, and (iii) combining sequences from different species in an attempt to obtain composite material properties. Specifically, we demonstrate the molecular ability to modulate material toughness, strength and resilience without compromising elasticity. These data inform structure/ disorder-function relationships of elastic proteins, and reveal great potential for fine-tuning material properties for elastic tissue engineering.

# Influence of nanometric substratum topography on human dermal and gingival fibroblast fibrillar adhesion formation

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**Introduction**: Contact guidance is the phenomenon where cells sense and respond to substratum topographic cues by orienting their adhesion formation, migration, and matrix deposition (Brunette & Chehroudi, 1999; Kim, Wen, Prowse, & Hamilton, 2015). Contact guidance is essential in development, pathological processes, repair and regenerative processes as observed within the process of wound healing (Britland et al., 1996; Brunette, 1988; Curtis & Clark, 1990). The dynamic nature of the extracellular matrix, specifically within the wound healing environment, presents cells with various topographic cues. Fibrillar adhesions are an important determinant of healing, but the role of these adhesion types on contact guidance on nanometric topographies has never been studied. The purpose of this study is to investigate the influence of nanometric topographies upon the establishment of fibrillar adhesion sites by human dermal fibroblasts (HDF) and gingival fibroblasts (HGF). In wound healing, HDF form large adhesion sites and result in scar formation. HGF have a regenerative capacity and their adhesion sites have not been studied in this capacity previously. We hypothesize that HGF will show a reduction in adhesion on certain topographical cues in comparison to HDF.

M**ethods**: The experiment was performed with both HDF and HGF below passage 6 and maintained within DMEM high glucose media. A glass non-topographic control and 12 dot, grid and line nanometric topographic substrates, varying in periodicity, were seeded with 20k cells and incubated at 37°C, 5% $CO_2$ for 24 hours. Immunofluorescence was performed for integrin β1 and tensin-1. Stained surfaces were imaged on a Zeiss Axio Imager M2m at 20X magnification. Adhesion site directionality was determined through fast Fournier transforms of the fluorescence intensity utilizing Image J software. Two-way ANOVA was utilized to determine significance.

**Results**: Analysis demonstrated that fibrillar adhesion protein directionality is controlled by various topographic substratum in both HDF and HGF. With increased topographic periodicity, directionality of fibrillar adhesions increases. Integrin β1 and tensin-1 upon dot and grid topographic patterns exhibit mixed directionality, similar to the flat control. As periodicity increases from 600 nm to 1200 nm, both dot and grid topographic patterns impart a linear directionality upon the integrin β1 and tensin-1 protein expression. Linear topographic cues demonstrate a very strong influence upon integrin β1 and tensin-1 protein expression directionality.

**Discussion and Conclusions**: The various substratum topographic cues investigated are able to influence fibrillar adhesion associated molecules integrin β1 and tensin-1. Directionality is influenced in both dermal and gingival fibroblast populations. The deposition and expression of integrin β1 and tensin-1 of the fibrillar adhesions upon the topographic substratum indicates there may be a hierarchical system in which the fibroblasts mechano-sense their substratum and impart stronger directionality towards linear cues. This preferred, unidirectional and linear, fibrillar adhesion protein alignment may elude to the cells preference for organized extracellular matrix and a mechanism of matrix deposition and remodeling. The use of topographic biomaterials has the potential to facilitate cell organization through adhesion formation, in addition to matrix deposition and cytoskeletal alignment – also currently being investigated, to influence tissue regeneration in wound healing.

**Investigating the role of CCCTC-binding factor in Osteoarthritis Pathogenesis**

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**Purpose:** Osteoarthritis (OA) is a degenerative disease in which joint cartilage breaks down as we age or as a result of injury. Despite its high prevalence, the etiology of OA remains elusive. Because the chromatin organizer, Ctcf (CCCTC-binding factor), plays an important role in skeletal development, this regulatory molecule may also play a role in OA pathogenesis. We have developed a mouse model for the genetic analysis of Ctcf and its function in order to study its effects on OA development. So far, preliminary results from our lab show that cartilage-specific deletion of Ctcf in mice results in severe skeletal defects and these mice die shortly after birth due to breathing problems. Since Ctcf knockout mice die at birth, we used heterozygote mice that have one functioning allele in order to study OA using a mouse aging model.

**Methods:** Spontaneous disease progression was analyzed in adult male and female mice using cartilage-specific Ctcf knockout generated using the Cre-lox system. Elbows, knees, and ankles were dissected at 21 months age to recapitulate spontaneous age-associated OA in humans. Joints were harvested and stained with Safranin-O/Fast Green and scored according to the OARSI histopathology scoring system. Immuno-histochemical analysis for Sox9, and osteoarthritis markers (i.e. MMP13 and type II collagen) were used to evaluate joint pathology. In addition, calcified tissue in knee joints was assessed by micro-computed (micro-CT) tomography.

**Results:** Cartilage thickness and proteoglycan staining of articular cartilage was significantly decreased in the knee joints of 21-month-old Ctcf heterozygous mice compared to controls. In addition, histological analysis showed the formation of chondro-osseous tissue in the knees of heterozygous mice. Further analysis of these chondro-osseous structures using micro-CT shows calcified-bone like tissue formation in these joints.

**Conclusions:** Ctcf heterozygote mice are susceptible to spontaneous OA development as a result of aging. Moreover, cartilage specific loss of Ctcf appears to result in the ectopic formation of calcified tissue in the knee joints of these mice. Our study is the first to examine the importance of Ctcf in joint health, further analysis will help elucidate the role of this chromatin organizer in the regulation of key genes involved in the development of OA.

**Senolytics to halt degeneration and pain in human intervertebral discs.**

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**Introduction:** Cellular senescence is a state of stable cell cycle arrest in response to a variety of cellular stresses including DNA damaging agents, oxidative stress, mitochondrial dysfunctions, adverse load and disruption of epigenetic regulation[1]. The accumulation of senescent IVD cells in the tissue suggests crucial roles of these cells in the initiation and development of painful IVD degeneration[2] through the secretion of an array of diverse cytokines, chemokines, growth factors, and proteases known as the senescence-associated secretory phenotype (SASP). The SASP promote matrix catabolism and inflammation in intervertebral disc thereby accelerating the process of its degeneration. The aim of this research is to quantify the levels of senescent cells in intervertebral discs and screen and select the most potent senolytic drug that remove senescent cells while preserving cells health, proliferation and overall matrix production of the remaining cells.

**Methods:** *Tissue collection and cell isolation:* Human IVDs were obtained from organ donors with no history of back pain through collaboration with Transplant Quebec. Pellet or monolayer cultures were prepared from freshly isolated cells in culture media (low-glucose DMEM, 5% FBS, Gentamicin, Glutamax). Then, cultured in the presence or absence of senolytics. Monolayer cultures were analysed after a 4-day and pellets after 21 days for the effect of senolysis. *Initial drug screening:* A cytotoxicity study was performed on monolayer cells treated and non-treated with senolytics using Alamar blue assay. Cells were plated in chamber-slides and treated with 3 different drugs at 8 concentrations. *Gene expression:* Following treatment, RNA was extracted and gene expression of senescence and inflammatory markers (p16, IL6, IL8, MMP3 and MMP13) and GAPDH, was evaluated by real-time quantitative-PCR using the comparative ΔΔCt method. *Protein expression:* Pellets or monolayer were fixed and P16, KI-67 and Caspase-3 expression were evaluated. Total number of cells was counted on consecutive sections using DAPI and five fields of view were analysed with an in-house developed MatLab script. SafraninO stain for proteoglycan synthesis and antibodies to stain for collagen type II expression were used to determine the percentage of senescent cells removal and if the remaining cells exhibit an improved matrix producing capacity.

**Results**: We observed a 40% higher level of senescent cells in degenerate compare to non-degenerate discs from the same individual. 3 drugs were evaluated for an effective senolytic dose that at the same time did not affect viability of non-senescent cells. Using the optimal respective dose, senolytics (S1, S2 and S3) cleared 35-40% of the senescent cells in monolayer and up to 80% in pellet cultures. mRNA and protein expression levels of SASP factors were decreased following treatment with senolytics.

**Discussion & Conclusions**: Elucidation of the complex and fine relationship between disc degeneration, tissue inflammation and the molecular mechanism of disc cell senescence appears to be critical to improve the current ineffective therapies. This work may identify novel therapy (senescent cells removal) for the treatment of IVD degeneration and new senolytic drugs that could lead to improved therapeutic interventions.

**References:** [1]Toussaint et al., 2000 Experimental-gerontology. [2] Le Maitre et al, 2007 Arthritis-Res-Ther.

**A mouse model of osteogenesis imperfecta, Col1a1Jrt/+, is protected against high-fat diet-induced weight gain.**

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**Objective**: Osteogenesis imperfecta (OI) is a genetic disorder most commonly caused by mutations in collagen type I, and characterized by low bone mass and high fracture rate. We have recently shown that young mice with severe form of OI, Col1a1Jrt/+ mouse, exhibit altered glucose/insulin metabolism and energy expenditure, as well as elevated levels of undercarboxylated osteocalcin, a novel bone-derived hormone that affects insulin production and sensitivity. Now we examined the effect of high-fat diet on weight gain, glucose metabolism, and bone properties in Col1a1Jrt/+ mice.

**Methods**: Starting at an age of 4 weeks, wild-type (WT) and OI mice were fed with either high-fat diet (60% fat, HFD) or a matched low-fat diet (10%, LFD) for 26 weeks.

**Results**: At 4-weeks of age, male and female OI mice had 20% lower body mass than WT littermates. WT male and female mice on LFD gained 0.65±0.03 g/week and 0.39±0.02 g/week, respectively, while on HFD males gained 1.00±0.05 g/week and females 1.10±0.06 g/week. Compared to WT mice, male and female OI mice showed significantly lower increase in body mass. Female OI mice gained about 0.28±0.02 g/week on LFD and 0.62±0.04 g/week on HFD. Male OI mice were protected from HFD-induced weight gain and showed gain in body mass of 0.44±0.02 g/week on LFD and 0.46±0.02 g/week on HFD. In WT mice, HFD-induced gain of body mass coincided with increase in white adipose tissue and liver weight, whereas in OI white adipose tissue and liver weight did not change. No diet-induced change in brown adipose tissue, heart, spleen, or pancreas weight was observed in WT or OI mice. Serum analysis revealed elevated cholesterol and high-density lipoprotein levels in WT and OI. Glucose tolerance test demonstrated HFD induced diabetic changes in glucose metabolism in both WT and OI mice. Trabecular structure as well as mechanical properties of long bones were significantly different between WT and OI but were not affected by diet.

**Conclusion**: OI mice, in particular males, are protected against HFD-induced obesity, but not from HFD-induced insulin resistance.

**Thyroid Hormone interacts with the sympathetic nervous system, via α2 adrenoceptor signaling, to regulate endochondral bone growth**

Manuela Miranda Rodrigues[1,2], Margaret Sunn[2], Patricia Brum[1], Cecilia Gouveia[1], Frank Beier[2]

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It is well known that thyroid hormone (TH) is essential for normal bone growth and development. However, the mechanisms by which TH regulates these processes are poorly understood. Recently, the sympathetic nervous system (SNS) was identified as a potent regulator of bone metabolism. In vivo studies by our group have shown that TH interacts with the SNS to regulate bone mass and structure, and that this interaction involves $\alpha_2$ adrenoceptor ($\alpha_2$-AR) signaling. We have also identified the presence of $\alpha_{2A}$-, $\alpha_{2B}$-, and $\alpha_{2C}$-AR subtypes in the epiphyseal growth plate (EGP) of mice. In addition, we have found that mice with isolated gene deletion of $\alpha_{2A}$-AR and $\alpha_{2C}$-AR ($\alpha_{2A}$-AR$^{-/-}$ and $\alpha_{2C}$-AR$^{-/-}$) show a disorganized EGP, shorter long bones and a delay in endochondral ossification (EO). In vivo studies revealed that the EGP of $\alpha_{2A}$-AR$^{-/-}$ and $\alpha_{2C}$-AR$^{-/-}$animals respond differently (than those of wild-type animals), to TH excess and deficiency, which strongly suggests that TH also interacts with the SNS to regulate bone growth and development. Through a long bone organ culture system, the present study had the goal of investigating if TH interacts with the SNS directly in the skeleton, to regulate the longitudinal bone growth and if $\alpha_2$-AR is involved in this process. Therefore, we evaluated the linear bone growth of tibias derived from 15.5-day-old WT and $\alpha_{2C}$-AR$^{-/-}$ mouse embryos (E15.5) for 6 days. We have seen that the KO tibias showed a lower longitudinal growth when compared to WT tibias, and that treatment with 10-8 M triiodothyronine (T3) significantly decreased the longitudinal growth of the WT tibias, which was not seen in the KO tibias. We found that the treatment with UK 14.304 (UK), a non-selective α2-agonist, induced the longitudinal growth only of the KO tibias. The expression of genes related to the terminal differentiation of chondrocytes (Col X, IGF-1, Wnt-4 and Runx2) was shown to be increased in the KO tibias (when compared to the expression in WT tibias). Treatment with T3, as expected, stimulated the expression of these genes in WT tibias, but decreased the expression in KO samples, highlighting the importance of these receptors in the modulation of T3 actions. We observed that the local $\alpha_2$-AR activation by UK blocked the expression of these chondrocyte differentiation-related genes, in addition to blocking the positive effects of T3 (UK + T3) in the expression of these genes. These findings show that α2 adrenoceptors act directly in the skeleton, to control the terminal differentiation of chondrocytes and, therefore, the longitudinal bone growth, in addition to allow an interaction with the TH signaling pathway to control these processes. In conclusion, this study shows that TH interacts with the SNS, locally in the skeleton, via α2 adrenergic signaling, to modulate the longitudinal bone growth.

**Cellular senescence is associated with increased expression of TIMP-1 and altered collagen remodeling in human gingival fibroblasts.**

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Cellular senescence corresponds to a cellular program characterized by an arrest in cell proliferation and altered matrix remodeling. However, the precise cellular mechanisms still remain to be elucidated. Collagen remodeling is a critical event in wound healing and previous studies have identified abnormal collagen phagocytosis in senescent human gingival fibroblasts. In the present study we have searched for molecules involved in matrix remodeling in senescent human gingival connective tissue cells.

Replicative cellular senescence was induced by cell passages in primary cultures of human gingival fibroblasts derived from young subjects. Proteins secreted by cell cultures were evaluated through antibody arrays. Cellular senescence was evaluated by SA-beta Gal staining, Ki-67 and actin staining. Collagen remodeling was evaluated through collagen gel contraction.

Cellular senescence was characterized by increased staining for SA-beta Gal, increased cell size, reduced cell proliferation and increased secretion of TIMP-1 within a series of secreted proteins (as determined through antibody arrays and ELISA). Using flow cytometry and immunofluorescence we confirmed the increased protein levels of TIMP-1 in senescent gingival fibroblasts. Cellular senescence was also associated with a defective collagen remodeling.

We propose that TIMP-1 is induced during replicative cellular senescence in human gingival connective tissue cells and is probably responsible for a decreased collagen remodeling in this cell phenotype.

**Biomechanical assessment of surgical maneuvers of alar cartilage performed in rhinoplasty**

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**Background:** A known complication of rhinoplasty is external valve collapse whereby the ala collapses, mechanically preventing air from entering the nasal cavity. Cephalic trimming of the alar cartilage is frequently performed in rhinoplasty procedures in an effort to reduce the size of the nasal tip. It is assumed that trimming the cartilage decreases the stiffness, thereby leading to greater valve collapse. However, the extent of this effect has not yet been studied. Numerous methods have been proposed to combat this weakening in order to maintain lateral crural stiffness (and hence ala stiffness) including the crural turn-in flap and the lateral crural strut graft, among others. The effectiveness of these routinely used techniques is not known. The purpose of this study was to quantify the effect of 1) standard surgical reduction (mucosal stripping, cephalic trimming), 2) re-enforcement using the cephalic turn-in technique, and 3) re-enforcement using lateral crural strut grafting technique on the stiffness (and therefore the resistance to collapse) of the lower lateral nasal cartilages.

**Methods:** *In situ* cyclic compressive loading was performed on eight lateral crura in 4 fresh frozen cadaveric head specimens. Testing was performed on the unaltered degloved cartilage (intact) and following each of the following interventions: mucosal stripping, cephalic turn-in flap, cephalic trimming, and lateral crural strut grafting. Linear regression of the generated force-displacement curves was used to calculate stiffness. Linear mixed modeling was used to analyze outcomes with the different maneuvers, with each maneuver compared to the intact cartilage.

**Results:** Alar cartilage of all of the specimens demonstrated a linear response to compressive loading. Intact lateral crural cartilage had a mean stiffness of 3.53 N/mm. Mucosal stripping and cephalic turn-in flaps yielded similar stiffness values to intact cartilage. Cephalic trimming reduced stiffness in all cases by a mean of 1.09 N/mm ($p = 0.003$). Lateral crural strut grafting significantly increased stiffness by a mean of 3.67 N/mm ($p = 0.0001$).

**Conclusions:** Cephalic trimming lead to decreased lateral crural stiffness in cadaveric specimens, suggesting a risk for external valve collapse in patients requiring trimming. Cephalic turn-in flap restored pre-trimmed stiffness, and lateral crural strut grafting significantly increased overall stiffness of the cartilage. The difference in outcomes with these two reenforcement techniques may be attributed to the structural differences between alar cartilage and the thicker, proteoglycan-rich matrix of septal graft cartilage. These findings should be considered in patients undergoing rhinoplasty, particularly if there are concerns regarding potential external valve collapse.

**An approach to metabolite signature selection and classification modeling for prediction of individuals with knee osteoarthritis**

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**Objective:** Age, sex, and BMI can help to predict knee osteoarthritis (OA) patients from healthy individuals. The metabolome, an overall output of metabolic processes occurring within an individual, and specifically the levels of select metabolites, can help diagnose disease. However, metabolite selection methods and modeling algorithms that best identify metabolites capable of predicting OA have not been established. We sought to determine a method that was capable of effectively identifying metabolite signatures predictive of OA in demographically-stratified populations and evaluate differences in signatures across strata.

**Methods:** Phosphatidylcholine (PC) and lyso(PC) analogues in plasma were measured by metabolomics from a Newfoundland cohort consisting of healthy volunteers (HV) and OA patients undergoing total knee replacement. The cohort was stratified by age, sex and BMI. Analogue signatures were determined by generating an empirical distribution of univariate area under the receiver operator curve (UAUC) values from 1000 randomly sampled training and test sets. Metabolites with UAUC > 0.5 at the 2.5% quantile were selected as capable of predicting OA from HV within strata. Three multivariate classification algorithms were tested using each signature, including principal component regression with logistic regression, partial least squares regression with logistic regression, and logistic regression alone. The most consistent algorithm was determined by the minimum difference between multivariate AUC values derived from 1000 resamplings, as above.

**Results:** The metabolite signature from males age > 50 years old encompassed the majority of identified metabolites in other strata, suggesting lysoPCs and PCs were dominant indicators of OA in older males. Principal component regression with logistic regression was the most consistent classification algorithm tested, having the smallest average difference between training and test sets across the 2.5%, 50% and 97.5% quantiles of all strata. Using signature-based modeling also improved prediction classification compared to sums of PC (broken down into two isotypes) and lysoPC analogues. Using this algorithm, the males age > 50 years old signature had fair power to classify OA patients from HV.

**Conclusions:** Individual levels of lysoPC and PC analogues may be indicative of individuals with OA in older male populations. Our metabolite signature modeling method is likely to increase classification power in validation cohorts.

**Characterizing murine strain-specific differences in osteoarthritis progression**

Anusha Ratneswaran[1], Bethia To[1], Simran Benipal[1], Frank Beier[1,2]

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Osteoarthritis (OA) is a chronic degenerative joint disorder with damage to one or more joint tissues. Rodent studies of OA often induce post-traumatic OA through surgery to study the disease process. However, post-traumatic OA only affects a subset of patients, and these results may not be generalized to other types of OA. Data from our laboratory and others have indicated genetic mutations in the same tissue of interest using different models of OA (such as post-traumatic vs age associated) can have dramatically different effects. The CD1 strain of mice are commonly used to test interventions, and for ex-vivo studies but little is known about their susceptibility to OA. Our objective was to compare pathological differences in spontaneous age-associated OA between CD1 and C57/B6 mice.

CD1, and C57/B6 were aged to 6, 12, 20, or 24 months and then subjected to gait analysis. Knees, elbows, and ankles were harvested for histology. Mice were compared through classical measures of OA progression including Toluidine-blue staining with OARSI scoring. Cartilage matrix breakdown products were assessed using immunohistochemistry. Picrosirius red staining was used to evaluate collagen structure and organization.

6 month old mice of either strain presented with minimal damage to joint structures. 12 month old CD1 male mice exhibited significant severe changes to the knee joint, including end-stage cartilage erosion to the Medial Femoral Condyle (MFC) in all animals, whereas C57/B6 male mice had little damage. Subchondral bone damage, and osteophyte formation pathological scores were also significantly increased in CD1 compared to C57/B6 male mice. Meniscal fissuring was evident in all CD1 male mice, with one half of these having completely degenerated menisci. CD1 male mice also had striking periarticular cartilage formation in the joint capsule. These significant changes in CD1 mice, were not present in the knee joints of C57/B6 male mice. Female mice presented with no significant differences at this age.

At 20 and 24 months of age, both male and female CD1 mice had severe OA in the medial compartment of the knee with persistent significantly elevated scores in MFC cartilage, meniscus, and periarticular cartilage formation. Conversely, male C57/B6 mice exhibited minor changes in articular cartilage at 20 and 24 months, consisting of focal proteoglycan loss and small fibrillations. At this age, there were also no observed differences in subchondral bone damage, and osteophyte formation between strains in male mice. All joint tissue histopathological parameters are significantly different between female CD1 and female C57/B6 mice.

Our results suggest differing underlying physiological processes leading to OA in C57/B6 and CD1 strains. CD1 mice appear to have more severe earlier-onset spontaneous OA, initiated by capsular cartilage formation and meniscal changes, whereas C57/B6 mice have more moderate changes largely in the cartilage and subchondral bone. These profiles are distinct, demonstrating the importance of genetic background in both time course and mechanisms of OA pathogenesis. These data also illustrate the necessity to carefully choose which model is most appropriate for investigating disease development and therapeutic targets, as well as the need to use multiple models.

**High-fat diet-induced or surgically-induced acceleration of osteoarthritis (OA) is attenuated by inhibition of autotaxin**

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**Objective**: Obesity increases the risk of developing Osteoarthritis (OA). Recent evidence suggests that after weight loss, a metabolic adaptation persists long after alteration of body composition. In high fat diet (HFD)-fed mice, which show acceleration of OA, we identified a signature rich in lysophosphatidyl cholines (lysoPC) analogues that was sustained up to at least 9-months of age. We also identified that changes in leptin in the HFD-fed mice knee joints are sustained and that leptin increases the expression of MMP13 by an autotaxin (ATX)-dependent mechanism. ATX is an enzyme responsible for the conversion of lysoPC to the inflammatory mediator lysophosphatidic acid (LPA). Presently, we sought to identify if local pharmacological inhibition of ATX can attenuate diet or surgically-induced OA pathogenesis in vivo and the contribution of LPA to the catabolic phenotype of chondrocytes in vitro.

**Methods**: Nine week-old mice were fed HFD for 18 weeks or 9 week-old mice were subjected to surgically-induced OA. ATX antagonist was injected intra-articularly in the knee joints and subsequent knee joint pathology was evaluated. Primary human chondrocytes were treated with various agents, including ATX antagonist, and the release/expression of selected metabolites or enzymes was determined.

**Results**: Local injection of ATX antagonist reduced the degree of OA pathogenesis surgically induced OA models compared to saline injected controls. In vitro, we found that LPA increased the expression of MMP13, consistent with our previous observations that ATX antagonist blocks MMP13 expression.

**Conclusion**: Inhibition of ATX attenuates surgically-induced OA, likely by modulating the production of catabolic MMP13 by blocking the conversion of local lysoPCs to LPA. We continue to examine pre-clinical efficacy of ATX inhibition in HFD-fed mice. Our data, to date, suggests pre-clinical efficacy of ATX to limit OA progression.

**A novel approach in measuring the frictional force of the tibiofemoral joint in a sheep model**

Mehdi Shekarforoush[1], Michael Scott[1], Gregory Muench[1], David Hart[1], and Nigel Shrive[1]

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**Introduction:** Osteoarthritis (OA) is a degenerative joint disease with pathological changes in bone and loss of articular cartilage. Previous clinical studies have shown that injury to the knee joint results in accelerated development of OA. It is thought that some type of injury-induced mechanical abnormalities of the injured joint may lead to cartilage damage. In this regard, alterations in frictional force on the articulating surfaces would play a significant role in joint degeneration. We performed a pilot study to investigate the frictional force in the complete tibiofemoral joint in a sheep model (N=2) by assuming dry condition.

**Hypothesis**: ACL/MCL transection in sheep results in variation in frictional forces on the articulating surfaces of the tibiofemoral joint.

**Methods**: According to the theory of dry friction, the magnitude of the frictional force is proportional to the normal force and its direction is defined from the relative sliding velocity of the surfaces. An instrumented spatial linkage system was used to define joint kinematics before injury, and 20 weeks after ACL/MCL transection. Following kinematic measurement, the in-situ normal loads on cartilages were measured using a robotic test system with a universal force-moment sensor. 3-Dimensional shapes of the articulated surfaces of the femur and tibia were obtained with a coordinate measuring system. The relative surface velocity of a joint after injury was defined from the previously published approach. The magnitude and direction of the frictional forces were defined on the surfaces and sum of the frictional force vectors through the gait cycle is used for analysis.

**Results:** For both animals, the maximum magnitudes of the frictional forces on the lateral tibial plateaus are higher than the medial tibial plateaus. The maximum magnitude of the frictional force in the anterior-posterior direction is much higher than in the medial-lateral directions on both medial and lateral tibial plateaus. The maximum magnitudes of the frictional force in the anterior-posterior and medial-lateral directions are almost the same for both the medial and lateral condyles. Interestingly, the magnitude of the frictional force decreased for both subjects at 20 weeks after ACL/MCL transection because of the reduction in the normal force on the articulating surfaces.

**Conclusion:** The most important conclusion that can be drawn is that the frictional forces on the surfaces are higher in some directions than others. This might explain the different orientations of the collagen fibres (split lines) on the cartilages. Further research needs to be performed to investigate the relation between directions of the frictional force on the surfaces and the split line orientations.

**Aquaporin 9 contributes to chondrocyte apoptosis in a murine model of osteoarthritis**

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**Objectives**: Osteoarthritis (OA) is characterized by a transition of articular chondrocytes towards hypertrophy and apoptosis. The chondrocyte channelome, comprising ion channels and porins, is believed to be important in guiding the differentiation process. AQP9 is a porin with relatively low water permeability that also transports solutes such as $H_2O_2$. $H_2O_2$ is an important intracellular signaling agent which has been associated with the development of apoptosis. We have discovered AQP9 immunostaining in human OA chondrocytes. Since AQP9 is expressed in the mouse pre-hypertrophic zone, we hypothesize that AQP9 may modulate chondrocyte hypertrophy and OA. Here, we investigate the impact of Aqp9 in cartilage utilizing two distinct preclinical mouse models of OA. We also explore if chemical inhibition of this channel changes the development of OA. Finally, we investigate whether Aqp9 contributes to the development of chondrocyte apoptosis.

**Methods**: Human knee cartilage obtained at the time of knee arthroplasty after informed written consent was analyzed by immunohistochemistry. An ageing (18-month-old mice) and a trauma induced knee OA model (medial meniscectomy on 8-week-old mice) in Aqp9null and WT C57BL/6 mice were used as animal models. An AQP9 antagonist (oral phloretin administered by gavage) was started on d1 post meniscectomy and continued for 8 weeks. Histopathological analysis included in situ hybridization (ISH), immunohistochemistry (IHC) assessments (Mmp13, Col10a1) and safranin O performed at 8 weeks for mouse knee OA models. WT and Aqp9 null mouse chondrocytes were cultured and treated with $H_2O_2$ to induce apoptosis, which was detected by annexin V. Expression of chondrocyte Mmp13 and Col10a1 were quantified through qPCR.

**Results**: Using IHC, we identified expression of AQP9 in human OA cartilage. qPCR revealed an up-regulation of AQP9 in damaged cartilage. Next, we studied the spatial distribution of Aqp9 in murine cartilage by ISH and found expression in articular and pre-hypertrophic growth plate chondrocytes. No abnormal musculoskeletal phenotype is found in Aqp9null mice. We confirmed a global loss of *Aqp9* has no effect on the skeletally mature murine skeleton. However, Aqp9null mice were protected against cartilage loss in vivo post meniscectomy surgery, as shown by reduced proteoglycan loss and OARSI scoring. Since age is an independent risk factor for OA, we utilized an ageing model to investigate the impact of Aqp9 on cartilage. We identified less proteoglycan loss and chondrocyte hypertrophy in 18-month-old Aqp9null mice. Phloretin is a known AQP9 antagonist and we show treatment with this agent significantly inhibits murine chondrocyte Aqp9 levels. Oral phloretin was protective against the development of cartilage loss post meniscectomy surgery in WT mice. Chondrocyte death is regarded as a major factor in the pathogenesis of OA and oxidative stress plays a major role through chondrocyte apoptosis. We found that Aqp9null chondrocytes were protected against apoptosis when treated with $H_2O_2$.

**Conclusion**: Our pre-clinical data strongly indicates that AQP9 is a modulator of OA and that treatment with an AQP9 antagonists may be a potential therapy to halt cartilage degradation after the development of knee OA. We are currently working to further understand the role of Aqp9 in chondrocytes.

**FIZZ1/RELMα is induced in oncostatin M-mediated lung inflammation and ECM accumulation**

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**Introduction:** Found in inflammatory zone 1 (FIZZ1)/resistin-like molecule alpha (RELMα) is a cysteine-rich secreted protein that has been previously implicated in bleomycin-induced models of lung fibrosis. FIZZ1 can be expressed by M2 macrophage phenotypes and type II alveolar epithelial cells. Regulation of FIZZ1 by gp130 cytokines such as Oncostatin M (OSM) or IL-6 is not known. Pulmonary transient overexpression of OSM in mice induces ECM accumulation in the lung, as well as the accumulation of Arginase-1$^+$ (Arg1$^+$) M2 macrophages in murine models. Here we examine whether FIZZ1 can be regulated downstream of OSM expression in C57Bl/6 and BALB/c mice.

**Methods:** Wildtype C57Bl/6 and BALB/c mice were endotracheally administered with empty adenoviral vectors (AdDel70), or those expressing mouse OSM (AdOSM) or IL-6 (AdIL-6) to induce transient overexpression of these cytokines. After 7 or 14 days, FIZZ1 protein in bronchoalveolar lavage (BAL) fluid and serum was assessed by Western blot and ELISA, and mRNA in lung homogenates by RT-PCR. Bone marrow cells were isolated from mice and differentiated into macrophages (M-CSF for 7 days). These bone marrow-derived macrophages (BMDM) were stimulated with various cytokines to skew them towards M1 or M2 phenotypes (24h). Culture supernatants were examined for FIZZ1 by Western blot and ELISA, and cell lysates were examined for cell signaling molecules STAT1, STAT3, STAT6, and Arg1 by Western blot.

**Results:** BAL from AdOSM-treated C57Bl/6 mice showed markedly increased levels of FIZZ1 compared to AdDel70 treatment. FIZZ1 levels were also elevated in AdIL-6-treated mice but were significantly lower than those upon AdOSM treatment, demonstrating that IL-6 alone is not sufficient for maximal induction of FIZZ1. However, IL-6 appears to be required for maximal responses to OSM since FIZZ1 was reduced in IL-6-deficient mice treated with AdOSM in comparison to AdOSM-treated wildtype mice. FIZZ1 mRNA expression in lung homogenates was upregulated in both C57Bl/6 and BALB/c mice treated with AdOSM, but not with AdDel70. BAL and serum FIZZ1 levels were elevated by AdOSM in both C57Bl/6 and BALB/c mice at day 7, consistent with changes observed at the mRNA level. The same trend was observed at day 14, where FIZZ1 expression remained elevated in BAL and serum of mice treated with AdOSM. BMDM stimulated with Th1-skewing cytokines (LPS and IFNγ) did not produce detectable FIZZ1 or Arg1, whereas those stimulated with Th2-skewing cytokines (IL-4 and IL-13) expressed increased Arg1 and secreted FIZZ1 into culture supernatants. The FIZZ1 elevation was associated with activation of pSTAT6 in BMDM stimulated with IL-4 and IL-13, but not associated with IL-6 (pSTAT3 activation) stimulation alone.

**Conclusion:** Thus, OSM can upregulate FIZZ1 mRNA and protein expression in lungs of C57Bl/6 and BALB/c mice. Lung inflammation and increased ECM accumulation induced by OSM *in vivo* may be mediated in part by FIZZ1/RELMα. (Supported by CIHR).

**TBC1D25 in human osteoclasts: impact on autophagy and bone resorption**

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Osteoclasts (OCs) are bone-resorbing cells which rely on strict control of endomembrane systems for their survival and functions including bone-resorbing activity (formation of the ruffled border by directional secretory vesicle trafficking), transcytosis and lysosomal degradation. These membrane traffic events are predominantly regulated by Rab small GTPases and their regulators, guanine nucleotide exchange factor (GEF) and GTPase-activating protein (GAP). Although vesicular trafficking is essential to OC biology, little is known about the key Rabs and their regulators in these cells. In a previous study, we identified in human OCs two spliced isoforms of *TBC1D25,* which encodes TBC1 domain family member 25, a RabGAP containing a LC3-interacting region, which had never been studied in OCs. Our hypothesis was that TBC1D25 might be a key player in OC biology, and we studied its role in survival and bone-resorbing activity of human OCs generated from cord blood monocytes.

We first investigated the expression of TBC1D25. Its location was studied by immunofluorescence, with a diffuse cytoplasmic pattern in basal conditions, and the formation of aggregates of TBC1D25 in presence of rapamycin, a potent autophagy inducer. We next studied the role of TBC1D25 in the autophagy flux of mature OCs. The expression of TBC1D25 (at protein and mRNA levels) slightly increased upon a 3-hour rapamycin stimulation. LC3B-II/LC3B-I levels which correlate with increased levels of autophagic vesicles, were evaluated in the presence of rapamycin or not, in cells transfected either with specific TBC1D25 DsiRNAs or with a negative control (scrambled DsiRNA), as well as in non-transfected cultures. Basal expression of LC3B-II/LC3B-I was higher in transfected cells. Compared to standard conditions, rapamycin strongly and significantly induced LC3B-II/LC3B-I expression, with a greater increase in transfected cells (Dsi scrambled or TBC1D25). In presence of milder stimulation of autophagy (nutritionally poor medium), LC3B-II/LC3B-I expression significantly increased compared to standard conditions in cells transfected with scrambled Dsi but not in Dsi TBC1D25-transfected cells, while a significant increase was observed in presence of chloroquine. Overall these data suggest did not suggest any defect in autophagosome clearance in Dsi TBC1D25-transfected cells, but TBC1D25 might play a role in autophagy induction in human OCs. Finally, while bone resorption was decreased in both transfected cultures, it was significantly higher in cells transfected with specific DsiRNAs compared to cells transfected with a scrambled DsiRNA.

In conclusion, TBC1D25 appears as a new player in OC biology. When TBC1D25 expression is decreased, bone resorption is increased and the induction of autophagosome formation is impeded in weak stimulatory conditions of autophagy. Therefore TBC1D25 might be involved in the induction of autophagy and to a greater extent in bone resorption, but further investigations are needed to determine the specific substrates of this GAP and the exact mechanisms in which it is a player in osteoclastic endomembrane system.

**The Development of Local Acting Biologics for the Prevention of Osteoarthritis**

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Cell and gene based therapies have great potential to contribute to novel treatment strategies for osteoarthritis (OA). The direct injection of mesenchymal stromal cells (MSCs) that have been engineered to deliver a local-acting anti-inflammatory biologic (interleukin-1β (IL-1β) sticky-trap) in a controllable manner may serve as a favourable strategy to attenuate destructive inflammatory processes and promote regeneration in OA joints.

MSCs were isolated from C57BL/6J mouse compact bones, cultured, sorted for MSC markers and confirmed for trilineage differentiation. An IL-1β sticky-trap was generated using the extracellular domain of IL-1 receptor 2 (IL-1R2), cloned into a constitutively expressing *piggyBac* transposons. Mouse MSCs were transfected with these constructs and in the presence of doxycycline, they inducibly expressed the IL-1β sticky-trap. Furthermore, MSCs were also transfected with luciferase constructs to enable in vivo tracking of injected cells. Our study included a total of 70 mice, 10 groups and 7 mice in each group. Seventy, 10 week old male C57BL/6J mice underwent sham or destabilization of the medial meniscus (DMM) surgery. One week after surgery, 5 x $10^4$ IL-1β sticky-trap expressing MSCs were injected intra-articularly into the right knees of the mice and they were administered doxycycline chow. At 10 weeks post-sham or DMM surgery, mice in all groups were sacrificed. Both hind limbs were collected for histology and for OARSI osteoarthritic damage and synovitis scoring.

Mouse MSCs showed trilineage differentiation. In the presence of doxycycline, IL-1β sticky-trap expressing MSCs expressed their luminescent green fluorescent protein tag. In vivo luciferase signal from the MSCs were present in mouse knees up to 6 weeks post injection. DMM model generation was effective (OARSI F=6, T=6) and IL-1β sticky-trap expressing MSCs (OARSI F=4, T=2) had an effect in reducing cartilage proteoglycan loss and less synovitis (S=1.5) compared to our sham and control groups.

IL-1β sticky-trap expressing MSCs may reduce the inflammation observed in OA and mitigate or prevent the disease.

**Mechanical education in vitro enhances regenerative capacities of human mesenchymal stem cells**

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**Background:** Mesenchymal stem cell (MSC) expansion is crucial to obtain sufficient cell numbers for tissue repair therapies. Conventional culture expansion on stiff surfaces reduces the regenerative potential and jeopardizes therapeutic outcomes by inducing scar features in MSCs. In contrast, we have shown that continued culture on skin-soft culture surfaces ('mechanical priming') preserves regenerative MSCs that improve the healing of rat wounds through yet unknown mechanisms.

**Objective/Hypothesis:** Mechanical priming on skin-soft culture surfaces stimulates secretory activities of MSCs that promote wound healing and suppress scarring.

**Methods:** Human umbilical cord perivascular cells (HUCPVCs) from three donors were explanted and primed for a minimum of 3 passages on soft or stiff culture substrates. Mechanical priming was verified using qRT-PCR and western blotting for markers of stromal cell activation. Primed HUCPVCs were tested for MSC criteria. Conditioned medium obtained from primed HUCPVCs was analyzed using cytokine arrays and transferred to fibroblast cultures in presence/absence of pro-fibrotic factor TGF-β1. Fibroblasts were analyzed for markers of myofibroblast-activation by western blot. Primed HUCPVCs were GFP-transfected using CRISPR/Cas9 technology for *in vivo* tracing to determine grafting success in a rat wound model.

**Results:** Soft-primed HUCPVCs were positive for CD44 and CD90 and able to differentiate into osteo- and adipogenic lineages. Primed HUCPVCs exhibited faster doubling times and reduced gene and protein expression of the scar markers α-SMA and ED-A fibronectin compared to stiff-primed HUCPVCs. Conditioned medium derived from primed HUCPVCs suppressed TGF-β1-induced fibrogenic activation of fibroblasts. Of 79 cytokines detected in conditioned medium, 48 were down-regulated and 4 were up-regulated in all soft- versus stiff-primed HUCPVCs. Of 10 most differentially expressed cytokines, 7 were related to inflammation and 3 to cell cycle regulation. We were able to stably label primed HUCPVCs with a fluorescent tag without affecting cell phenotype and viability.

**Conclusion:** Soft-priming enhances regenerative character of human MSCs by preserving cell proliferation, suppressing scarring features, and creating distinct paracrine profiles. We are currently transplanting primed HUCPVCs to splinted rat full thickness wounds to trace the cells for grafting success and analyze wound tissue for cell and matrix composition.

**Significance/Impact**: Understanding how soft-primed MSCs modulate the wound environment will enable the design of more efficient MSC therapies to treat fibrocontractive diseases. Long term benefits of the research will be improved health of patients who are impacted by scarring of large area burn wounds.

**A new mechanism of latent TGF-beta1 presentation by lung macrophages**

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**Background:** Accumulation of scar tissue in fibrosis diminishes organ function. Fibrosis is characterized by the chronic co-existence of macrophages (MΦ), which produce pro-fibrotic growth factors and myofibroblasts (MFs), which secrete and contract collagen. Our recent data show that MΦ express latent TGF-β1, which is only activated in co-culture with MFs upon direct contact.

**Objective:** We here investigate the mechanism how MΦ present latent TGF-β1 to MFs for local activation. Regulatory T cells are known to extracellularly present latent TGF-β1 using the transmembrane protein glycoprotein A repetitions predominant (GARP).

**Materials and Methods:** To test whether tissue MΦ also express GARP, we immuno-colocalized GARP with MΦ marker CD68 in normal, inflamed and fibrotic human lung. Primary cultures of human MΦ at different polarization states were tested for GARP expression by Western blotting, flow cytometry and RT-PCR.

**Results:** Our results show that GARP is expressed in MΦ of normal (n=17) and inflamed lung (n=32) and on the surface of *in vitro* polarized pro-inflammatory MΦ (6%). Western blots performed with cultured MΦ under non-reducing conditions detect GARP, LAP and TGF-β1 at 250 kDa, identifying the GARP-LAP-TGF-β1 complex. To test whether MFs release latent TGF-β1 from MΦ-GARP, we measured active TGF-β1 levels in MF co-cultures with GARP-expressing and -depleted MΦ. The release mechanism is currently under investigation.

**Conclusion:** Collectively, our results indicate that GARP serves as novel surface anchor on MΦ to provide a local TGF-β1 source in inflammation and fibrosis. Interfering with local TGF-β1 presentation and/or activation are potential novel anti-fibrotic strategies.

**Matricellular proteins in the context of kidney injury and repair**

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Kidney disease is a major public health concern, affecting 1 in 10 Canadians. Chronic maladaptive repair, known as fibrosis, is the common pathophysiological phenomenon of a majority of progressive chronic kidney diseases (CKDs). It is defined by excessive deposition of extracellular matrix (ECM), interfering with organ function to the point of death. A more precise understanding of early kidney repair prior to fibrotic CKD is needed for successful treatments, as current methods of intervention to mitigate CKD are imperfect and still retain the persistent injury signal that elicits chronic repair (ie. Fibrosis).

The ECM is the structural microenvironment necessary for the support of cells embedded within it. It is also vital to providing signals necessary for controlling cellular outcomes, including cell proliferation, differentiation and survival. The status of the ECM is a major signal for cellular processes that drives tissue repair but can shift a constructive restoration towards a destructive remodelling of the tissue; however, the molecular mechanisms governing such events have yet to be fully elucidated. Matricellular proteins (MPs) are a recently classified group of proteins known to regulate events that affect ECM processing, as well as modulate ECM-cellular communication. MPs exhibit context-specific effects, typically induced transiently during repair and sustained in chronic pathologies. Therefore, MPs offer a novel approach to understand the details of the kidney repair mechanism. Using RNA sequencing data from two mechanistically different mouse models of kidney injury, we recently identified several MP genes that were significantly upregulated and downregulated. Our candidates will be used to successfully validate the approach of studying MPs to understand the mechanisms that transition from kidney repair to fibrosis.

Overall, our research will clarify the fundamental role of MPs in the ECM, which is severely understudied. It will also describe MPs as potentially druggable targets since they are specifically regulated in response to kidney injury signals, minimizing the side-effects typically observed with pleiotropic targets. Therefore, our study on MPs is expected to have a significant impact because it will provide an important rationale to target MPs for preventative strategies against kidney fibrosis.

**Glycosaminoglycans accelerate biomineralization of dentin in biomimetic tissue-based *in vitro* model.**

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Teeth are attached to the jawbone through an exquisitely controlled mineralization process. Phosphoproteins have been studied extensively as controlling factors in crystal nucleation and growth, however glycosaminoglycans (GAGs) are also suspected to play a role in controlling mineralization. Here, we explore the role of GAGs in collagen mineralization, using a previously developed mouse peridontium tissue-based *in vitro* model of biomineralization. To determine the role of matrix GAGs in control of mineralization, demineralized mouse periodontal sections were digested chondroitinase ABC and hyaluronidase to remove most abundant GAGs. The GAG removal was quantified using a modified DMMB assay, histology, and electrophoresis. Enzymatic treatment removed 76±7% of GAGs compared to untreated section. Histology showed that GAGs were removed from dentin and ligament. Proteomic analysis shown additionally that enzymatic GAG removal does not change protein content in tissue sections. TEM analysis of remineralized tissue sections has shown that GAG removal reduced the rate of remineralization compared to the untreated control. The selectivity of remineralization was maintained in all cases; only dentin and cementum remineralized, while the ligament remained unmineralized. The acidic character of GAGs implies possible roles in calcium ions binding, nucleation and control of crystal growth. Our results show that GAGs, along with acidic phosphoproteins, are responsible for promoting mineralization in dentin/cementum rather than inhibiting crystal formation in ligament. Our findings indicate that GAGs might play a crucial role in control of nucleation during collagen mineralization.

**Palmar fascia mimetics as novel models of Dupuytren's disease cord development**

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Dupuytren's disease (DD) is a common fibro-proliferative disease of the palmar fascia, characterized by high myofibroblast proliferation and a disorder of the extracellular matrix (ECM). During the contracture stage, the affected area becomes abnormally thick causing shortening and progressive loss of finger and hand function. Despite its central biochemical role in DD, most in vitro studies have been performed in the absence of a contracted ECM. The aim of this study was to determine the viability of "bioengineered" cord constructs with primary fibroblasts derived from fibrotic cords or non-fibrotic palmar fascia, to create more physiologically relevant models of DD disease progression.

Primary fibroblasts derived from the explant palmar fasciae of patients with or without DD were used to construct type-1-collagen based bio-artificial tissue (BATs), using the Flexcell Tissue Train Tension System. Histological analysis of fibrillar ECM components was carried out by Pricosirius red staining and Masson's trichome after 14 days of culture. Sequential image of BATs to measure changes in total area, analysis of cellular orientation and collagen fiber thickness was performed to describe BAT remodeling.

Consistent and significant reduction in total area was evident in BATs containing primary palmar fascia fibroblasts relative to cell-free controls. Fibroblasts aligned their cellular extensions along the long axis of the construct. Collagen fiber number and thickness were increased in BATs containing fibroblast derived from patients with DD relative to BATs containing normal palmar fascia and to cell-free controls.

Palmar fascia fibroblasts derived from patients with DD remodel BATs into 3D structures that have histological similarities to DD contracture cords and are distinguishable from BATs remodeled by normal palmar fascia fibroblasts. Palmar fascia mimetics may serve as physiologically relevant models of DD progression and have potential as high-throughput drug discovery systems to facilitate the development of new treatments for DD.

**Short Link N promotes repair of disc degeneration, but more may not be better**

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**Introduction:** The degeneration of the intervertebral disc (IVD) is characterized by proteolytic degradation of the extracellular matrix, and its repair requires the production of an extracellular matrix with a high proteoglycan to collagen ratio characteristic of a nucleus pulposus (NP)-like phenotype *in vivo*. Currently, there is no medical treatment to reverse or delay disc degeneration. In our study we evaluate the potential dose dependency of Short Link N (sLN) to promote extracellular matrix regeneration in a rabbit annular needle puncture model of IVD degeneration.

**Methods:** Adolescent New Zealand white rabbits received an annular puncture in 2 noncontiguous discs with an 18-gauge needle to induce disc degeneration. Two weeks later, either saline (10 µL) or sLN (25 µg or 200 µg in 10 µL saline) was injected into the center of the NP. The effect on radiographic, biochemical and histologic changes were evaluated.

**Results:** Following needle puncture, disc height decreased by about 25-30% over the next 2 weeks, and although this was partially restored by sLN injection, the 200 µg sLN injection was always less effective than the sLN 25 µg, indicating that increase in disc height does not correlate with sLN concentration. sLN injection at 25 µg or 200 µg led to an increase in GAG content 12 weeks post-injection in both the NP and anulus fibrosus (AF). There was a trend towards maintaining control disc collagen content at 25 µg sLN, whereas at 200 µg sLN, there was a significant increase in collagen content when compared to the non-punctured discs. The amount of collagen synthesized in the presence of 200 µg sLN was higher than that with 25 µg sLN. For the 25 µg sLN treated groups, the GAG to collagen ratio in the NP increased when compared to the saline group to an average of 3.0:1. For the 200 µg sLN treated groups, the GAG to collagen ratio in the NP was on average 0.9:1.

**Discussion:** When administered to the degenerate disc *in vivo*, the 200 µg sLN injection leads to an increase in proteoglycan, but it also increases collagen content making it harder for the disc to swell. Hence, even though both the 25 µg and 200 µg sLN treatments eventually reach a similar disc height, the 200 µg treatment takes longer to achieve this even though it has more GAG. Thus, supplementation with the 25 µg sLN could be a better dose for treating disc degeneration as it leads to high GAG synthesis and swelling potential but minimizes fibrosis and the consequent delay in swelling rate.

**Significance:** sLN proves effective as a therapy in disc repair.

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**Enhanced decelluarized extracellular matrix hydrogel for skin tissue engineering**

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Trauma including burn injuries damages the skin and without adequate treatment the wound will result in adverse clinical outcomes. Recently, advanced skin substitutes using 3D printing technology have been developed to treat skin injuries. This is a novel and exciting avenue to wound closure. One of the challenges to using 3D print technology is developing a matrix that has the structural integrity and biocompatibility. Thus, we aimed to develop a hybridized bioink containing decllularized extracellular matrix (dECM), alginate, and oxidized dextran (O-dex). We hypothesized that dECM from porcine dermis containing alginate and O-dex will offer accurate physical strength and suitable environment for cell viability after gelation.

**Methods:** dECM was prepared by using 0.25% trypsin and 1% triton X-100 with buffers to remove cells in porcine dermis. Dextran was modified by using periodate to crosslink the primary amine in dECM. A novel bioink was prepared by hybridization of biomaterials including dECM/O-Dex/Alginate at a low temperature. A multitude of combinations of bioink with different ratios were studied with various crosslinking conditions to enhance mechanical strength. To evaluate cell viability, human fibroblasts were incorporated into the hydrogel and were cultured. A commercially available bovine collagen matrix served as a control.

**Results:** The removed nuclei residue in decellularized ECM was confirmed by histological H&E staining and the 3.5% of remained dsDNA in solubilized dECM was quantified. The 70% oxidized dextran was confirmed by titration method using 0.25 N of hydroxylamine hydrochloride. Fabricated dECM hydrogel substantiated a higher ultimate strength (79.95 kPa) than the control matrix (75.21 kPa). In addition, the incorporated human fibroblasts showed more than 90 % cell viability.

**Summary**: We developed a biocompatible and physically enhanced hybridized d-ECM hydrogel and it demonstrated a potential for 3D printing and skin tissue engineering.

**Promotion of dermal regeneration by a pullulan/gelatin scaffold**

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Insults to the skin such as deep cuts, burns or ulcers can cause damage to the skin and further compromise the clinical outcome. Conventional treatments such as autografts, allografts, and xenografts have been restricted by the limitation of donor sites and the high risk of immunological rejection. With the rapid development of regenerative medicine, the use of engineered skin substitutes to treat skin damage and promote dermal regeneration has become a promising alternative. Due to their biocompatibility, excellent support on cell growth and low cost, pullulan/gelatin (PG) scaffold has been recognized as a good candidate for skin substitutes.

**Purpose and hypothesis**: The instability during transportation and handling as well as the unsatisfied cell delivery of our first generation PG scaffold (PG1), particularly when tested on large animals, hinders its application in the clinic. Thus, the purpose here is (1) to fabricate a more cell-friendly PG scaffold (PG2) with enhanced physical properties that allows easy handling of the large scaffolds through optimization of P/G ratio and dual-crosslinking; (2) to study PG2 by comparing to clinically used Integra® dermal regeneration template (Control).

**Methods**: (1) Material characterization of PG2; (2) *In vitro* human dermal fibroblast incorporation into the skin substitutes; (3) Grafting the two skin substitutes to a mouse model with a full-thickness wound.

**Results**: Material evaluation and *in vitro* cell incorporation showed PG2's excellent properties and biocompatibility as a dermal skin substitute. *In vivo* grafting of PG2 led to a high degree of neo-tissue formation and more cell infiltration followed by a fast disintegration of PG2. While the wound bed of the PG2 scaffold contains a lower number of F4/80 positive macrophages, more of these macrophages infiltrated into the scaffold, contributing into a higher degree of scaffold degradation in compare with the commercial control. This likely enhances a rapid myofibroblastic transition which leads to a lower number of α-smooth muscle actin positive cells in the PG2 scaffold.

**Conclusion:** PG2 is a promising dermal skin substitute that accelerates healing kinetics while attenuating scar formation.

# Poster Presentations Session 2A

**Deletion of menin early in the osteoblast lineage affects mineralization of dense collagen gels by primary osteoblasts**

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**Introduction:** Menin, the product of the Multiple Endocrine Neoplasia Type 1 (*MEN1*) gene, is predominantly a nuclear protein that facilitates cell proliferation and differentiation control. *In vivo* studies have shown the importance of menin for proper functioning of the mature osteoblast and maintenance of bone mass. In this study, we use three-dimensional (3D) dense collagen hydrogel scaffolds, which better mimic the physiological bone microenvironment, as a cell-culture model to investigate the differentiation and mineralization capabilities of primary osteoblasts of conditional knockout mice in which the *Men1* gene is deleted early in the osteoblast lineage.

**Methods:** Primary calvarial osteoblasts were isolated from control wild-type (*Men1f/f*) and *Prx1-Cre;Men1f/f* mice which represent knockout of the *Men1* gene at the level of the mesenchymal stem cell. The cells were then seeded into plastic compressed dense collagen gels and cultured for 21 days in an osteogenic medium to induce differentiation.

**Results:** The number of viable primary calvarial osteoblasts within the dense collagen scaffolds of knockout mice was identical to that of control, as assessed by confocal laser scanning microscopy. Proliferative capabilities of calvarial osteoblast isolated from *Prx1-Cre;Men1f/f* and control mice were similar throughout the 3 weeks of differentiation. Scanning electron microscopy (SEM) revealed the presence of cell mediated mineralization at day 21 in both control and knockout samples. In contrast to collagen gels seeded with wild-type primary osteoblasts, collagen fibres of *Prx1-Cre;Men1f/f* seeded gels appeared covered by overall smaller calcium phosphate based mineral quantities, suggesting a different cell mediated mineralization process carried out by knockout cells compared to wild-type. Through Fourier Transform Infrared Spectroscopy (FTIR) analysis, the absorption band intensities attributable to phosphate and carbonate species were lower in collagen gels seeded with primary osteoblasts of knockout mice in comparison to wild-type. Diffractograms obtained by X-Ray Diffraction (XRD) analysis showed a switch from an amorphous phase to a more crystalline structure after 21 days of culture in both control and knockout seeded collagen gels. However, in comparison to wild-type primary osteoblast seeded gels, diffractograms obtained from knockout seeded gels were characterized by the presence of lower intensity peaks attributable to hydroxyapatite, the main mineral component of bone tissue.

**Conclusion**: Using 3D dense collagen hydrogel scaffolds, both a better alternative to conventional two-dimensional systems of primary cell cultures and a more sensitive model to study cell-mediated mineralization processes, we show that early expression of menin is important for controlling osteoblast mineralization.

**Characterizing AQP9: a regulator of epiphyseal plate chondrocyte proliferation, hypertrophy, and long bone growth**

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In children, epiphyseal plate (EP) injuries disrupt long bone growth and cause limb length discrepancies. Long bone length occurs through ossification at the EP, secondary to the migration of resting chondrocytes from the top of the EP to the distal end. In this primary process, chondrocytes proliferate, undergo hypertrophy, and mineralize to extend bone length. Many factors control chondrocyte migration and growth in the EP, especially cell membrane channels. Previous data show Aquaporin-9 (*Aqp9*) expression in the EP pre-hypertrophic zone. Newborn *Aqp9* -/- mice have longer limbs compared to +/+ littermates. Furthermore, Aqp9 transports H2O2, which may influence chondrocyte proliferation and hypertrophy. Aqp9 may therefore control chondrocyte differentiation, influencing long bone growth.

The data suggests that ***Aqp9* is a regulator of EP chondrocyte proliferation, hypertrophy, and long bone growth**. To test my hypothesis, I proposed:

1. To substantiate growth differences by comparing long bone lengths of *Aqp9* +/+ and -/- mice at P5 and P21. Femur, tibia, and humerus bones were stained with Alizarin red and Alcian blue to produce skeletal preps. Lengths were measured manually via digital calipers and digitally via photography and software analysis with ImageJ.

2. To compare cellular activity and phenotypical differences between *Aqp9* +/+, +/-, and -/- EP chondrocytes. P5 chondrocytes were extracted and cultured to measure proliferation differences with cell counting and alamarBlue assays. P5 knees were fixed and stained with H&E to investigate cell density and length differences in the EP zones.

3. To investigate transcriptome differences between *Aqp9* +/+ and -/- EP chondrocytes. Microarray analysis was performed to investigate genes with fold changes. Phloretin and insulin (reputed *Aqp9* inhibitors) were used to treat *Aqp9* +/+ and ATDC5 chondrocytes to observe expression changes in proliferation and hypertrophy genes.

P5 *Aqp9* -/- mice presented with longer femur, tibia, and humerus bones than their +/+ littermate counterparts. P21 *Aqp9* - /- presented with slightly shorter long bones than their +/+ littermates. *Aqp9* -/- EP chondrocytes were shown to proliferate at higher rates than their +/+ and -/- counterparts. Proliferating chondrocytes in the EP proliferative zone were at higher cell density in *Aqp9* -/- mice than +/+ mice. Microarray analysis did not show significant changes in common chondrocyte differentiation genes between +/+ and -/- mice. Phloretin decreased *Aqp9* and *Mmp13* (hypertrophy marker) expression in *Aqp9* +/+ chondrocytes. Insulin increased *Aqp9* but decreased *Col2* (proliferation marker) expression in ATDC5 chondrocytes.

P5 *Aqp9* -/- mice may exhibit early growth spurts due to a proliferative phenotype of *Aqp9* -/- EP chondrocytes. *Aqp9* -/-chondrocytes proliferate faster than their +/+ counterparts and appear denser in the EP, suggesting

higher division rates. The concomitant decrease of *Aqp9* and *Mmp13* expression suggest that *Aqp9* may contribute to hypertrophic activity, while the increase of *Aqp9* expression alongside the decrease of *Col2* expression suggest that *Aqp9* opposes proliferative activity. These observations suggest that loss of *Aqp9* promotes chondrocyte proliferation in the EP and advances early long bone growth. *Aqp9* may be of therapeutic value in treating limb length discrepancies.

**Positive effects of intermittent PTH on growing bone and dystrophic muscle in Mdx mouse model of Duchenne Muscular Dystrophy**

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Duchenne Muscular Dystrophy (DMD) is a progressive muscle disorder caused by genetic mutations on dystrophin encoding gene. With an absence of functional dystrophin, DMD boys show not only muscle wasting and inflammation, but also compromised bone health with high risk of fracture. The use of high dose glucocorticoids as the gold standard therapy also contributes to bone fragility in DMD boys. Our previous study showed that the prophylactic use of anti-resorptive bisphosphonates improved bone mineral density (BMD) but did not improve mechanical properties of bone in the Mdx mouse model of DMD and dramatically decreased bone turnover. Thus, this study examined the effects of daily administered parathyroid hormone (PTH), the only available bone anabolic therapy, on growing bone and dystrophic muscle in the presence of slow-release pellet of prednisone using the Mdx mice. Five-week prednisone treated Mdx mice showed decreased cortical bone thickness and area ($p<0.001$), which was significantly improved by PTH treatment ($p<0.001$). This bone increase by intermittent PTH was through suppression of osteoclasts on endocortical surfaces ($p<0.001$). While there was no significant effect of muscular dystrophy on cortical bone, Mdx mice showed significantly less volumetric BMD and bone mass of trabecular compartments in lumbar vertebrae (L6). Prednisone or intermittent PTH by themselves did not have significant effect, but the combination of two treatments resulted in significant increase in volumetric BMD ($p<0.001$) and percent bone volume ($p<0.01$). Moreover, prednisone treatment resulted in significantly improved grip strength and endurance in treadmill running, which were maintained and further improved, respectively, in co-treated Mdx mice. All together, our study demonstrated that intermittent PTH significantly improved glucocorticoid-induced bone loss and maintained or further enhanced dystrophic muscle function that is already improved by prednisone treatment. These findings could give an insight into the use of teriparatide in DMD boys, and also other forms of glucocorticoid-induced osteoporotic patients.

**Assessing individuals with and without hand arthritis to track the hand forces produced using a new wearable sensor technology while playing golf**

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With diagnosed arthritis affecting 1 in 10 adults in Canada and with an increasingly aging population, playing sports such as golf becomes difficult. The grip of a golf club is the only contact point between the player and the club, making the player's gripping force and the golf grip itself an important element of the game. Comprehensive examinations have not been done on current golf grips and a small amount of arthritis golf grips have been developed but are not based on empirical measurements or been properly tested to determine their effectiveness at reducing joint pain in a players hands. The purpose of this study is to systematically analyse the hand forces produced from different styles of golf grips along the distal phalanges at the hand-grip interface using a new wearable sensor technology of varying skill level golfers, with and without hand arthritis while performing a standard golf shot with a mid-iron club.

In order to study the forces that occur at the hand-grip interface of a golfers grip, a new way of measuring forces in the fingers has been developed at St. Joseph's Hospital, London, ON, in the Surgical Mechatronics Laboratory at the Roth McFarlane Hand and Upper Limb Centre (HULC). A small strain gauge sensor embedded into an acrylic substrate is mounted on the distal phalanges (fingernails) of the hands. The sensors provide an alternative technique of measuring strain and therefore forces in the hands without interfering with the hand-grip interface. Preliminary tests were performed on an avid left hand golfer (M, 36-50). Graphs were created to demonstrate the variation in micro-strain of the index, middle, and pinky of the left hand (bottom hand) and the ring and pinky fingernails of the right hand (top hand). The CP2 Pro Standard, CP2 Pro Jumbo, and the Tacki-Mac Arthritis grips were evaluated. The data demonstrates that the standard grip produced the largest micro-strain values in the left middle, left pinky and right ring finger with the jumbo grip producing the largest micro-strain values in the left index and right pinky fingers. These preliminary results demonstrate that standard sized golf grips produce larger strain values on the hands then other grips. However, preliminary results suggest that large diameter grips designed for players with hand arthritis may not be the most effective at reducing the strain and therefore the force in a players hands.

The hand is a very complex structure and has not been fully studied when relating arthritis to sports. Therefore, a thorough understanding of the effects that various materials and geometries of golf grips have on the forces occurring at the hand-grip interface is important. The intent of this project is to determine the most suitable grip for a golfer with arthritis and to develop a joint protection golf grip that will minimise the damaging forces at the hand-grip interface to enable individuals with arthritis to continue to golf and to decrease the potential risk of developing arthritis in the hands.

**Dynamic mechanostimulation of live cells with high-frequency oscillatory fluid shear during realtime microscopy**

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**Introduction**: Mechanotransduction is the process by which cells sense – and respond to – the local mechanical environment. This ability to react to external loads and forces is a critical component of physiology and is essential for normal functioning of our bones, lungs, and blood vessels; yet, the underlying mechanisms are not fully understood. A form of mechanical stimulation that is commonly implicated in mechanotransduction is fluid shear stress. Recently, high-frequency oscillatory fluid shear (HFOFS) has been identified as a physiologically relevant stimulus, but the equipment does not yet exist to apply HFOFS to live-cells during real-time monitoring. Our goal is to observe the immediate responses of cells to high-frequency OFS. Here, we describe the development and validation of a microfluidic platform for applying OFS to livecell cultures at frequencies up to 90 Hz, with shear up to 3 Pa; this platform is compatible with real-time optical microscopy and photometry.

**Methods**: The system was comprised of 3 main components: a microfluidic device with an on-chip pump, an electromagnetic (EM) actuator to interface with the on-chip pump, and a motorized micro-manipulator to precisely position the EM actuator; all of which were assembled on an aluminum plate and then mounted on an inverted microscope. The microfluidic device was of a novel design and fabricated from the silicone polymer polydimethylsiloxane using a method described previously (Lorusso *et al.* Biomed. Microdevices 18:78, 2016). The channel height was nominally 300 µm in all cases, and the width tapered from a nominal maximum of 1252 µm, to 751 µm, and to 500 µm at its narrowest. Fluid dynamics within the channels were modeled with Abaqus CFD. Steady flow was introduced to the device with a syringe-pump and the flow waveform was modulated with a sinusoidal oscillation using the on-chip EM pump. To observe flow velocities, marker beads were pumped through the channels and imaged at high-speed with a micro-particle image velocimetry system. These velocities were then fit to a sine curve using non-linear regression in GraphPad Prism statistical software package. To validate the application to cells, MC3T3-E1 osteoblast-like cells were seeded into the device, and evaluated for compatibility with fluorescence microscopy.

**Results**: During operation above 30 Hz, sinusoidal flow waveforms were observed, with velocities in the millimeters per second range. At 1 mL/min steady flow, from the narrowest to widest channel, velocities of 22.6, 15.4, and 7.8 cm/s were predicted, resulting in wall shear stresses of 3.0, 2.1, and 1.0 Pa, respectively. During preliminary studies at 30 Hz oscillatory flow simultaneous with 15 µL/min steady flow, peak velocity was 7.64 ± 0.14 mm/s, with an average frequency of 30.14 ± 0.09 Hz ($R^2$ = 0.95). Cells were successfully seeded in the device and remained adherent and viable, while maintaining compatibility with fluorescence microscopy.

**Conclusions**: We have developed and tested a microfluidic system capable of – for the first time – delivering physiologically relevant high-frequency oscillatory fluid shear stress to live-cells during real-time microscopy and photometry.

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**TGFalpha deficiency does not affect the phenotype of cartilage-specific MIG6-knockout mice**

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**Purpose:** Osteoarthritis (OA) is a prevalent joint disease that involves the degeneration of smooth articular cartilage found at interfacing bone surfaces in synovial joints. The mRNA for transforming growth factor-alpha (Tgfa, a potential ligand of epidermal growth factor receptor (EGFR)), has been seen upregulated in a subset of OA cases, both in rat models and human patients (*Appleton et al., 2007*). Mitogen-inducible gene 6 (Mig-6) is induced by EGFR signaling and acts as a negative feedback regulator by sterically inhibiting downstream signaling and targeting EGFR for internalization and degradation. In previous studies, mice with a cartilage-specific knockout (KO) of *Mig-6* have exhibited anabolic increases in articular cartilage thickness, suggesting that decreased EGFR signaling can mitigate OA effects (*Pest et al, 2014*). However, development of chondro-osseous nodules (CONs) within the joint capsule in this phenotype restricts movement of the joint, which can be debilitating. We aimed to attenuate EGFR signaling by inhibiting TGFα production in Mig-6-KO mice to further understand the role of this pathway in joint homeostasis.

**Methods:** Male mice displaying cartilage-specific Mig-6 deletion with both homozygous *Tgfa* wild type (*Mig-6$^{fl/fl}$Col2a1-Cre$^{+}$ Tgfa$^{+/+}$*) and *Tgfa*-null (*Mig-6$^{fl/fl}$Col2a1-Cre$^{+}$ Tgfa$^{-/-}$)* genotypes, along with male mice displaying control genotypes (Mig6$^{fl/fl}$Col2a1-Cre$^{-}$ Tgfa$^{+/+}$ and Mig-6$^{fl/fl}$Col2a1-Cre$^{-}$ Tgfa$^{-/-}$), were generated and raised to 12 weeks of age. Mice from different litters were age-matched in order to achieve N=5. Joint structure was then assessed using toluidine blue histological staining. Average articular cartilage thickness and cell density were obtained from sagittal sections of the elbow and knee joint, as well as from frontal sections of the knee. Immunohistochemical analysis was also performed for SOX9, a transcription factor and key marker for both chondrocyte development and phenotype maintenance.

**Results:** Regardless of the absence or presence of TGFα, a significant increase in articular cartilage thickness was found at the surfaces of the humerus, ulna, femur, and tibia in Mig-6-KO models compared to controls. Similarly, cartilage-specific Mig-6-KO, but not whole-body *Tgfa* KO mice, significantly increased cell density in mice 12 weeks of age. Formation of CONs were found in the knee joints of Mig-6-KO mice regardless of the presence of TGFα. Unexpectedly, *Tgfa*-null Mig-6-KO mice appeared to develop more severe CONs that were larger in area with increased proteoglycan staining compared to Mig-6KO mice with TGFα. Immunochemical staining for SOX9 also appeared more intense in Mig-6-KO mice without TGFα compared to controls.

**Conclusions:** This study confirms that Mig-6 plays an integral role in regulating articular cartilage growth in synovial joints and deletion of *Mig-6* results in an anabolic phenotype. However, at the time point examined, TGFα did not have an effect on articular cartilage thickness or cell density. This suggests that the anabolic phenotype in Mig6-deficient mice arises from the absence of Mig-6 inhibition in other non-EGFR specific mechanisms, or through alternate ligand activation of EGFR.

**Endothelial cell expression of ephrin B2 and ephrin B4 signalling in pulmonary fibrosis**

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**Preliminary Data:** Pulmonary fibrosis yields poor clinical outcome and features in multiple diseases, most prominently in idiopathic pulmonary fibrosis and systemic sclerosis (SSc). Currently, mechanisms to explain the cause or development of fibrosis are unclear. Previously, we have identified a novel shedding phenomena of the receptor tyrosine kinase ephrin B2 (EfnB2) within the lung following ADAM10 cleavage. Soluble ephrin B2 (s-EfnB2) levels increase with the onset of fibrosis in mice undergoing bleomycin-induced pulmonary fibrosis; decreases in s-EfnB2 through inducible genetic ablation of EfnB2 in fibroblasts reduce lung fibrosis in mice. However, fibroblast-specific knockout of EfnB2 is not sufficient in removing sEfnB2 from mouse BAL or lung homogenate. EfnB2 in the lung binds the receptors ephrin B3 and ephrin B4 (EphB4); it is possible that contribution of s-EfnB2 from other cell types such as endothelial cells is significant enough to permit fibrotic change despite ablation in fibroblasts. The cell type that interacts with s-EfnB2 to facilitate lung fibrosis still remains unknown. Notably, endothelial cells are a key feature of SSc-related pulmonary arterial hypertension, which is a distinct pathology related to lung fibrosis which contributes significantly to mortality.

**Objectives:** This study seeks to evaluate the effects of EfnB2 knockout as well as EphB4 knockout in endothelial cells for a lung fibrosis mouse model.

**Methods:** C57BL/6J mice expressing either floxed EphB4 or floxed EfnB2 were crossed with C57BL/6J mice expressing TekCre. To initiate genetic deletion, 4 week old mice underwent tamoxifen treatment (1 mg in 100 µL corn oil daily for 5 days) or corn oil as control. Bleomycin sulphate (1.2 U/kg) was administered to mice through intratracheal instillation and mouse lungs were collected after 14 days.

**Results:** Mouse breeding yielded EphB4 f/f Tek-Cre mice and EfnB2 f/f Tek-Cre mice which are endothelial specific knockouts inducible through tamoxifen treatment. Knockout was confirmed through exon-1 genotyping and IHC. Bleomycin treatment and dosage was shown to cause moderate to severe fibrosis at the selected time point. 6-week old KO or control mice will undergo non-surgical intratracheal instillation with bleomycin to induce pulmonary fibrosis and sacrificed following 14 days. Fibrosis will be qualitatively assessed through histology and Masson's trichrome staining and then quantified through hydroxyproline analysis of either whole lung homogenate or BAL. Endothelial cells or fibroblasts will be extracted from mouse lung homogenate and cultured for further biochemical analysis.

**Localalized delivery of doxorubicin using nanobeads in a 3D-tumor model**

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**Introduction:** The spine is one of the most common sites of metastasis, with 80% of prostate, lung, and breast cancers metastasizing to the spine. Chemotherapy is an important treatment modality, with Doxorubicin (Dox) being one of the most effective options. The high systemic doses required for the drug to be effective prevent its prolonged use due to the significant heart failure it causes with cumulative doses. Mesoporous silica nanobeads have been proven to be an effective vehicle for sustained and prolonged release of drugs locally. However, no current study has assessed the ability of nanobead release of Dox on cancer cells inside a bone-like 3D culture model. We hypothesize that nanobeads loaded with Doxorubicin will allow for adequate and sustained local delivery of the drug for treatment of prostate, lung, and breast metastasis inside a 3D model. We first test the prostate cancer cell line LAPC4, as this cell line has never been studied with Dox and has no established IC50 dose. The objectives of this study are 1) Test the effect of Dox on LAPC4 viability in a 2D culture and 3D bone-like model to establish an IC50 dose. Objective 2) Study the release mechanics of Dox from nanobeads to be able to achieve sustained drug levels in the range of the IC50 dose determined in the first objective.

**Methods:** LAPC4 prostate cancer cell line has been acquired from Dr. Lapointe at McGill University. The effect of dox on cell metabolism (AlamarBlue®), proliferation (vybrant® MTT), migration (Boyden Chamber), invasion (3D spheroid invasion), and viability (LIVE/DEAD™ assay) were determined. Mesoporous silica nanoparticles were loaded with fluorescent Dox and the release amount was quantified over 14 days using a Tecan fluorescence plate reader. Ongoing work to generate the 3D matrix model uses a combination of alginate and gelatin, supplemented with primary osteoblast cells, nano-hydroxyapatite, and collagen to mimic a bone-like environment.

**Results:** An IC50 dose for Dox on LAPC4 metabolic activity was established at 5.4 uM (+/- 0.96 uM) (n=4). After loading the silica nanoparticles with ~100 ng of Dox, the beads were shown to release 58% of the loaded drug over a 14-day period (n=2). Bone marrow-derived MSCs grown for over 28 days in the alginate/gelatin-3D model can achieve 90% viability and produce calcified matrix under osteogenic conditions (n=1).

**Conclusion:** These data have established an IC50 dose for LAPC4 cells, which has not previously been reported. It was demonstrated that nanoparticles can effectively release Dox over a two-week period, providing potential for further study on sustained release of the drug directly onto cells. Using nanoparticles for controlled and sustained local delivery of Dox can allow for development of novel therapeutics to prevent the severe side effects caused by the high systemic doses currently administered. This will in turn lead to better treatment and outcomes for patients suffering from spine and bone metastases.

**Articular facet joint cartilage degeneration in adolescent idiopathic scoliosis**

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Adolescent Idiopathic Scoliosis (AIS) is a progressive bending of the spine which affects up to 4% of children between the ages of 10 to 16. The 3-dimensional deformation of the spine causes a biomechanical misbalance on the load bearing tissues such as the intervertebral discs (IVD) and the facet joints. It has previously been reported that abnormal loading can lead to degenerative pathways in articular cartilage and IVDs, but scoliotic facet joint cartilage has not been characterized yet. In this study, we analyzed facet joint cartilage from consenting AIS patients undergoing corrective surgery through histology and western blotting to assess proteoglycan content, cell density, secreted degenerative factors such as proteases and inflammatory cytokines as well as extracellular matrix component fragmentation. Healthy facet joint cartilage from familial consenting cadaveric donors was used as a control group. Safranin O – Fast green histological staining revealed that scoliotic cartilage had significantly less proteoglycan content and higher osteoarthritic phenotype assessed by OARSI score. Interestingly, cell density within the cartilage varied significantly between the two facet joints forming a pair in AIS patients but was constant in healthy donors. To support this, KI-67 immunopositivity revealed that higher proliferation was present in AIS cartilage. Furthermore, the proteases MMP3, MMP13 and the pro-inflammatory cytokine IL-1b were overexpressed in IHC compared to non-scoliotic controls. Western blots of extracellular matrix components showed protein fragments of chondroadherin, decorin, fibromodulin and biglycan which were not seen in aged-matched non-scoliotic cartilage. Interestingly, the amount of fragmentation seen in chondroadherin and decorin correlate with the severity of the scoliotic curve. These findings indicate that AIS patients suffer from early cartilage degeneration of the facet joints, which we speculate is originating from the abnormal biomechanics. As with age-related osteoarthritis, the joint degeneration in AIS patients is likely to contribute to the pain perceived in these individuals. We are currently conducting a study to investigate the link between the biomechanical loading, joint degeneration and pain.

**MicroRNA 27b-3p promotes extracellular matrix production in osteoarthritis synovial fibroblasts**

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**Purpose**: We have recently shown that microRNA 27b-3p (miR-27b-3p) expression is significantly elevated in the synovial fluid of patients with late-stage osteoarthritis (OA; Kellgren-Lawrence score 3 & 4) compared to early-stage knee OA (Kellgren-Lawrence score 1 & 2). Our explant studies further identified that miR-27b-3p was released from synovium, rather than cartilage, into the synovial fluid in response to inflammation. Therefore, in the present study we focused on synovial fibroblasts as the major cell type in the synovial lining in order to characterize the roles and regulation of miR-27b-3p in synovial inflammation and fibrosis associated with OA.

**Methods**: Synovial specimens were obtained with informed written consent from late-stage OA patients who underwent total knee replacement. Primary synovial fibroblasts were isolated and passages 4-6 were subjected to interleukin-1beta (IL1β, the main proinflammatory cytokine) and transforming growth factor beta (TGF-β, the major profibrotic mediator) treatments. Synovial fibroblasts from the same passages were transfected with miR-27b-3p mimic or miR-27b-3p inhibitor and control mimic or control inhibitor. Real-time PCR or Western blot were performed to measure the expression of the main mediators of synovial inflammation, fibrosis, and cell adhesion. For synovial inflammation, the expression of interleukin 6 (IL-6) and tumor necrosis factor alpha (TNFα) were measured. To explore synovial fibrosis, the expression of TGF-β, Collagen1A (COL1A) and a number of adhesion molecules including intercellular adhesion molecule 1 (ICAM-1), vascular adhesion molecule 1 (VCAM-1), epithelial cadherin (CDH1), and neural cadherin (CDH2) were examined.

**Results**: We first investigated the regulation of miR-27b-3p in synovial fibroblasts and observed a significant decrease in miR-27b-3p expression levels with IL-1β treatment. We then characterized the functional roles of miR-27b-3p in synovial inflammation; our results indicate that IL-1β was undetectable in most of miR-27b-3p mimic/inhibitor transfected synovial fibroblasts. At the mRNA level, IL-6 showed a trend of upregulation and TNFα was significantly upregulated with miR-27b3p overexpression. When synovial fibroblasts were subjected to TGF-β treatment, there was no effect on the expression of miR-27b-3p. Further, evaluation of ECM and adhesion markers in the miR-27b-3p mimic/inhibitor transfected synovial fibroblasts demonstrated that overexpression of miR-27b-3p significantly increased the expression of VCAM-1 mRNA, COL1A mRNA and protein, and upregulated both fibronectin 1 and CDH2 mRNAs while CDH1 remained undetectable. Interestingly, suppression of miR27b-3p led to a significant decrease of COL1A protein expression.

**Conclusions**: Our data for the first time shows that miR-27b-3p is involved in the elevation of TNFα expression and the regulation of ECM by promoting collagen production as well as the expression of adhesion molecules VCAM-1 and CDH2 in OA synovial fibroblasts. Furthermore, inhibition of miR-27b-3p results in the suppression of collagen production, suggesting a crucial role of miR-27b-3p in mediating inflammation and ECM production . Current efforts are underway to further investigate if miR-27b-3p modulates OA synovial fibroblast functions such as migration, adhesion and proliferation to further establish its role in mediating synovial inflammation and fibrosis during OA.

**MLO-Y4 osteocyte response to in-vitro mechanical unloading in a 3D scaffolding**

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The negative effects of spaceflight on astronaut bone homeostasis is currently limiting our ability to undergo long-term manned missions beyond low-Earth orbit. Here, we studied an osteocyte cell line, MLO-Y4, under simulated microgravity achieved with the Synthecon Rotary Cell Culture System (RCCS) to investigate how the lack of mechanical forces influences the transcriptome and morphology of these bone cells. The study also aimed to validate the RCCS with a novel 3D physiologically relevant collagen and mineral scaffolding for the cells and compare their gene expression and morphology to studies conducted in true microgravity. This work will lead to a better understanding of the osteocyte's role in astronaut osteoporosis and provide a more 3D-relevant platform for studying bone cells in a ground-based microgravity simulator. Gel scaffolds composed of collagen I and calcium phosphate were used to produce a bone-like environment for the cells. The gel was inoculated with a cell suspension and mixed to ensure homogenous cell distribution. The mixture was then formed into droplets to allow compatibility with the RCCS. Our results of cell distribution in the scaffolding indicate that cell viability is maintained in the core of the droplets. We also show that the droplets coalesce in the RCCS and achieve stable free-fall in the culture media. Quantitative Real-Time PCR data from cells exposed to free-fall for 3 days in the scaffolding will be used to determine if MLO-Y4 cells in free-fall are being mechanically stimulated or not. Once an optimized simulated microgravity environment is achieved, a transcriptome analysis will be performed. The data obtained will be used to determine novel genes affected by mechanical unloading in osteocytes which can be further studied as potential therapeutic targets to combat astronaut bone loss. We will also observe the changes in morphology between cells in simulated microgravity and static control conditions to better inform the mechanisms responsible for osteocyte dysregulation in unloaded environments.

## Characterizing TβL1/TβLR1 SUMOylation in Dupuytren's Disease

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**Background:** Dupuytren's disease (DD) is a chronic fibrosis of the palmar fascia, a fibrous layer of tissue found underneath the skin of the palm and digits that can cause permanent finger contractures. Treatments are mainly limited to surgery, however disease recurrence rates are high and new therapeutics need to be developed. One molecular characteristic of DD is increased cytoplasmic and nuclear β-catenin levels in fibroblasts derived from DD (DD cells). Nuclear translocation of βcatenin is implicated in the trans-activation of gene expression in DD, but the specific molecular mechanisms facilitating βcatenin nuclear translocation are unclear. Recently, conjugation of small ubiquitin-like modifiers (SUMO1) to Transducin βlike 1 (TβL1) and Transducin β-like receptor 1 (TβLR1) have been implicated in aberrant β-catenin nuclear localization in cancer. BC2059 (Tegatrabetan, β-Cat Pharma), a small molecule inhibitor, has been developed to competitively inhibit SUMOylated TβL1/TβLR1 interactions with β-catenin as an anti-cancer drug. Our preliminary findings demonstrated that DD cells treated with BC2059 show significantly attenuated β-catenin levels, whereas palmar fascia fibroblasts derived from patients without a history of DD (control, CT cells) remain relatively unaffected.

**Rationale:** Based on the findings that 1) there are increased levels of cytoplasmic and nuclear β-catenin in DD, and 2) BC2059 treatment attenuated β-catenin levels in DD cells but not in CT cells, we hypothesize that increased β-catenin nuclear localization in DD is a result of increased SUMOylated TβL1/TβLR1 levels. The purpose of this ongoing study is to determine any parallels in the molecular characteristics associated with aberrant β-catenin nuclear localization between cancer and DD, in order to develop a strong rationale for cross-purposing anti-cancer therapeutics such as BC2059 for the treatment of DD and other fibrotic diseases.

**Methods and Results:** DD and CT cells were isolated and cultured from the palmar fascia of patients with and without DD, respectively. Cells were collected and all SUMOylated proteins were immunoprecipitated using an anti-SUMO1 antibody, and the immunoprecipitate was immunoblotted for TβL1. Preliminary data suggests a trend towards increased levels of TβL1 SUMOylation in DD cells relative to CT cells. Immunocytofluorescence microscopy was also performed on cultured CT and DD cells, to characterize TβL1 and TβLR1 localization. Preliminary findings suggest that TβL1 localization was predominantly nuclear in CT cells, but both cytoplasmic and nuclear in DD cells. Furthermore, preliminary findings suggest that although TβLR1 localization was nuclear and cytoplasmic in both CT and DD cells, TβLR1 localization was observed at a higher intensity in DD cells.

**Discussion:** Our preliminary data suggest that increased β-catenin levels and transcriptional transactivation in DD may be the result of interactions with SUMOylated TβL1/TβLR1, as seen in cancers. This ongoing study will elucidate these and other molecular mechanisms that DD and cancers have in common to determine the utility of cross-purposing anti-cancer drugs for treatment of DD and other fibroses.

**Characterization of Wilms' tumour 1 (WT-1) as a fibrotic biomarker for Duchenne muscular dystrophy**

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**Background:** Duchenne muscular dystrophy (DMD) is a progressive neuromuscular disorder. DMD has no cure, and most patients succumb to the condition in their mid-twenties. It is characterized by muscle wasting and muscle weakness, which initially presents at three to five years of age. DMD results from loss of the cytoskeletal protein dystrophin. Poor dystrophin function, or reduced production, leads to a decrease in structural support in muscle cells. This causes cell death and necrosis, resulting in remodeling of muscle into fibrous and fatty connective tissue, impeding overall muscle function. Fibrosis is profound in DMD, generating a microenvironment that is a significant impediment to both endogenous muscle repair and potential regenerative strategies. At present, there are few therapies that specifically target fibrosis and microenvironment improvement, but one possibility rests in targeting Wilms' Tumour 1 (WT-1) protein. WT-1 is a zinc finger transcription factor commonly found in nephroblastomas, and recently shown to be expressed in fibrotic conditions such as Dupuytren's disease and pulmonary fibrosis.

**Hypothesis :** The objective of this research project is to characterize WT-1 expression in DMD muscle tissue. We hypothesize that WT-1 is upregulated in DMD muscle tissue following onset of fibrosis.

**Methods:** To provide an understanding of WT-1 function in DMD, our methodology makes use of wildtype control mice and mdx DMD model mice. Subtypes of these mdx mice, with one or both alleles for utrophin knocked out, were also used to provide models for progressively more acute fibrosis forms. We utilized immunohistochemistry to quantify WT-1 protein expression in muscle tissue taken from the diaphragm and gastrocnemius muscles of these mice. One-way ANOVA with Tukey's post-hoc test was used to identify significant differences between groups.

**Results:** WT-1 expression was found to be elevated in the diaphragm of mdx mice compared to wildtype controls, but not in the gastrocnemius muscles of these same mice. Furthermore, WT-1 expression was found to be elevated in both diaphragm and gastrocnemius muscles of mdx/utrn+/- and mdx/utrn-/- mice compared to wildtype controls.

**Discussion and Conclusions:** Of the tissues examined, only the gastrocnemius muscles of mdx mice have been previously identified as non-fibrotic, with all others having been identified as fibrotic. Therefore, the hypothesis that WT-1 is upregulated in DMD muscle tissue following onset of fibrosis was supported, as WT-1 was only found upregulated in fibrotic tissue. Furthermore, that WT-1 is upregulated in this form of fibrosis suggests that WT-1 is a broad fibrotic marker, rather than being limited to conditions such as Dupuytren's disease or pulmonary fibrosis. With regards to future work, the impact of WT-1 on the progression of fibrosis still requires investigation. Clinical applications involving WT-1 are numerous, most notably the foremost application that WT-1 may be used as a biomarker to detect fibrosis onset. Additionally, immunotherapies currently exist which target WT-1 positive cancer cells for destruction by the immune system. Should WT1 contribute to fibrosis rather than only acting as a biomarker for it, whether the application of these therapies impedes fibrosis overall can be examined.

**Ultrafast pulsed laser ablation of articular cartilage for applications in orthopedic surgery**

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**Background:** Articular cartilage defects can significantly decrease patient quality of life. Cartilage grafts can correct tissue damage while becoming permanently incorporated into the surrounding tissue, thus delaying total joint replacement. However, major constraints of grafting capabilities are the limitations in producing grafts of diverse shapes and dimensions. Suboptimal fitting can lead to mechanical weakness, bone cysts, and a poor healing environment. Current mechanical-based graft preparation methods cause substantial collateral tissue damage from mechanical tearing while nanosecond pulsed lasers produce significant thermal injury and charring. Standard size pre-prepared cartilage plugs lack modularity and do not precisely fit injury sites. However, the use of an ultrafast burst-mode fibre laser to customize grafts and graft beds would allow for the precise fitting of grafts to damaged cartilage while minimizing collateral tissue damage, thereby improving patient outcome.

**Method:** Porcine cartilage underwent laser ablation *ex vivo* with an ultrafast burst-mode ytterbium fibre laser system (FiberLAST Inc., 1037 nm wavelength, 350 fs pulse duration, 60 pulses per burst, 20 µm spot). Subsequently, samples were stained using live/dead fluorescent dye and imaged by confocal microscopy to illustrate local cell survival. Optical coherence tomography (OCT) imaging allowed for volumetric calculations of material removed during ablation. Histological sections were prepared and stained with hematoxylin and eosin (H&E) to reveal cellular structure and safranin-O to illustrate proteoglycan content.

**Results:** Large volumes of cartilage tissue up to ~1 $mm^3$ were ablated in predetermined geometries to demonstrate the potential of the laser to produce cartilage graft beds. OCT imaging characterized the 3-dimensional shapes and volumes of each graft bed. Ideal laser ablation settings produced graft beds that had straight, smooth edges and reached the predetermined target depth. Confocal microscopy demonstrated minimal collateral tissue damage to the host tissue with cell death limited to a few cell-lengths from the cut. This is substantially less damage compared to that reported from use of mechanical cutting and nanosecond laser ablation. Histological examination illustrated changes in cellular structure directly adjacent to the ablation site, however these changes were highly localized. By optimization of laser parameters, damage was consistently limited to < 5 cell lengths from the cuts.

Ongoing work includes evaluating changes to collagen fibres using second harmonic generation imaging and immunohistological staining to indicate mechanism of cell death post-laser ablation. Long-term cellular effects of the ultrafast laser are also under investigation by evaluating irradiated cartilage explants that have been cultured *in vitro* for several days.

**Conclusions:** Improvement in the precision of graft cutting and graft bed preparation could potentially affect millions of patients with osteoarthritis. This preliminary work demonstrates the advantages of using an ultrafast fibre laser over longer-pulse lasers and mechanical tools, highlighting the potential clinical benefits of using such a technique for orthopedic surgery.

**FoxD1 lineage derived fibroblasts as contributors to skin development, homeostasis, and wound healing**

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Central to dermal repair is recruitment of fibroblasts and their subsequent differentiation towards a myofibroblast phenotype, the latter of which produces excess and inappropriately organized ECM to repair damaged tissue. With critical roles in fibrotic processes, including fibrosis and wound repair, there has been a recent focus on identifying the progenitor cells which give rise to this expansive population. In the present study, we are investigating a population of Foxd1-positive-progenitor derived (Foxd1 lineage positive; FLP) fibroblasts, a lineage which originates during development, and has been reported to contribute to myofibroblast populations in kidney and lung fibrosis in adult mice. It is unknown whether a similarly derived population contributes to dermal wound repair, which would suggest developmental commonalities between myofibroblast precursors across tissues. To investigate FLP contributions to skin development, homeostasis, and tissue repair, we have employed a genetic cross between transgenic mice expressing Cre recombinase downstream of the Foxd1 promoter sequence, and a reporter line expressing loxP-stop-loxP-tdTomato under the ubiquitous Rosa26 and CAG promoters. During murine embryogenesis, we have observed Foxd1 expression from embryonic day 9.5 (E9.5) to E14.5 within the developing dorsal tissue, where it is expressed in tissues ventral to the developing dermis, but not within the dermis itself. Conversely, FLP cells are present within the developing dermis. In adult mouse skin, FLP cells are present throughout the dermis, and hypodermis, but not within the epidermis. Notably, we have found that Foxd1 is not expressed within adult skin, suggesting that dermal FLP cells represent a developmentally distinct lineage from the Foxd1 lineage negative (FLN) population. Next, we investigated the contributions of FLP and FLN cells to excisional wound repair. FLP cells are seen in low abundance, near the wound edges 3-days post wounding (dpw), increasing in frequency to 10 dpw, and remaining, with similar frequency, up to 21 dpw. We have identified that FLP cells constitute 54% (±10%; n=6) of fibroblasts within the adult dermis, and 63% (±8%; n=6; $p>0.05$) of fibroblasts isolated from granulation tissue at 10 dpw. Gene expression of FLP and FLN fibroblasts, from uninjured skin and 10 dpw granulation tissue, was assessed using a qPCR array to investigate genes related to fibrosis. Interestingly, FLP fibroblasts were enriched in genes associated with matrix production and remodeling, whereas FLN fibroblasts were enriched in signaling related genes. As these cells appear to maintain phenotypic differences during homeostasis and repair, we are currently investigating whether these differences can be maintained *in vitro* to allow further comparison of these distinct fibroblast populations. Preliminary gene expression data show a rapid change, and loss of unique phenotype when sorted FLP and FLN fibroblasts are seeded onto 2D surfaces of varying stiffness. In contrast, when seeded within a 3D collagen matrix, FLP and FLN fibroblasts partially maintain their *in vivo* expression profiles. The data collected support several recent findings regarding the heterogeneity of fibroblast populations, and that this heterogeneity originates during development. Further investigation may provide mechanisms through which to alter the healing response through selective activation or inhibition of these populations.

**The roles of dysregulated Wilms tumor 1 in Dupuytren's disease development**

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Dupuytren's disease (DD) is a common and heritable connective tissue fibrosis of the palmar fascia. Characteristics of DD include excessive secretion of extracellular matrix molecules, fibrotic nodules and palmar-digital contractures. Despite surgical intervention, the recurrence rate of these debilitating contractures is high and this condition is currently considered incurable. Previous research in our laboratory has identified an increase in *WT1* mRNA transcripts which encodes Wilm's Tumor 1 (WT1) proteins in primary fibroblasts derived from diseased DD connective tissue. Unlike DD fibroblasts, "pre-fibrotic" (PF) and normal control (CT) fibroblasts expression levels of *WT1* are very low or undetectable in the absence of pro-fibrotic stimuli (M1 cytokines). *WT1* can theoretically encode more than 30 different transcripts and is commonly known for its roles in cancers. Four major isoforms of WT1 are differentiated by the inclusion or exclusion of exon 5 and a Lysine-Threonine-Serine (KTS) motif in exon 9. Inclusion of exon 5 was reported to act as a transcriptional regulator, whereas KTS+WT1 isoforms bind RNA and have roles in RNA splicing. Preliminary data from our adenoviral-mediated gain of WT1 function studies in CT fibroblasts indicate that constitutive expression of WT1 isoform B (exon 5+/ KTS-) promotes a proliferative phenotype relative to vector-transduced cells.

We hypothesize that the sustained abnormal expression of *WT1* promotes the development of DD in normal palmar fascia repair. With ethics approval and patient consent, we derive primary fibroblasts from palmar fascia connective tissue that are either visibly fibrotic (DD) or normal (control). We aim to delete specific *WT1* transcripts by deleting exon 5 and/or sections of exon 9 containing KTS motif via CRISPR/CAS9 adenoviral transduction and cloning of CRISPR edited fibroblasts to achieve homogenous populations. We anticipate that Chromatin Immunoprecipitation Sequencing (ChIP-Seq), RNA Immunoprecipitation Sequencing (RIP-Seq) and total RNA sequencing analysis will identify novel WT1 regulated genes and interacting RNAs in normal and abnormal palmar fascia repair.

**Development of a new biological substitute for alveolar filling**

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Loss of bone volume after tooth extraction may occur in the maxillary alveolar tissue limiting the success of the dental implant. A common solution is to fill the cavity by an alveolar preservation product generally based on acellular biomaterials. However, subsequent inflammatory and / or infectious reactions can affect the bone regeneration and osteointegration of the dental implant. The overall goal of this project is to develop a new alveolar preservation substitute composed solely of mesenchymal stem cells, autologous cells extracted from adipose tissue (ASCs). Bone morphogenetic proteins (BMPs) such as BMP-9 play a key role in the osteogenic differentiation of mesenchymal stem cells *in vitro* and bone repair *in vivo*, hence the following hypotheses: i) Addition of BMP-9 will significantly improve the level of osteogenic differentiation and mineralization of ASCs and reconstructed tissues *in vitro* ii) Self-assembled co-culture of endothelial cells with ASCs will allow the formation of a new model of bone substitute possessing a capillary micro-network, free of exogenous biomaterials.

The first objective was to verify whether BMP-9 (1 nM) can improve the osteoblastic differentiation of ASCs cultured in an osteogenic medium for 3, 6 and 10 days (d). The BMP-2, a clinical standard, was used as a control. Quantitative RT-qPCR analyses on ASCs incubated in osteogenic medium showed a greater activation of genes encoding osteogenic markers such as *Runx2* (3d) and *Osterix* (3 and 6d) in the presence of BMP-9 compared to BMP-2. Moreover, a greater activation of *Alkaline phosphatase (ALP)* gene (3, 6 and 10d) was also detected in ASCs incubated in osteogenic medium with BMP-9 compared to all other experimental conditions. No expression of genes encoding markers of adipogenic lineage was detected. Moreover, the activation of gene encoding *Angiopoietin-1,* a key factor for angiogenesis, was also enhanced in cells incubated for 10 days in osteogenic medium supplemented with BMP-9 compared to BMP-2. Thus, the osteogenic medium enriched with BMP-9 appears to be a promising approach to improve osteogenic differentiation and secretion of angiogenic factors by ASCs.

The second objective was to engineer reconstructed tissues using the self-assembly approach. ASCs grown with or without osteogenic medium were either stimulated with BMP-9 or co-cultured with human endothelial cells from the umbilical vein expressing the green fluorescence protein (GFP). Increases in ALP activity were only observed in ASCs grown in osteogenic medium after the 15 and 21 days, especially when stimulated with BMP-9. Fluorescence monitoring of GFP endothelial cells also revealed the spontaneous formation of a capillary micro-network in the ASC-E co-culture within 21 days.

In a future *in vivo* study, we will determine the bone healing kinetics of ASCs bone substitutes obtained by BMP-9 treatment and prevascularization approaches on alveolar defects created in rats.

**Cell interactions of fibulin-4 and latent transforming growth factor-β binding protein-4**

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Elastic fibers are abundant extracellular matrix components that provide tissues such as skin, lungs, and blood vessels with elasticity. Mature elastic fibers consist of two distinct regions, the highly crosslinked elastic core surrounded by fibrillin-containing microfibrils. The cell surface located elastic fiber synthesis represents a highly complex process that requires the recruitment of several associated molecules including fibulin-4 (FBLN4) and latent transforming growth factor beta binding protein-4 (LTBP4). Mutations in FBLN4 cause autosomal recessive cutis laxa (ARCL), characterized by a severe and diverse array of defects including loose skin, aortic aneurysms, and emphysema. Mutations in LTBP4 were reported in patients with ARCL combined with arterial tortuosity syndrome. The mouse models of FBLN4 and LTBP4 also highlighted the roles of these two proteins in elastogenesis. Despite their importance in human and murine physiology and pathology, very little is known how FBLN4 and LTBP4 function on the molecular level.

Previously, we have shown that FBLN4 interacts with primary smooth muscle cells and fibroblasts. However, cell interaction with LTBP4 has not been demonstrated. In this study, we show that skin fibroblasts bind strongly to LTBP4. We further demonstrate the functionality of FBLN4 multimerization in cell binding. FBLN4 multimers, but not monomers, interact with cells. With a set of FBLN4 deletion mutants, we found two cell interaction epitopes, one located in cbEGF2-3 and a second one in the C-terminal domain. Additionally, we have investigated potential cell receptor(s) for FBLN4 and LTBP4. Neither FBLN4 nor LTBP4 contains an arginine-glycine-aspartic acid (RGD)-integrin binding sequence, and both FBLN4 and LTBP4 have high affinity for heparin. This suggests heparan sulfate proteoglycans may mediate FBLN4 and LTBP4 cell interaction. We demonstrate that FBLN4 cell interaction is exclusively mediated by cell surface-located heparan sulfate. In the presence of heparin, cell binding to FBLN4 is entirely abolished, and binding to LTBP4 is reduced. Treatment of cells with heparinases II and III reduces cell attachment to both, FBLN4 and LTBP4. These data suggest a new cell-interaction role for FBLN4 and LTBP4 role in elastogenesis.

**Role of fibrillin-1 in obesity**

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Fibrillins are large extracellular glycoproteins ubiquitously expressed in elastic and non-elastic tissues throughout the body. Type I fibrillinopathies are heritable connective tissue disorders caused by mutations in the fibrillin-1 gene. Marfan syndrome (MFS) is the most common type-I fibrillinopathy. Obesity is common in adults with MFS and it increases the risk of cardiovascular complications. Fibrillin-1 expression correlates with obesity and increased adipocyte size. It is unclear how fibrillin-1 deficiency derails fat metabolism in in MFS. The aim is to understand the role of fibrillin-1 in adipogenesis and the impact of nutrition using the MFS mgR mouse model. *Fbn1*$^{mgR/mgR}$ male mice show significantly higher fat deposition compared to the litter matched wild-type control mice. However, the mutant mice are not significantly different to wild-type controls in the whole-body weight. Gene expression studies of adipogenic markers such as PPARG (adipogenic transcription factor) and adiponectin (protein hormone involved in regulating fatty acid break down) demonstrated that male *Fbn1*$^{mgR/mgR}$ mice are characterized by higher levels of these markers, indicating that fibrillin-1 deficiency in *Fbn1*$^{mgR/mgR}$ mice leads to increased adipogenesis. To study the impact of nutrition on obesity, *Fbn1*$^{mgR/mgR}$ mice were fed with a high fat diet and a matched control diet. The feeding schedule started at 4 weeks and ended at 16 weeks. *Fbn1*$^{mgR/mgR}$ male mice tended to have a higher body weight compared to litter matched wild-type controls. The relative fat mass of the mice was determined by X-ray. Brown and white adipose tissue showed an significant increase in male *Fbn1*$^{mgR/mgR}$ mice, but not in female mice. Therefore, fibrillin-1 deficiency in male mice alters the adiposity and increases the fat mass as observed in obese patients with Marfan syndrome. The impact of nutrition on *Fbn1*$^{mgR/mgR}$ mice clearly demonstrates a link between fibrillin-1 and fat metabolism.

**Development of a 3D microenvironment model for human bone metastases.**

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**Background:** The bone is a dynamic tissue that is constantly broken down and repaired through a balance between osteoclasts and osteoblasts activity. However, when various cancers invade the bone, they can disrupt the physiological balance leading to various deleterious changes in bone structure and function. At the moment, radiotherapy, chemotherapy and surgical resection are the main clinical approaches to bone metastases. Studying isolated bone metastases cells molecularly at the *in vitro* level relies on 2D monolayer cultures which in no way represents the physiological in vivo tissue microenvironment. Therefore, preclinical and translational cancer research trends are moving toward organoid studies and 3D biomimetic models to better simulate the physiological tumor microenvironment and provide more clinically relevant drug screening. We believe that a combination of nano-crystal hydroxyapatite with primary human osteoblasts in an osteogenic media will result in the most robust 3D bone-like microenvironment which will allow better understanding of interactions between cells and tumors.

M**ethods:** We set out to generate a bone-like microenvironment to better model human bone metastasis. A working 3D hydrogel (1% alginate; 7% gelatin) model for cancer cell-migration was modified to incorporate nano-crystal hydroxyapatite, primary human osteoblasts and primary human bone marrow derived stromal cells. The constructs were cultured for 28 days in either normal growth medium (DMEM + 10% FBS) or osteogenic medium (OM) with and without 0.5 mg/mL hydroxyapatite (HA). Live/Dead assays were performed to quantify viability at 28 days of culture. Fixed samples were cryo-embedded and 12 µm sections were stained with Alizarin red for calcified matrix deposition.

**Results:** Live/Dead analysis revealed strong primary human osteoblast viability in all conditions after 28 days of culture: 91.3 ± 3.18 % for DMEM/$HA^+$, 92.5 ± 2.5 % for DMEM/$HA^-$, 88.6 ± 0.38 % for OM/$HA^+$ and 85.9 ± 6.2 % for OM/$HA^-$. Primary human MSCs showed approximately 85.1, 69.4, 87.8 and 90% viability in the same four conditions. Alizarin red staining showed that cells grown in DMEM without HA had the least amount of bone mineralized matrix. The combination found to have the most amount of bone mineralized matrix was OM/$HA^+$, followed by DMEM/$HA^+$, and OM/$HA^-$.

**Conclusion:** Based on these results, the combination of 0.5mg/mL nano-HA with OM produces a favorable microenvironment for primary human osteoblasts and MSCs to generate a bone-like matrix. The generated model will be ideal for screening therapeutics against patient-derived tumor cells cultured within a bone-like microenvironment. Ongoing work is assessing migration of fluorescently labeled tumor cells within this construct in the presence or absence of various chemotherapeutics.

**Validation of a new strategy to repair jaw bone voids in the presence of anti-resorptive induced osteonecrosis**

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**Introduction**: Bisphosphonate related osteonecrosis in the jaw (BRONJ) is caused primarily by trauma, such as a tooth extraction, in patients who have received high doses of bisphosphonate agents to treat osteoporosis or bone cancer metastases. Currently no effective treatment for BRONJ exists, leaving patients with a debilitating condition that causes chronic pain and affects the ability to eat and talk. The combination of 3D printing technology of biocompatible materials to generate better bone substitutes to treat this disease, is a strategy that is currently under intensive exploration. The goal of my research is to determine if 3D printed poly-caprolactone (PCL) scaffolds and PCL scaffolds coated with chitosan nanoparticles (PCL/c) lead to more effective tissue regeneration than Norian® bone cement, which is the current standard of care. Our hypothesis is that 3D printed scaffolds will be more effective than commercial cements promoting bone regeneration and efficient reconstruction of critical size mandibular defects.

**Methods**: Skeletally mature rats (n = 12) received weekly injections of either of 0.13 mg/kg zoledronic acid + 3.8 mg/kg dexamethasone (ZA+DX) or sterile PBS for 4 weeks before extraction of 2 molars on each side of the mandible. The rats continued receiving medication or PBS for 4 more weeks before removing the necrotic tissue to generate a critical size bone defect measuring 5 mm long, 3 mm deep and 2 mm wide, which were filled with one of the biomaterials. The animals were euthanized 6 weeks later to quantify bone regeneration and microvascular ingress using micro CT. Osteoblast, osteoclast, macrophage and vascular cell distribution pattern and activity will be evaluated by histology in regenerating bone and soft tissue. Comparisons will be made by ANOVA and Tukey post-hoc tests at 95% confidence interval.

**Results**: Bone regeneration between ZA+DX and placebo treated animals differed significantly. While Norian® bone cement promoted the highest rate of regeneration in ZA+DX treated animals, it caused the least amount of regeneration in placebo treated animals. PCL scaffolds promoted the best regeneration in placebo treated rats, while it produced the worst outcomes in ZA+ DX treated animals. We found no significant differences between PCL and PCL/c scaffolds in the latter group.

**Conclusions**: A challenge for the treatment of BRONJ is recognizing that the strategies used to treat normal bone defects may not be the best to treat diseased bone. PCL is a novel material that is easy to 3D print and manipulate, and has the advantage of being FDA approved. Our preliminary results show that it may be a promising strategy to treat BRONJ patients. This bone tissue engineering strategy can further be adapted to models of impaired long bone healing, such as those induced by infection, poor vascularization or old age, but more research is needed.

**Effects of Chondroadherin peptides LEAK and CKF on matrix production and MSC differentiation**

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**Background:** Chondroadherin (CHAD) is a leucine-rich repeat protein found in cartilaginous tissues including the intervertebral disc (IVD). It is involved in cell attachment and cell signaling. Two peptides of CHAD mediate this cell attachment, CQLRGLRRWLEAK (LEAK) which binds to the α2β1 integrin, and CKFPTKRSKKAGRH (CKF) which binds to heparin sulfate proteoglycans such as syndecan. Previous work has demonstrated a role for these peptides in chondrocyte cell adhesion and spreading. Role and mechanisms of their effects on matrix assembly in the IVD has not been characterized. By selectively enriching media with either peptide, or a combination of the two (MIX), we aim to examine if the peptides can preserve and promote an IVD like matrix production. We are also evaluating a possible role for the peptides in Mesenchymal Stem Cell (MSC) differentiation to a IVD-like phenotype.

**Methods**: *Pellet culture*: To test the effects on IVD homeostasis, inner annulus fibrosus (IAF) cells were grown in pellet culture with media supplemented with CHAD peptides (20 µM) of their treatment group (CKF/LEAK/MIX) over a three week growth period. Pellets were fixed cryosectioned and stained with Safranin-O for proteoglycan content, or DAB stained for Collagen I & II content. MSC Differentiation: MSCs were cultured in monolayer with media supplemented with CHAD peptides (20 µM) of their treatment group (CKF/LEAK/MIX) over a three-week growth period. Each experiment with either complete, chondrogenic differentiation, or osteogenic differentiation media. Wells containing respective mixes of media/enrichment were fixed and stained for proteoglycan content with Safranin-O as a measure of chondrogenic differentiation, or calcium stained with Alizarin-Red for osteogenic differentiation.

**Results:** Preliminary results have suggested a difference in matrix assembly for peptide enriched IAF pellets. In absence of CHAD peptides matrix composition appears more dense and fibrotic in contrast to enriched replicates resulting in a less dense matrix composition similar to in-vivo IAF. Possible differences in proteoglycan content or collagen I and collagen II content are pending further analysis, fibrosis will be further investigated using α-smooth muscle actin staining. Examination will also focus on collagen to proteoglycan ratios and the presence of other IVD cell surface markers and matrix components.

In MSC differentiation, after one week, LEAK supplemented replicates appear to grow less dense in monolayer in both chondrogenic and osteogenic media. CHAD peptide treatment during osteogenic MSC differentiation appears to alter cell morphology. Gene expression analysis will be performed to determine the effect of the peptides on osteogenic and chondrogenic differentiation. In addition, the presence of IVD cell surface markers and matrix components will be evaluated.

**Discussion:** Preliminary data indicated that in absence of supplementation with CHAD peptides, IAF appear denser and more fibrotic. Supplemented cultures appear looser, more gelatinous and hydrated. Given the prevalence of disc degeneration, and their poor regenerative capacity, advances in culturing techniques to promote retention of in-vivo traits will further support efforts toward cell-based treatments. There is no benign site to harvest autologous IVD cells therefore, enhancing the ability to generate IVD cells from multi-potent cells will circumvent complications from allografts.

**Cardiomyocyte shape control improves contractility tests in drug screening applications**

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**Background**: Pharmaceutical companies must perform pharmacology and toxicology pre-clinical trials to commercialize drugs. Over 90% of drugs that are effective in preclinical assessment later fail in clinical trials because of poor efficacy or adverse effects. Frequent causes of drug failure and/or market withdrawal are adverse cardiovascular effects. To address this gap, human induced stem-cell-derived cardiomyocytes (CMs) are used in cell-based high throughput drug screening (HTS). However, contraction force -a central CM function- is not measured in current HTS. We have developed a novel HTS device that measures changes in CM contractile force and frequency by quantifying visible deformations in silicone-based culture substrates. The device has been successfully benchmarked against existing technologies using a panel of drugs. However, the heterogeneity of stem-cell derived CM populations introduces data variability.

**Objective/Hypothesis**: We hypothesize that geometrically confining CMs on compliant silicone substrates into their physiological shape improves force production and beating frequency. By patterning the silicone surface of our contraction measurement device with arrays of adhesive islands with the footprint of CMs, we aim to standardize CM behavior for automated analysis in drug screens.

**Methods**: It is a major challenge to transfer adhesive proteins onto soft surfaces. To achieve patterns that determine CM shape on very soft recipient surfaces, we developed microcontact printing with fluorescently tagged proteins. Non-printed areas of the silicone substrate were passivated to prevent cell attachment, followed by seeding of CMs. To evaluate protein patterning success and effect on CM beating behavior, we used live imaging and fluorescence microscopy. Computational scripts were developed to automate data analysis.

**Results**: With improved microcontact printing, we achieved protein patterns of 120×20 µm rectangles on soft silicone substrates. CMs adhered and were geometrically confined to the island shape and exhibited visible contractility. Automated image data analysis was successfully implemented and demonstrated to greatly reduce analysis time.

**Conclusion**: The improved microcontact printing improved our CM-based HTS platform by achieving distinct CMs patterns without compromising CM contractility.

**Significance/Impact**: Controlling CM shape and position with protein patterning increases the potential of our HTS platform. We are currently analyzing whether CM cell shape confinement generates homogenous CM maturation states, regular beating, and increases CM contraction forces.

# Poster Presentations Session 2B

**Calcium phosphate precipitation in biologically relevant systems: Scoping review**

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**Introduction:** Biologically guided precipitation of calcium phosphates is important for the formation of calcified human tissues, such as bone and teeth. In addition, it is of practical significance in a number of processes relevant to industry and agriculture, such as wastewater treatment and dairy ultrafiltration. Mineral precipitation is a physicochemically complex process, which becomes significantly more complex in the presence of biological materials. The theoretical foundation of phase transition in general has been developed and is vital for many applications, such as metallurgy and weather prediction.

**Objective:** The goal of this scoping review was to identify established mathematical approaches developed to describe the formation of calcium precipitates in biological systems.

**Method:** A scoping review was conducted using MathSciNet, Scopus, and Web of Science databases to retrieve eligible mathematical modelling papers on calcium precipitates (CPs) in biological systems.

**Results:** From the 2096 studies screened, 114 studies were included. The major biological systems of interest were tissues of the human body (48/114), water research (38/114) and agricultural applications. The majority of studies described precipitation of calcium phosphate (78/114), followed by calcium carbonate (22/114). Mathematical modelling of CPs was dominated by classical nucleation (64/114) and kinetic (38/114) theories. Overall, the choice of modeling approach appeared to be guided by the time scale of interest for the researcher.

**Conclusion:** This scoping review suggests that broad theories, such as classical nucleation theory and kinetic, may be adapted for modelling calcium precipitation in biologically relevant systems.

**A systematic review and meta-analysis of mechanically-stimulated ATP release: quantitative estimates, mechanisms and role in pathophysiology**

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Body tissues are exposed to a complex combinations of shear forces, strains, and pressures, which are perceived by cells and converted to biochemical signals. Extracellular ATP released by mechanically stimulated cells is one such signal, and is involved in diverse tissue-specific functions including bone remodeling, wound healing and inflammation. We have conducted a systematic large-scale data synthesis aimed to quantitatively characterize the amounts, kinetics and mechanisms of mechanically-stimulated ATP release under normal and pathological conditions. From 278 systematically selected studies, we extracted 123 estimates of absolute and 212 estimates of relative amount of ATP released, 74 kinetic time-series, 592 pharmacological and 89 genetic intervention outcomes, and 51 pathophysiological comparisons. Using a meta-analytic methodology, we have established that mechanically-stimulated mammalian cells release 38.6 (CI: 18.2 to 81.8) amoles ATP/cell, with a characteristic time constant of 32 s (CI: 16 to 66 s) measured using real-time recording methods. We have found that mechanically-stimulated ATP release is a universally conserved phenomenon in mammalian cells, and that cells from different species, embryonic origin and most organ systems release similar amount of ATP when mechanically stimulated. Our data-driven summary of mechanically-stimulated ATP release mechanisms allowed us to infer tissue-level generalizations that suggest the existence of general and tissue-specific routes of ATP release, and to identify conserved cell type-independent signaling patterns. We have found that inflammation and injury were associated with increased mechanically-stimulated ATP release, while studied hereditary and metabolic conditions commonly attenuated ATP release. Importantly, several lines of evidence, including differences in release kinetics, stimulus-specific involvement of release routes and responses in pathophysiological conditions suggest that cells can discriminate between stretch- and shear-related forces. Thus, consolidating and quantifying over 25 years of basic research data generated in 64 unique cell types derived from 12 organ systems and stimulated by 9 distinct force applications allowed us to generate novel testable hypotheses, and provide evidence-driven recommendations for translational studies.

**Osteoblast-specific overexpression of Gαs and Gαq/11 leads to differential fracture healing responses.**

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Bone exhibits a remarkable ability for self-repair. Despite its astounding regenerative powers, however, approximately 4% of Canadians who sustain a bone fracture every year require additional medical care for complications of bone repair with additional medical costs and loss of productivity. Clearly, advances in unraveling the signaling pathways that regulate fracture repair have considerable clinical and economical potential. Considerable evidence from both basic and clinical research has implicated GPCR signaling in osteoblasts as a key regulator of skeletal development and homeostasis. GPCR signaling can be modulated by alterations in G protein α-subunit levels, which in turn lead to profound changes in bone mass, quality, and rate of turnover. Of particular interest are $G\alpha_s$ and $G\alpha_{q/11}$. Constitutive signaling of $G\alpha_s$ in osteoblasts causes a dramatic increase in bone mass in mice and osteoblastic differentiation defects leading to McCune Albright Syndrome in humans. Conversely, mice expressing constitutively active $G\alpha_q$ develop osteopenia with reduced bone formation. Altogether the evidence implies that precise regulation of $G\alpha_s$ and $G\alpha_{q/11}$ levels in bone cells is essential for normal bone development and potentially fracture repair. Interestingly, a large variation in the expression levels of $G\alpha_s$ and $G\alpha_{q/11}$ has also been observed within normal human populations, possibly making some people more susceptible to poor bone health and impaired bone healing. The aim of the study is, therefore, to elucidate the functional roles of $G\alpha_s$ and $G\alpha_{11}$ on bone formation and remodeling during fracture repair in vivo using two transgenic mouse models with different levels of $G\alpha_s$ ($G\alpha_s$-Tg) and $G\alpha_{q/11}$ ($G\alpha_{11}$-Tg) expression in osteoblasts. The basal skeletal phenotypes of these transgenic mice have already been characterized in our laboratory. $G\alpha_s$-Tg mice showed increased bone mass due to a disproportionate increase in bone formation by osteoblasts over bone resorption by osteoclasts at the expense of bone quality .Conversely, $G_{11}$-Tg mice demonstrated an osteopenic phenotype with decreased bone formation rate and increased osteoclast number. Here, we test our hypothesis that $G\alpha_s$ and $G\alpha_{11}$ signaling pathways play disparate roles during fracture repair and therefore lead to differential fracture healing rates and strengths. A transverse osteotomy with intramedullary pin stabilization was performed in male $G_{11}$-Tg, $G_s$-Tg and wild type control FVB mice. At 2 and 3 weeks post-fracture, the fractured tibiae were collected for Micro-CT analysis, histomorphometry, quantitative gene expression analysis, and biomechanical testing. Our Micro-CT analyses revealed significantly enhanced callus mineralization and bone formation in Gs-Tg mice at all investigated time points suggesting that increased $G\alpha_s$ signaling in osteoblasts leads to increased rate of fracture healing. In contrast, early transition into the remodeling phase was evidenced by a significant reduction in calcified cartilage at 3 weeks post-fracture in $G_{11}$-Tg mice. Together, these results show that overexpression of $G\alpha_s$ and $G\alpha_{q/11}$ in osteoblasts induces different fracture healing responses, which may explain individual differences in fracture healing rates and outcomes.

**Novel in vitro microfluidic platforms for osteocyte mechanotransduction studies**

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**Introduction:** Bone remodelling is an important process that is responsible for bone growth and recovery. Osteocytes are the major bone cells embedded in bone matrix with important mechanosensing functions (Ref. 1). Current research has focused on observing bone cell mechanotransduction under different simulated physiological conditions (e.g., shear stress, strain, pressure, etc.) using macro-scale devices. However, these devices often require large sample volumes, low through-put, extensive setup protocol, as well as very limited designs only suitable for general cell culture (Ref. 2). On the other hand, *in vitro* microfluidic devices provide an optimal tool to better understand this biological process with its flexible design, physiologically-relevant dimensions, and high-throughput capabilities. Recent work on co-culture platform has demonstrated the feasibility of building more complex microfluidic devices for osteocyte mechanotransduction studies, while maintaining its biological relevance (Ref. 3). However, there lacks a robust system where multi-physiological flow conditions are applied to bone cells to study their intercellular communication. This project aims to fulfill this gap by designing and fabricating a multi-shear stress, co-culture platform to study interaction between osteocytes and other bone cells when exposed to an array of physical cues.

**Current Progress and Goals:** The project will rely on standard microfluidic principles in designing devices that utilize changing geometric parameters to induce different flow rates that are directly proportional to the levels of shear stress. All channels within the same device will share a common inlet, while adjusting the resistance of each individual channel will result in a different flow rate. Devices are fabricated using PDMS, and bonded to glass slides of equal sizes. MLO-Y4 osteocyte like cells seeded in the device are stimulated with oscillatory fluid flow (2 Pa, 1 Hz, 2 hours) with a custom in-house pump. Significant differences in RANKL levels are observed between channels, demonstrating that proper cellular response to flow can be elicit from each distinct shear stress channels as designed.

Furthermore, we aim to pair these multi-shear stress channels with corresponding culturing chambers connected through perfusion pores. Through perfusion between the multi-shear stress channels and culturing chambers, different cell population can communicate to each other as they are stimulated by varying levels of shear stress. Using this platform, we will be able to mimic the interaction between osteocytes and other bone cells *in vitro*. Due to the advantage of using microfluidic devices, various analytical methods can be used on-chip to determine cellular response, such as staining for biomarkers and differentiation factors.

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**Chondrocytes obtained from the upper and lower regions of deep zone articular cartilage form cartilaginous tissue in vitro that mineralizes differentially**

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**Introduction:** Articular cartilage (AC) has a depth dependent zonal organization. Its deep zone (DZ) merges into a zone of calcified cartilage (ZCC). Mechanisms regulating AC calcification, as yet undocumented, are expected to involve alkaline phosphatase (ALP) and polyphosphate (PP), both located in the DZ.

**Hypothesis:** *In vitro* models of the upper and lower aspects of the DZ can be developed and used to characterize ALP and PP during mineralization.

**Methods:** Full thickness AC was obtained from calf metacarpo-phalangeal joints. In designated experiments, cartilage was harvested from the upper and lower DZ regions. Chondrocytes from these regions, isolated by sequential enzymatic digestion, were grown in 3D culture. PP and ALP distribution, PP levels, and ALP activity were assessed.

**Results:** Distinct distributions and levels of PP and ALP in native full thickness AC suggested that the DZ can be divided into upper and lower regions. Cells obtained from native upper and lower DZ cartilage, cultured in non-mineralizing and mineralizing conditions, produced upper DZ (UDZ) and lower DZ (LDZ) cartilage tissues. Both tissues mineralized in the presence of ß-glycerophosphate, but UDZ mineralization was delayed. ALP activities of LDZ tissues were greater than those of UDZ tissues at day 0. All ALP activities approached those found in respective native AC regions by day 10. In mineralizing LDZ and UDZ, $P_i$ levels rose with time and by day 10 were significantly higher, compared to day 0. $P_i$ levels in mineralizing UDZ increased slower and were significantly lower at day 2 and day 4 than in mineralizing LDZ, but by day 10 $P_i$ contents of UDZ and LDZ tissues were similar. PP levels did not change significantly in either mineralizing tissues over 10 days.

**Discussion:** *In vitro* models of upper and lower DZ were generated and showed differences in ALP activities and time to mineralization. ALP activity was found in mineralizing and non-mineralizing tissues. $P_i$ levels rose during mineralization but more slowly in UDZ. PP levels were constant in UDZ and LDZ *in vitro* formed tissues, suggesting that PP may play other roles in addition to regulating mineralization.

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**Mechanical regulation of breast cancer bone metastasis via osteocytes' signaling to endothelial cells**

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**Introduction:** Bone metastases, the migration of cancers to the bone, occur in 65-75% of patients with advanced breast cancer (Ref. 1) and significantly increase the morbidity and mortality of patients. Bone mechanical loading during exercise, an intervention strategy commonly suggested for breast cancer patients to improve their quality of life, is known to stimulate osteocytes, the major population of cells in the bone. We have previously shown that mechanically stimulated osteocytes affect breast cancer cell migration and apoptosis both directly through chemokines and indirectly through osteoclasts and endothelial cells (Ref. 2). Since endothelial cells comprise the important first barrier encountered by metastasizing cancer cells in blood vessels, we will be investigating more into the ***mechanism underlying mechanically stimulated osteocytes' regulation on endothelial cells and how this further affect breast cancer cells***.

M**ethods:** To simulate the mechanical stimulation experienced by osteocytes during mild exercise, MLO-Y4 osteocyte-like cells (gift of Dr. Lynda Bonewald, Indiana University) on glass slides were placed in flow chambers and subjected to oscillatory fluid flow (1Pa; 1Hz; 2 hours). Osteocytes were then cultured for 24 hours and medium was extracted (conditioned medium; CM). Controls were glass slides not placed in flow chambers. Human umbilical vein endothelial cells (HUVECs; gift of Dr. Young, University of Toronto) were conditioned in osteocyte CM. Endothelial permeability was measured with fluorescein-dextran, 40 kDa. MDA-MB-231 breast cancer cells' adhesion to endothelial cells was tested by allowing adhesion for 30 minutes then counting the number of cells that remained after 30 minutes of oscillatory fluid flow (1Pa; 1Hz). Gene expression profile of the MDA-MB-231/1833 bone-metastatic breast cancer cells (gift of Dr. Seth, University of Toronto) conditioned in CM from endothelial cells conditioned in osteocyte CM were determined using RNA-sequencing.

**Preliminary Results:** Preliminary results suggested that CM from mechanically stimulated osteocytes reduces the adhesion of breast cancer cells (by 18%) to endothelial cells by lowering endothelial cell' expression of ICAM-1 (intercellular adhesion molecule 1). This aligns well with our previous result demonstrating the reduction of breast cancer cell extravasation towards CM from mechanically stimulated osteocytes (Ref. 2). Furthermore, CM from endothelial cells conditioned in CM form mechanically stimulated osteocytes lowered the expression of MMP-9 (matrix metallopeptidase 9; important for metastasizing breast cancer cells to degrade the bone matrix during invasion into the bone) by bone-metastatic breast cancer cells (by 38%). More genes that are differentially expressed when osteocytes are mechanically stimulated are being identified with RNA-sequencing.

**Conclusion:** We have demonstrated that signaling from mechanically stimulated osteocytes reduces the ability of breast cancer cells to adhere to endothelial cells and degrade the bone. This is a novel research that provides insights into the impact of exercises on bone metastases through osteocyte and endothelial cell signaling. Examining the effect of mechanical loading on metastases and its mechanism will assist in designing cancer intervention programs that lowers the risk for bone metastases.

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**The role of actin binding protein adseverin in regulating chondrocyte cellular morphology and secretion**

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**Introduction:** Actin polymerization status and distribution is critical for chondrocyte phenotype. Chondrocyte dedifferentiation is associated with a spread shape, increased amount of actin and development of stress fibers. Adseverin (ads), an actin binding protein, was previously shown to be significantly upregulated in normal chondrocytes compared to dedifferentiated chondrocytes suggesting a role for this molecule in mediating chondrocyte function. This study will provide insights to ads' role in regulating actin to promote chondrocyte phenotype.

**Hypothesis:** Adseverin regulates chondrocyte cell morphology and secretion through actin cytoskeleton modulation.

**Methods/Results:** Chondrocytes were isolated from bovine articular cartilage by enzymatic digestion. Cells were transfected with ads siRNA and knockdown of gene and protein expression was confirmed. The effect of ads knockdown (KD) on cell shape and volume, phenotype and secretion was evaluated. Chondrocytes were grown in monolayer culture and passaged twice to cause dedifferentiation. These P2 cells were transfected with ads-GFP plasmid. Protein overexpression was confirmed by western blot. Ads KD in primary cells resulted in increased cell area, surface area, cell volume and decreased circularity and shape factor compared to cells transfected with scrambled siRNA. These cells acquired actin stress fibers, decreased G/F-actin ratio, and increased actin free barbed ends relative to control. Ads overexpressed in P2 cells resulted in decreased cell area, surface area, cell volume and increased circularity and shape factor compared to cells with no overexpression of ads. These cells also showed increased G/F-actin ratio relative to control, and re-established a cortical actin distribution in areas where ads was highly localized. Overexpression of ads in P2 cells resulted in increased proteoglycan secretion.

**Conclusions:** Ads plays a role in regulating cellular morphology and secretion through modulating actin polymerization status. This was confirmed by overexpressing ads in P2 chondrocytes that have little ads. These findings provide insights to a potential regulator of actin that may promote chondrocyte function. Future work will aim to determine ads' effect on chondrogenic gene expression and thus cell phenotype.

**EGFR/Mig-6 signalling in osteoarthritis**

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**Purpose:** OA is a heterogeneous and multifactorial disease characterized by the degeneration of joint that involves mechanical, structural, genetic and environmental factors. The pathophysiology of OA is not well understood and therefore no treatments exist to stop the progression of cartilage breakdown. Our laboratory has been interested in the role of the epidermal growth factor receptor (EGFR) in articular cartilage function through the Mitogen-inducible gene 6 (Mig-6) which has been identified as negative regulator of receptor tyrosine kinases EGFR and c-Met. Our initial investigation using ablation of Mig-6 in cartilage showed anabolic buildup of articular cartilage and formation of chondro-osseous nodules (Pest et al. 2014). Therefore, EGFR is required to regulate chondrocyte homeostasis and may play a role in OA development. However, it is possible that deregulation of other growth factors such as HGF/c-MET by Mig-6 play an important role in tissue repair and may be partially responsible to the Mig-6 KO phenotype. Since our studies suggest dosage-dependent roles of EGFR signalling in joint homeostasis and OA, we planned to examine whether overexpression of Mig-6 alters these processes.

**Methods:** Genetically modified mice for cartilage-specific overexpression of Mig-6 were generated and harvested at 6 and 11 weeks of age. Frontal knee sections were stained with toluidine blue to evaluate glycosaminoglycan (GAG) distribution and structural changes in the joints of Mig-6 over (*Mig-6$^{over/over}$; Col2-Cre$^{+/-}$)* and control (*Mig-6$^{over/over}$; Col2-Cre$^{-/-}$)* animals. Immunohistochemistry (IHC) was performed on frontal sections of knees from 11 weeks-old female and male Mig-*6$^{over/over}$* mice for detecting SOX9.

**Results:** Our results show strong toluidine blue staining in the articular cartilage for both groups of Mig-6 overexpressing and control female animals at 11 weeks-old. However, articular cartilage thickness appears decreased in the medial femoral condyle (MFC) and medial tibial plateau (MTP) of Mig-6 overexpressing mice. The lateral femoral condyle (LFC) and lateral tibial plateu (LTP) showed no difference. SOX9 immunostaining was similar in the Mig-6 over (*Mig-6$^{over/over}$; Col2-Cre$^{+/-}$)* and control (*Mig-6$^{over/over}$; Col2-Cre$^{-/-}$)* groups of male and female mice.

**Conclusion:** Our findings show that decreased EGFR signaling through Mig-6 overexpression may play a role in articular cartilage growth. Further examination of articular cartilage of Mig-6 overexpressing mice in spontaneous models of OA is underway. Evidently, our understanding of the EGFR/Mig-6 network in cartilage remains incomplete, but evidence suggests that this pathway is critical for joint homeostasis and might present a promising therapeutic target for OA.

# Defining the minimal pharmacophore for positive allosteric modulation of PTH1 receptor signaling by extracellular nucleotides

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**Background**: Parathyroid hormone (PTH) activates the PTH/PTH-related peptide receptor (PTH1R) on osteoblasts and other target cells. Mechanical stimulation of cells, including osteoblasts, causes release of nucleotides such as ATP into the extracellular fluid. In addition to its role as an energy source, ATP serves as an agonist at P2 receptors and an allosteric regulator of many intracellular proteins. We recently investigated the effects of extracellular ATP on PTH1R signaling. We found that extracellular nucleotides increase PTH affinity, efficacy or both. Moreover, nucleotide-induced potentiation of PTH1R signaling is independent of P2 receptors. These studies established that extracellular ATP enhances PTH1R signaling through a previously unrecognized allosteric mechanism (*Cell Signal* 46:103-112, 2018).

**Objective**: The goal of the present study was to identify the minimal pharmacophore for positive allosteric modulation of PTH1R signaling by extracellular nucleotides.

**Methods**: UMR-106 cells, a rat osteoblast-like cell line that endogenously expresses PTH1R, were transfected with a plasmid encoding a luciferase-based biosensor, which allowed us to monitor cytosolic cAMP levels in real time. We also used a luciferase complementation assay to follow recruitment of β-arrestin to PTH1R in live HEK293H cells. In both cases, cells were stimulated by PTH in the presence and absence of extracellular nucleotides, potential allosteric modulators, or control compounds.

**Results**: Extracellular ATP produced no measurable effects on its own, but markedly enhanced PTH-induced cyclic AMP signaling in HEK293H cells transfected with PTH1R and in UMR-106 rat osteoblastic cells. ATP also enhanced β-arrestin recruitment to PTH1R in HEK293H cells. ATP (1.5 mM) reduced the concentration of PTH required to produce a half-maximal cyclic AMP or β-arrestin response, with no evident change in maximal receptor activity (for cAMP, $pEC_{50}$ for PTH alone was 8.75 ± 0.08 and for PTH + ATP was 10.51 ± 0.09; for β-arrestin, $pEC_{50}$ for PTH alone was 8.11 ± 0.10 and for PTH + ATP was 8.94 ± 0.07). Importantly, these effects of ATP were mimicked by i) CMP, another nucleotide; ii) ribose 5-phosphate, contained within all ribonucleotides; and iii) certain other phosphorylated monosaccharides. Though neither glucose nor inorganic phosphate on their own enhanced PTH-induced signaling, glucose 1-phosphate caused striking enhancement of β-arrestin recruitment (potentiating the effect of PTH [5 nM] by 4.1 ± 0.4 fold). Interestingly, its constitutional isomer glucose 6-phosphate also enhanced PTH1R signaling, but to a significantly lesser extent (potentiating the effect of PTH [5 nM] by 2.1 ± 0.3 fold). As well, fructose 6-phosphate and fructose 1,6-bisphosphate both enhanced PTH1R signaling (potentiating the effect of PTH [10 nM] on β-arrestin recruitment by 1.9 ± 0.1 and 2.9 ± 0.4 fold, respectively).

**Conclusions**: These observations suggest that the minimal pharmacophore includes a phosphorylated monosaccharide. At least in some cases, it appears that extent of positive allosteric modulation depends on the position of the phosphate group. Knowledge of the pharmacophore may permit the future development of bitopic ligands that interact concomitantly with both the orthosteric and allosteric sites to increase therapeutic efficacy of PTH1R agonists.

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**Extracellular matrix fragment expression profiles in human intervertebral discs**

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**Introduction:** Low back pain is one of the most prevalent medical conditions and is caused by intervertebral disc (IVD) degeneration. Degeneration is characterized by an increase in inflammatory mediators and matrix-degrading enzymes. It is known that some of these inflammatory molecules can signal through toll-like receptors (TLRs) in the immune system; TLRs are also present in IVDs. Moreover, some extracellular matrix fragments have been shown to activate TLRs in macrophages, but not yet in disc cells. Here, we set out to determine which extracellular matrix fragments are found in degenerate human IVDs and which can cause an increased inflammatory response. We hypothesize that extracellular matrix fragments will increase with increasing level of disc degeneration, and samples from degenerate discs (containing high fragment levels) will drive inflammatory responses in culture IVD cells.

**Methods:** Human lumbar spines were harvested from organ donors through cooperation with Transplant Quebec. IVDs were isolated using standard laboratory protocols. Biopsy punches (4mm) were taken from each region of the disc and free protein fragments are extracted through a series of buffers. Western blots were used to detect fragments of matrix proteoglycans, such as biglycan, chondroadherin, and PRELP. Positive control PAMcsk2 and extracted fragments were then applied to HEK293 TLR cells—these cells stably overexpress TLRs and alkaline phosphatase, which turns its media blue upon TLR activation. Activation is quantified at 630nm with a TECAN plate reader via absorbance. In ongoing work, extractions that show positive responses will be separated with chromatography and sequenced and quantified with mass spectrometry.

**Results:** In initial experiments, we have shown that protein fragments are able to move freely within the disc. Western blotting showed fragments were present and able to freely move out of the tissue. Blots were compared between young, healthy disc extracts and older, degenerate ones, with an increase in fragmentation in the more degenerate disc. We successfully produced a standard curve for Pam2CSK4-treated HEK cells. Treatment of HEK cells with fragments gave signal below the linear range of the standard curve, indicating that optimization of fragment concentration is necessary to ensure accurate measurements. In future experiments we anticipate degenerate extracts giving more signal from the increase in protein fragments. We also expect to find bigylcan and fibromodulin fragments, among others, in fractions that we sequence with mass spectrometry.

**Conclusion:** The endogenous signaling capabilities of IVD extracellular matrix fragments show the importance of toll-like receptors and their associated pathways in disc degeneration. By preventing the signals, further matrix degradation could be inhibited. Future work will need to use current induced degeneration models, combined with an inhibitor for this pathway, and analyze if and how the degeneration profile changes. By identifying inflammation-producing matrix fragments, we will show that disc degeneration can be caused, at least in part, due to endogenous signaling, regardless of the external factors.

**Exosomal release of plastin-2 by breast cancer cells leads to increased osteoclast differentiation**

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Bone is a common site of breast cancer metastasis causing tumor-induced bone degradation. We have previously shown that cancer derived factors from conditioned medium (CM) of MDA-MB-231 human breast carcinoma cells induce osteoclast differentiation from late osteoclast precursors in a RANKL-independent pathway. Using proteomics, we identified one of the cancer-derived proteins, plastin, which has three isoforms exhibiting a high degree of homology. Using immunoblotting, we demonstrated that plastin-2, also known as L-plastin, was present in MDA-MB-231 CM in high amount. When L-plastin in MDA-MB-231 cells was silenced using siRNA, the expression for L-plastin was significantly reduced and the CM displayed a significant reduction in osteoclast formation compared to naïve CM. We hypothesized that L-plastin is transported from cancer cells to osteoclast precursors by cell-derived vesicles, exosomes. Exosomes from MDA-MB-231 CM were isolated through differential centrifugation. Using nanoparticle tracking analysis, we found a particle size distribution in CM of around 100 nm and the presence of 50-100 nm exosomes in CM was confirmed with transmission electron microscopy. Using immunoblotting, L-plastin presence in the exosomes were verified using two exosomes markers and a specific antibody against L-plastin. Addition of recombinant L-plastin to osteoclast precursors promoted elevation of intracellular [Ca2+]i, increased NFATc1 nuclear translocation, and stimulated osteoclast formation for RANKL-primed precursors. These data suggest that L-plastin is released by breast cancer cells and transported by exosomes to osteoclast precursors, where it can induce osteoclastogenesis by mimicing signaling from osteoclast co-stimulatory receptors.

**Establishment and image based evaluation of a new preclinical rat model of osteoblastic bone metastasis**

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**Purpose:** Breast, prostate, lung and renal cancers are the most prevalent primary tumours to metastasize to bone. The metastasis of cancer to bone impacts the normal bone remodeling cycle via increasing bone resorption (osteolytic), bone formation (osteoblastic) or a combination of the two. The vertebral column is the most common site of bone metastases, diagnosed in one third of cancer patients. Established rat models of osteolytic and mixed metastases have been extensively used to identify structural changes to the skeleton caused by bone metastases. The aim of this study is to develop and establish an osteoblastic preclinical rat model and characterize its progression through image-based evaluation.

**Methods:** Intracardiac injection of one million human ZR-75-1 breast cancer cells into a preclinical athymic rat model (n=10) was used to develop osteoblastic metastases. The osteoblastic lesion development in the bone was followed in four rats exclusively via µCT imaging (Locus Ultra µCT, GE, 154µm resolution). After confirmation of lesion development, ZR-75-1 cells were transfected with luciferase to allow for monitoring of the progression of tumour involvement via bioluminescence imaging (n=6, IVIS Spectrum, Perkin Elmer). Only one in-vivo µCT image was obtained from these rats at the end of the experiment. Imaging was conducted every 3 weeks for 4 months. At 4-months post injection, ex-vivo µCT images were acquired of the excised limbs (femur and tibia) and spine (L1-L3) at 20µm and 7µm resolution respectively (µCT 100, Scanco Medical). High spatial resolution imaging of the motion segments allows for quantification of changes in tissue-level stereological features to be evaluated using an in-house developed algorithm. Following parameters are established in stereological analysis: trabecular bone volume ratio, trabecular number, trabecular spacing, trabecular thickness, bone mineral density and tissue mineral density. After obtaining the µCT images, the bone was decalcified (10% EDTA) and prepared for histology. Histology sections were stained, with hematoxylin and eosin, to confirm osteoblastic involvement.

**Results:** All rats exhibited osteoblastic involvement in the appendicular bones and vertebrae 3 to 4-months post inoculation, with the highest frequency of metastases detected in Th13-L6. Bioluminescent signals from the osteoblastic metastases however were variable; signals were not seen in some involved bones, or initial signals seen at early timepoints disappeared over time, in areas of large well established osteoblastic lesions confirmed by µCT. Histological evaluation confirmed µCT findings of osteoblastic bone formation. Stereological analysis of trabecular involvement is ongoing.

**Conclusions:** Intracardiac injection of ZR-75-1 breast cancer cell line in rats induces purely osteoblastic metastases evident on µCT imaging and histology. This now established osteoblastic rat model can be used for future preclinical studies.

**Mutations in or near a putative lipid binding motif contained within the V-ATPase "a" isoforms suggests the molecular mechanism underlying different human diseases**

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Vacuolar ATPases (V-ATPase) are conserved multi-subunit ATP–dependent proton pumps necessary for numerous cellular functions including pH regulation and membrane fusion. V-ATPases in specialized cells, such as epididymis, kidney and bone cells, are targeted to the plasma membrane for extracellular pH regulation, or in the case of tumour cells, for extracellular acidification required for invasiveness. The V-ATPase complex is composed of 14 subunits. The 100kDa *a* subunit, thought to be involved in the differential targeting of V-ATPases to multiple locations, has 4 isoforms in humans, *a*1-*a*4. Expression of the different isoforms is tissue/organelle-specific. Recent evidence suggests that interaction of V-ATPase *a* subunit with membrane signaling lipid phosphatidylinositol 3,5-biphosphate (PI3,5$P_2$) regulates membrane recruitment of the V-ATPases complex in yeast. We generated a homology model of the amino terminal domain of human *a4* (*a4*NT) and proposed a non-canonical pleckstrin-homology-like phosphoinositides (PIPs) binding domain structurally homologous to a PIP binding domain in Osh3, a characterized PI4P-binding protein. A critical sequence motif for PIP interaction, $KX_nK(R)IK(R)$, is conserved in four *a* isoforms. Interestingly, mutations in this putative motif result in human diseases. The K237_V238del mutation in the Golgi specific *a2* isoform result in cutis laxa. Deletions adjacent to the motif in the osteoclast-specific *a3* isoform result in osteopetrosis. Finally, the K237del mutation in the kidney specific *a4* isoform causes distal renal tubular acidosis (dRTA). We recreated the human a4 mutation K237del, and a double mutation in the motif K234A/K237A. Protein lipid overlay assay showed wildtype *a4*NT preferentially bound to PI(4,5)$P_2$, and both mutants K234A/K237A and K237del showed comparable reduced binding to PI(4,5)$P_2$. These results were consistent with pull-down experiment with PI(4,5)$P_2$-enriched liposomes. Circular dichroism (CD) spectra of the mutant protein were comparable to the wildtype, indicating that the mutation disrupted the lipid binding, without altering protein structure. When expressed in HEK293, wildtype *a4*NT co-purified with microsomal membrane and the mutant showed reduced membrane association, suggesting that *a4*NT is exclusively sufficient to retain in the membrane and that the lipid binding capacity was involved in the membrane retention of the V-ATPases. This study is the first to identify a PIP binding domain in the NT of the *a* subunits and to suggest the molecular mechanisms underlying mutations that result in cutis laxa, osteopetrosis, and dTRA.

**Modelling post-surgical Cutibacterium acnes infections of connective tissue in joints**

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Infection of connective tissue presents major complications to orthopedic surgery. *Cutibacterium acnes* is an aerotolerant anaerobe that remains the most common cause of post-surgical infection of joint connective tissue. *C. acnes* infections can lead to implant loosening and tissue necrosis. These infections are difficult to treat because *C. acnes* can form biofilms on implant surfaces that confer resistance to most host immune responses and antibiotic therapy. At present, there is no standard approach for studying the progression of *C. acnes* infections in connective tissue due to the poor clinical translatability of known animal models. We aim to develop *in vitro* culturing system to model the progression of post-surgical *C. acnes* joint infections in a controlled environment. In brief, isolated shoulder cells were infiltrated onto a collagen-coated strip and wrapped around a *C. acnes*-coated mandrel. After a period of co-culture, the bio-composite strip would be unrolled for layer-by-layer analysis of *C. acnes* biofilm formation and its consequences to host cell physiology. Expression analysis of hypoxic indicators showed the interior layers of the wrapped scaffold provide a low oxygen environment favorable for *C. acnes* proliferation. Currently, we are optimizing culturing conditions to support *C. acnes* biofilm formation between the mandrel-scaffold interface. We hypothesize that mandrels made of harder metals will have fewer surface scratches that provide favorable microenvironments for the proliferation of anaerobic bacteria, and result in decreased *P. acnes* biofilm formation. If confirmed, our findings would provide unique insights into the selection of materials used for surgical implants.

**Bone remodeling in microgravity: Insights from mathematical modeling**

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Bone adapts to changes in mechanical loading, resulting in bone gain in response to exercise and bone loss due to unloading, such as in microgravity. We quantified available data on the changes in bone mass in astronauts that demonstrate a persisting decrease in bone density over 200 days of space travel. Serum and urine biochemical markers allow tracking the activities of bone resorbing osteoclasts and bone forming by osteoblasts, which mediate changes in bone mass. Quantification of bone marker data demonstrates that osteoclastic activity increases during the first 20-40 days of space travel, after which it stabilizes at the level approximately 2 times higher than pre-flight. Osteoblast markers do not change during the first 30-50 days of spaceflight, after which they gradually and slowly increase. We employed a mathematical model to 1) examine which assumptions regarding the effects of microgravity on bone cells will result in the observed dynamics of bone markers; and 2) test how markers of bone resorption and formation predict overall changes in bone mass. In the model of osteoblast-osteoclast interactions, we incorporated the assumption that microgravity results in an independent increase in RANKL/OPG and decrease in Sclerostin, which can be sustained for the duration of the flight, or transient accounting for bone adaptation. We have found that bone resorption markers are best explained by a sustained increase in RANKL/OPG, while bone formation markers by a transient decrease in Sclerostin followed by slow adaptation. However, based on the bone cell population dynamics, the model predicts slow recovery of bone mass by the end of 200 days of spaceflight, in contrast to observed continuous bone loss. These differences can potentially be explained by uncoupling of the population dynamics, or by significant differences in bone remodelling across skeletal sites, as the bone markers data encompasses the entire skeleton whereas bone loss data comes only from the lower half of the body. These findings contribute to our understanding of the effects of gravity on bone remodelling, and suggest concrete experiments which may further our knowledge of the mechanisms of sustained bone loss in microgravity.

**Characterizing the microtubule organizing centres in osteoclasts**

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Osteoclasts are highly specialized, multinucleated cells that are primarily tasked with the purpose of degrading bone via selective resorption of bone matrix components. These cells are derived from monocyte/macrophage precursors which, upon reception of receptor activator of nuclear factor kappa-B ligand, initiate fusion and differentiation. Our present study aims to analyze the origins and organization of their microtubular cytoskeleton during differentiation as well as how microtubules contribute to bone resorption. Microtubule nucleation is restricted to specific subcellular sites called microtubule organizing centres, which in mitotic animal cells is primarily fulfilled by the centrosome. While osteoclast precursor cells that fuse together during differentiation contain centrosomes, previous work has observed a lack of viable centrosomes in mature osteoclasts. This suggests that centrosomes donated to multinucleated cell bodies during differentiation are inactivated upon maturation, at which point microtubules could be nucleated at non-centrosomal sites in order to more efficiently carry out their primary function. Using drug-induced microtubule repolymerization assays and immunofluorescence, we attempt to track centrosomal behavior as well as their ability to organize microtubules in osteoclasts during differentiation and bone resorption.

**Using non-adhesive microfluidic wound dressing for optimal wound healing in burn patients**

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**Purpose:** Burn injury remains a serious problem leading to morbidity and mortality. Effective wound closure is crucial for the survival of burn patients but is limited by skin graft thickness and availability. Currently, synthetic skin substitutes are used but exhibit slow cell infiltration and slow incorporation of the dermal analog layer into the skin. Current wound dressings play a passive role in wound healing by protecting the wound and absorbing excess wound exudate but are not actively involved in wound healing. We report a novel non-adhesive wound dressing which participates actively and dynamically in wound repair. The purpose of the study is to evaluate the effectiveness of a novel method of cell delivery through a newly developed non-adhesive microfluidic wound dressing. We hypothesize that the skin cells will be delivered in clumps and show enhanced proliferation compared to randomly distributed cells.

**Methods:** The microfluidic wound dressing is manufactured from polydimethylsiloxane (PDMS), which is an inexpensive and biocompatible material. An array of channels (0.1 - 1mm wide) is built within the wound dressing with one inlet diverging into several outlets. Cells were injected *in vitro* through the microfluidic channels into collagen-coated wells. The seeded cells were examined for distribution and proliferation using toluidine blue staining and confocal microscopy.

**Results:** Fibroblasts and keratinocytes were shown to segregate into clumps, illustrating cellular distribution through the microfluidic device. The delivery of fibroblasts through the device in clumps resulted in enhanced proliferation of fibroblasts compared to randomly dispersed cells ($P<0.05$) as shown by BrdU staining.

**Conclusion:** This device could be used to enhance wound healing, by delivering different cell types at a controllable rate to enhance wound repair dynamically, in addition to their traditional role of protecting and maintaining the wound.

**Wound healing myofibroblast-derived microvesicles target fibroblasts and promote collagen production.**

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**Introduction:** Wound healing is a complex mechanism among which extracellular matrix (ECM) reconstruction and remodeling is crucial. Myofibroblasts are cells differentiating from fibroblasts that appear during healing to produce new ECM. They also express alpha smooth muscle actin (A-SMA) that participate to the contraction of the wound edges. In presence of serum, myofibroblasts from normal wound (Wmyo) produce microvesicles (MVs) that play a crucial role during angiogenesis. The results of the proteomic study showed that MVs isolated from Wmyo also contain proteins involved in the ECM remodeling. These suggest that MVs could contribute to the production or remodeling of the new tissue.

**Methods:** Confluent human Wmyo were cultured during 48h with medium containing serum depleted from extracellular vesicles. MVs were isolated from conditioned medium by differential centrifugation (300g, 10 min then 20 000 g, 20 min). The protein concentration of MVs was measured using a spectrophotometry method (NanoDrop 1000). Aspect and size distribution of MVs were evaluated using transmission electron microscopy and dynamic light scattering (Nanosizer), respectively. Some Wmyo populations were transduced with fluorescent protein gene and cultured to obtain fluorescent MVs. Human skin fibroblasts (Fb) were then treated with fluorescent MVs during 6h and quantification of Fb fluorescence was performed by flow cytometry. In parallel, Fb were cultured in presence of different concentration of MVs until confluency. Following the treatments, parameters linked to the ECM were studied: cell growth by cell counting, pro-collagen I dosed by ELISA and MMP activity level determined using an enzymatic test. A-SMA cell expression was evaluated using flow cytometry.

**Results:** MV proteins produced during 48h were 5.9±3.4 pg proteins/cell/48h (N=4 populations of Wmyo). Transmission electron microscopy pictures showed a well-defined structure of MVs with a diameter of 396.56 nm ± 51.16 (N=3 populations of Wmyo). Incubation of Fb with fluorescent MVs increased cell fluorescence, demonstrating the binding of MVs on Wmyo. Fb treatment with MVs significantly stimulated cell growth (fold change of 1.17±0.40 versus control). A-SMA expression did not change in presence of MVs while no MMP activity was detected in culture medium following stimulation with MVs. Pro-collagen secretion quantification showed a significantly increase of its level in Wmyo culture medium when MVs were added (fold change of 1.91±0.53 versus control).

**Conclusion:** Wmyo derived-MVs can potentially regulate ECM production by Fb via a slight increase of cell number but mostly via an increase of collagen production. Uncovering the mechanisms of MVs actions on ECM synthesis and remodeling would be a great advance in MVs domain and tissue regeneration.

**CD109 is an important regulator of chondrocyte function: Negative regulation of TGF-beta Receptor expression, signaling and ECM protein production**

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**Objective**: Transforming growth factor-beta (TGF-β) is a pleotropic growth factor that maintains the integrity and function of cartilage and dysregulation of TGF-β action is implicated in joint tissue diseases such as osteoarthritis (OA). Our group has previously reported CD109 as a novel TGF-β co-receptor and has shown that CD109 is a potent negative regulator of TGF-β signaling and action in the skin. The objectives of the current study were: (i) to determine whether CD109 is expressed in human articular chondrocytes, and (ii) to examine whether CD109 regulates TGF-β signaling and ECM protein expression in human chondrocytes.

**Methods**: Primary articular chondrocytes were isolated from normal cartilage harvested from adult patients with traumatic open joint injury who have no history of joint diseases. CD109 expression was determined by western blot and CD109 function was analyzed by blocking CD109 expression using CD109-specific siRNA.

**Results**: Our results show that CD109 is expressed in human articular chondrocytes and that loss of CD109 expression markedly enhances ALK5 levels, TGF-β1-induced Smad2 and Smad3 phosphorylation, Smad3-driven transcriptional activity, type II collagen and plasminogen activator inhibitor-1 (PAI-1) protein levels and aggrecan gene expression. On the other hand, loss of CD109 expression decreases ALK1 levels, TGF-β1-induced Smad1 phosphorylation and inhibits MMP13 expression at both mRNA and protein levels. Conclusion: Our findings suggest that CD109 differentially regulates TGF-β signaling pathways and inhibits ECM protein production while promoting proteases expression in human articular chondrocytes. We conclude that CD109 may play an important role in maintaining cartilage function and integrity.

**Development of an in vitro outer annulus fibrosus-cartilage endplate interface model**

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**Introduction:** The intervertebral disc consists of annulus fibrosus (AF) tissue that surrounds a centrally placed nucleus pulposus. It is integrated to adjacent vertebral bodies through a cartilage endplate (CEP). Disc degeneration can lead to chronic neck or lower back pain. Current surgical treatments include discectomy, disc fusion and prosthetic disc implants; however, these treatments are not completely restorative. Attention has turned to developing a biological disc replacement that mimics native tissue and does not induce further degeneration. Integration of an engineered disc with adjacent vertebral bodies through a cartilage endplate is required to ensure mechanical stability of the implant replacement.

**Hypothesis:** *In vitro* formed OAF tissues will integrate with *in vitro* formed cartilage to generate a mechanically stable interface model that resembles the composition of native OAF-CEP interface. This model will allow for investigation of mechanical factors that influence integration.

**Methods:** OAF cells and articular chondrocytes were isolated from bovine caudal discs and synovial joints respectively. OAF cells were cultured on multilamellar electrospun nanofibrous polycarbonate urethane scaffolds in bioreactors for 2 weeks. Chondrocytes were placed onto collagen type II coated membranes and cultured for 3 days. Resulting OAF and cartilage tissues were co-cultured in direct contact for up to 4 weeks to form an OAF-cartilage interface. The interface was evaluated histologically by H&E and toluidine blue staining, and the composition was determined by immunohistochemical staining for collagen type I, collagen type II, and aggrecan. To determine if chondrocytes migrated into the OAF tissue, in select cultures chondrocytes were labelled with green fluorescent CFDA dye prior to culture. Interface strength was evaluated by a pull-apart test.

**Results:** When 2-week old OAF tissues were co-cultured with 3-day old cartilage, integration occurred within one week for all cultures. OAF cells near the interface showed directional alignment parallel to the cartilage layer. The interface stained positively for collagen type I in the OAF and in the pericellular regions of the chondrocytes within the cartilage. Collagen type II and aggrecan were localized to the cartilage layer. Chondrocytes remained localized to the cartilage tissue and did not appear to migrate up into the OAF. This was similar to that seen for the native bovine OAF-CEP interface. Preliminary results demonstrated that the interface between the OAF and cartilage had mechanical strength.

**Conclusion:** This study demonstrated that it is possible to engineer an OAF-CEP interface model *in vitro* that has mechanical strength. Future study will focus on identifying factors (e.g. dynamic compressive loading) that may affect interface strength.

**Human chondrocytes derived from intact or severely osteoarthritic cartilage form hyaline cartilaginous tissues of similar quality in the presence of TGF-beta in vitro**

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**Introduction**: Osteoarthritis (OA) is a disease of joints that involves progressive articular cartilage (AC) degradation. Biological therapies to repair AC are being explored as a better alternative to prosthetic joint replacement. Chondrocytes, the cells of AC, are one cell type which may be used for this purpose. However, due to the inherent low cellularity of AC, chondrocytes are serially passaged to increase the number of cells, a process leading to de-differentiation of the chondrocytes and impaired tissue formation. We have shown in previous studies that passaged human chondrocytes derived from macroscopically undamaged OA cartilage can be induced to form phenotypically stable AC in vitro, when the media is supplemented with transforming growth factor beta 3 (TGFβ3). However, it is still unclear whether chondrocytes derived from severe OA will respond similarly. Studies assessing the transcriptome of human chondrocytes from moderately and severely diseased cartilage have demonstrated differentially activated pathways (Dunn, S. L. et al. Osteoarthr. Cartil. 2016). Furthermore studies in animal models of OA have demonstrated a sustained loss of the TGFβ receptors with disease progression (Blaney Davidson, E. N. et al. J. Immunol. 2009). As the cells in these studies were not de-differentiated, it is unknown if these distinctions will play a role in cartilage tissue formation after passaging. We hypothesize that chondrocytes obtained from cartilage taken from OA joints, with either severe or no changes macroscopically, will form cartilaginous tissue of similar quality after cell passaging.

**Methods**: Human AC tissue was examined macroscopically for indications of OA. Areas showing no osteoarthritic changes (Low OA) or more severe osteoarthritic changes (High OA) were separately dissected. Representative samples of tissue were fixed in formalin for histological analysis. Chondrocytes were isolated enzymatically and passaged twice (P2). P2 cells were seeded onto collagen type 2 coated membrane inserts (MillicellTM) at 2 million cells per membrane and cultured for 3 weeks in chondrogenic media with 10ng/ml TGFβ3. In vitro formed tissues were assessed histologically, biochemically, and by immunohistochemistry. Flow cytometry was used to assess expression of TGFβ receptors (ALK1 and ALK5) and co-receptors (CD105 and TGFβRIII) in primary and in passaged (P2) chondrocytes.

**Results**: Histological grading of native AC revealed that tissues isolated from areas of High OA were significantly different than Low OA. After serial passaging and tissue formation, no significant differences were observed in the amount of sulphated proteoglycan or collagen accumulated in the in vitro formed tissues. Furthermore, when assessed via immunohistochemistry the in vitro formed tissues from both cell types accumulated hyaline-like cartilage matrix (rich in collagen type 2 and aggrecan) and did not accumulate collagen type X and collagen type 1. Flow cytometry revealed no significant differences in CD105 and TGFβRIII.

**Conclusions**: This study demonstrated that chondrocytes obtained from severely osteoarthritic cartilage form cartilaginous tissue of similar quality to cells obtained from more normal appearing cartilage. This suggests that chondrocytes obtained from any site in the OA joint are suitable for this use, an important finding given the limited amount of cartilage available in the OA joint.

# Autologous nucleus pulposus cell-seeded hydrogel promotes tissue repair of human intervertebral discs in an ex vivo, physiological culture model.

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**Introduction:** Intervertebral disc (IVD) degeneration has been directly linked to low back pain. Current treatment options for painful IVD degeneration are either physiotherapy and pain management or invasive surgical procedures to remove the painful degenerate disc. Over the past decade, several high-level in vitro and in vivo animal studies have paved the way for novel tissue engineering strategies to repair or reverse painful IVD degeneration. However, translation to human IVD repair has not been as rapid. We previously developed a whole disc organ culture system for physiological loading of human IVDs and showed feasibility of monitoring cell injection therapy. Here we use our unique platform for determining NP cell biocompatibility and matrix production within a hydrogel. We also assess human disc repair whereby intact lumbar IVDs are harvested from organ donors and cultured under physiological conditions in a bioreactor system.

M**ethods:** Human lumbar IVDs were isolated from lumbar spine with institutional ethics approval. NP cells were isolated and expanded for one passage. Cells were first tested for viability and matrix production within hydrogels in vitro in a custom silicone/agarose model within bioreactors system for 3 weeks. Human discs were scanned using a custom quadrature volumetric coil with T1ρ pulse sequence MRI. Discs were then injected with ~500 µL of either HA-pNIPAM hydrogel alone or hydrogel seeded with $2x10^6$ cells/mL and placed in the bioreactor. After 5 weeks of physiological culture, discs underwent follow-up MRI in the same orientation. Disc sections were stained with safranin-O and antibodies against collagen types I and II. T1ρ image quantification was performed using MIPAV software (NIH). Statistical analysis using paired t-tests in Microsoft Excel.

**Results:** The silicone/agarose model showed suitable mechanical properties. The bioreactor system proved its consistency by providing constant compression throughout culture period. The initial in vitro physiological culture of human NP cells seeded in hydrogel showed high levels of viability in both loaded and unloaded conditions. The loaded constructs generated more proteoglycan formation than the unloaded controls. After 5 weeks of culture, post-treatment T1ρ scans revealed increased signal in the treated discs corresponding to injected regions. Histological analysis with safranin-O showed positive proteolglycan staining and signs of neo-matrix production in samples treated with cell-seeded hydrogel. Discs injected with cell-seeded gel displayed collagen type II staining surrounding implanted cells.

**Discussion:** This study presents an *in vitro* physiological culture tool to assess any number of hydrogel and cell combinations for tissue engineering and drug delivery applications. It focused on implantation of autologous NP cells within an injectable thermoresponsive hydrogel, which would theoretically produce disc matrix proteins at a high level. Our current work demonstrates that this unique culture platform provides a pre-clinical *ex vivo* model system where numerous parameters such as growth factors, $O_2$ tension, glucose levels, bioactive therapeutics and mechanical properties can be evaluated. Obtained T1ρ values suggest appropriate quantification of proteoglycan content pre- and post-treatment, which may have clinical significance. Since most current clinical work focuses on delivery of mesenchymal stem cells, future studies will assess MSC-NP co-culture using this approach.

**Characterization of the effect of Periostin deletion on palatal wound healing**

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Cleft lip and cleft palate are two of the most frequently occurring birth defects in Canada, affecting between 400 and 500 newborns every year. Healing after the surgical repair of cleft palate typically results in excessive scar formation, due to the increased mechanical tension related to the localization of bone under the mucoperiosteum, However, the characteristics and the behavior of palatal mucoperiosteum during healing have been poorly investigated. Current data suggests that significantly more contractile myofibroblasts exist in palatal mucoperiosteum wounds than is evident in skin wounds in a rat model, an observation that has been attributed to the different contractile properties of the cells present in these tissues. Palatal mucoperiosteum is tightly attached to the underlying bone and, therefore, contraction generates higher mechanical tension, which feeds back by inducing increased myofibroblasts. Our lab has previously identified that the matricellular protein Periostin (Postn) is a central driver of cell contraction, promoting myofibroblast differentiation. Postn's physiological role has been delineated mainly in collagen-rich biomechanically active tissues. Although Postn has been shown to influence many aspects of skin healing, its role during palatal wound healing processes has not yet been investigated. **Our overall hypothesis is** that the genetic deletion of Postn will impair palatal wound healing which will manifest in alterations in cellular processes associated with the proliferative phase of healing.

1.5mm full-thickness excisional wounds were created on the hard palate of periostin-knockout (KO) and wild-type (WT) mice. Wound healing kinetics, histological analysis, in situ hybridization and Reverse-Transcription-quantitative-Polymerase-Chain-Reaction (RTqPCR) were performed assessing several Extracellular Matrix components, such as fibronectin, βigh3, αSMA/acta2, and the presence of fibroblasts and inflammatory cells. In WT mice, periostin mRNA increased by 6 days after wounding, peaking at day 12. Genetic deletion of periostin significantly reduced wound closure rates compared to WT mice. This alteration corresponds with the onset of periostin expression in WT animals, as well as with the peak of βigh3 and acta2 expression. βigh3 is upregulated during palatal healing but its expression is reduced in KO wounds. Absence of periostin resulted in reduction in the mRNA levels of pivotal genes in wound repair, including as αSMA/acta2, fibronectin and βigh3. Impaired recruitment of fibroblasts and inflammatory cells in KO wounds was also observed, visualized by immunofluorescent staining for fibroblast specific factor-1 and macrophages markers Arginase-1 and iNOS. Palatal fibroblasts isolated from WT and KO mice were seeded on substrates with varying stiffness and the expression of βigh3, acta2 and FN was evaluated using RTqPCR and immunofluorescence. Our results show that the expression of these genes is depended on the surface compliance, and that all of these genes are downregulated in KO cells when compared to WT. By elucidating the role of periostin in palatal wound healing the findings of our study further contribute to the better understanding of the underlying healing mechanisms and could provide new insights that can be used towards the development of novel approaches to accelerate and enhance the healing process after dental and maxillofacial surgical procedures.