23rd CANADIAN CONNECTIVE TISSUE CONFERENCE

May 18 – 20, 2017 | Montreal, QC

PROGRAM & ABSTRACTS

CANADIAN CONNECTIVE TISSUE SOCIETY

http://connective-tissue-canada.com

The Canadian Connective Tissue Conference 2017

Welcome to the 23rd Canadian Connective Tissue Conference (CCTC) 2017.

The organizing committee is pleased to welcome you to the CCTC 2017 hosted at the McGill New Residence Hall in Montreal, Quebec, on May 18-20, 2017. The CCTC, representing the major outreach activity of the non-profit Canadian Connective Tissue Society aims to fill the gaps in current scientific and clinical understanding of connective tissues in both health and disease.

CCTC is the premiere annual conference for scientists and clinicians interested in connective tissue healing and repair in Canada and provides an excellent forum to interact and exchange knowledge and ideas and network with industry. It also encourages and recognizes the work of young scientists, postdoctoral fellows, graduate students, and undergraduates in the field of connective tissue research by providing them the opportunity to present their work at scientific sessions.

This year's conference includes national and international researchers as invited speakers. The program also features 27 oral presentations selected from submitted abstracts, as well as two poster sessions. Prizes will be awarded for best trainee oral and poster presentations.

The CCTC 2017 organizing committee members look forward to seeing you all in Montreal in May 2017!

Anie Philip
Conference Co-Chair

Stéphane Roy
Conference Co-Chair

The Canadian Connective Tissue Society

President *Lisbet Haglund (Montréal)*

Secretary *Boris Hinz (Toronto)*

Treasurer *Dieter Reinhardt (Montréal)*

Dear Colleagues and Friends,

Welcome to Montreal and the 23rd Canadian Connective Tissue Conference. The CCTC has been held yearly since 1994, and is now the flagship of the Canadian Connective Tissue Society, which was founded in 2012. The Organizing Committee for CCTC 2017 is co-chaired by Anie Philip (McGill Univerity) and Stephane Roy (Université de Montreal) and includes members from other Canadian Universities. The conference is attended by approximately 200 researchers and trainees from across Canada. The 2017 conference is organized by researchers at McGill University and Université de Montréal and will be attended by approximately 150-200 researchers and trainees from across Canada.

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. Importantly, it has encouraged and motivated the next generation young Canadian scientists by giving them the opportunity to present their research as well as to interact with leading Canadian and international researchers in this field.

The Canadian Connective Tissue Society has now 200 members and is providing a platform for exchange of scientific knowledge. The specific objectives and anticipated impact of the society and the conferences a are:

- To support new emerging themes and teams involving multidisciplinary research.
- To encourage and recognize the work of young scientists, postdoctoral fellows and graduate students in this field of research through travel awards and excellence awards.
- To further establish national and international networks between leading Canadian and international scientists and newcomers interested in connective tissue research.
- To improve health of Canadians by stimulating knowledge transfers and by giving the opportunity to basic scientists and clinicians to exchange with representatives of industries and pharmaceutical societies.
- To encourage the participation of patient advocate groups and other non-academic stakeholders in connective tissue research.

On behalf of the board, it is my pleasure to welcome you to Montreal.

President, Canadian Connective Tissue Society

Canadian Connective Tissue Society Conference 2017

Organizing Committee

Anie Philip, PhD (Conference co-chair)

Professor, Department of Surgery, McGill University

Stéphane Roy, PhD (Conference co-chair)

Professor, Faculties of Dentistry and Medicine, University of Montreal

Lisbet Haglund, PhD

Associate Professor of Surgery, Associate Director, Experimental Surgery, McGill University

Boris Hinz, PhD

Professor, Laboratory of Tissue Repair and Regeneration, Matrix Dynamics Group, Faculty of Dentistry, University of Toronto

Dieter P. Reinhardt, PhD

Professor, Faculties of Medicine and Dentistry, McGill University

Event Manager

Padma Madiraju, MSc.
McGill University

Local Trainee Organizing Committee

Fadi Sader *(University of Montreal)*

Jean François Denis *(University of Montreal)*

Muthu Lakshmi Muthu *(McGill University)*

Rongmo Zhang *(McGill University)*

Heena Kumra *(McGill University)*

Joseph Di Paolo *(McGill University)*

Shufeng Zhou *(McGill University)*

Faegheh Ghanbari Divshali *(McGill University)*

Emily Buck *(McGill University)*

Rayan Fairag *(McGill University)*

23rd Canadian Connective Tissue Conference EVENT PROGRAM	
THURSDAY, May 18	
6:00- 9:00pm	Registration Reception and Refreshments
FRIDAY, MAY 19	
7:45 - 8:30am	Light Breakfast
7:45 - 3:00pm	Registration desk open
8:30 - 8:45am	Welcome remarks from co-chairs of organizing committee. ***Anie Philip**, McGill University* ***Stephane Roy**, Université de Montréal*
SESSION I	
BONE AND CARTILAGE DISEASE: MECHANISTIC AND REGULATORY ASPECTS	
Moderators: Lauren Flynn and Jean Vacher	
8:45 - 9:30am Keynote	"Influence of Surface Topography and Micromotion on Healing at the Bone-Implant Interface." ***Antonio Nanci**, Université de Montréal*
9:30 - 10:30am	**Selected Abstracts**
9:30am	"MicroRNA-34a: Role In The Development Of Osteoarthritis During Obesity." ***Helal Endisha**, University of Toronto, ON.*
9:40am	"Cartilage-Specific ULK-1 Knockout Induces Inhibition Of Autophagy Pathway And Increased Cartilage Destruction With Secondary Inflammatory Effects On Synovium." ***Brain Wu**, Krembil Research Institute, University Health Network, Toronto, ON.*
9:50am	"High-Fat Diet-Induced Acceleration Of Osteoarthritis Is Associated With Sustained Metabolite Signature Regulated By Leptin/Autotaxin Pathway." ***Poulami Datta**, University of Toronto, ON.*
10:00am	"Modulation of Parathyroid Hormone Receptor 1 Signaling By Extracellular Nucleotides." ***Brandon H. Kim**, Schulich School of Medicine & Dentistry, Western University ON.*
10:10am	"Role of Osteoblast Menin In Bone Metabolism: In-Vivo Studies Of Knockout Mice." ***Jad Abi-Rafeh**, Division of Experimental Medicine, McGill University, Montreal, ON.*
10:20am	"Mechanically-Evoked ATP Release Is Regulated By Facilitated Membrane Resealing In Murine Osteoblasts." ***Nicholas Mikolajewicz**, Shriners Hospital for Children, McGillUniversity*
10:30 - 10:45am	Coffee and Networking Break

SESSION II	
CONNECTIVE TISSUE DISEASE CHARACTERISTICS: CLINICAL AND FUNDAMENTAL ASPECTS	
Moderators: Fackson Mwale and David O'Gorman	
10:45 - 11:15am Invited talk	"Elucidating the Role of POC5 in Idiopathic Scoliosis." ***Florina Moldovan**, Université de Montréal, CHU Sainte-Justine Research Center, Montreal, QC.*
11:15- 12:15pm	**Selected Abstracts**
11:15am	"Characterizing Degeneration Of Facet Joint Cartilage In Adolescent Idiopathic Scoliosis." ***Daniel Bisson**, McGill University & Shriner's Hospital for Children, Montreal, QC.*
11:25am	"Effect Of Tissue-Specific Extracellular Matrix On The Adipogenic And Chondrogenic Differentiation Of Human Adipose-Derived Stromal Cells." ***Danielle Heinbuch**, University of Western Ontario, London, ON.*
11:35am	"Characterization Of Proteins In The Specialized Basal Lamina Associated With The Tooth." ***Aurélien Fouillen**, Université de Montréal, Montréal, QC.*
11:45am	"Use Of Unfixed Histology To Visualize And Characterize Meniscus Inter-Tie Coil Structures In Intact Knees And Knees Following Surgical Procedures." ***Colleen Mathieu**, Ecole Polytechnique de Montreal, Montreal, QC.*
11:55am	"Role Of Phex-Fgf23 Axis And Γ-Carboxylation On Vascular Calcification Caused By Mgp Deficiency." ***Abhinav Parashar**, McGill University, Montréal, QC.*
12:05pm	"Male Mice Are More Susceptible To Whole-Body Vibration Induced Joint Damage Than Age-Matched Female Mice." ***Geoffrey Kerr**, Schulich School of Medicine and Dentistry, The University of Western Ontario, London, ON.*
12:15 - 2:00pm	LUNCH/ Poster viewing
12:15 - 2:00pm	CCTS BOARD MEETING
SESSION III	
BONE AND CARTILAGE: GENETIC AND DEVELOPMENTAL ASPECTS	
Moderators: *Svetlana Komarova and Showan N Nazhat*	
2:00 - 2:30pm Invited talk	"Building articular cartilage: A new blueprint." ***Rebekah S. Decker**, Genomics Institute of the Novartis Research Foundation.*
2:30 - 3:00pm	**Selected Abstracts**
2:30pm	"Identifying Gli Transcriptional Targets In Chondrosarcoma Using Next Generation Sequencing." ***Amanda Ali**, University Health Network, Toronto, ON.*
2:40pm	"Undercarboxylated Osteocalcin And Metabolic Phenotype In The Mouse Model Of Osteogenesis Imperfecta." ***Iris Boraschi-Diaz**, Shriners Hospital for Children, McGill University, Montreal, QC.*

2:50pm	"Biological Repair Of Osteoarthritic Cartilage Using Short Link N." ***Muhammad Albesher**, Lady Davis Institute for Medical Research, McGill University, Montreal, QC.*
3:00 - 3:15pm	Coffee and Networking Break
SESSION IV	
3:15 - 4:15pm	**Selected Abstracts**
Moderators: Rebekah S. Decker and Morris Manolson	
CONNECTIVE TISSUE REPAIR AND FIBROSIS: CELLULAR AND MOLECULAR ASPECTS	
3:15pm	"Toll-Like Receptor Activation Induces Human Intervertebral Disc Degeneration." ***Emerson Krock**, McGill University, Montreal, QC.*
3:25pm	"Investigating The Role Of Pannexin 3 As A Regulator Of Intervertebral Disc Health." ***Meaghan E. Serjeant**, Schulich School of Medicine and Dentistry, Bone and Joint Institute, University of Western Ontario.*
3:35pm	"Functional relevance of Fibulin-4 interactions with latent transforming growth factor beta binding protein- 4 in the context of fibronectin and fibrillin-1." ***Heena Kumra**, McGill University, Montreal, QC.*
BONE AND CARTILAGE DISEASE: TRANSLATIONAL AND CLINICAL ASPECTS	
3:45pm	"Synovial Membrane Nerve Fiber Density Decreases With Osteoarthritis In Horses." ***Raymond Pujol**, University of Montreal, St. Hyacinthe, QC.*
3:55pm	"Lactosylceramide Enhances Chondrocytic Differentiation." ***Lilit Antonyan**, Research Centre, Shriners Hospitals for Children, McGill University, Montreal, QC.*
4:05pm	"Novel Electromechanical Arthroscopic Device For The Objective And Quantitative Evaluation Of Cartilage Quality." ***Insaf Hadjab**, Institute of Biomedical Engineering, Polytechnique Montreal, Biomomentum Inc.*
4:15 - 5:45pm	**Poster Presentations and Viewing**

23rd Canadian Connective Tissue Conference

Banquet Event

May 19, 2017 - 6:00pm - 9:00pm

Venue

McGill La Citadel 26th Floor Lounge

410 Sherbrooke St W, Montreal, QC. H3A 1B3

Plenary Talk

"Mechanistic Modeling: The Pathway to Precision Medicine"

V. A. Shiva Ayyadurai, Chairman and CEO, CytoSolve, Inc.

SATURDAY, MAY 20	
8:00 - 9:00am	**Light Breakfast**
8:30 -10:30am	**Registration desk open**
8:30 -9:00am	**CCTS BUSINESS MEETING/GENERAL ASSEMBLY**
SESSION V	
TISSUES REPAIR, REPLACE OR RE-ENGINEER	
Moderators: Rene Harrison and Julie Fradette	
9:00 - 9:45am Keynote	"Connective Tissue Signaling Through Cell Adhesions in Matrix Homeostasis and Fibrosis." ***Christopher A. McCulloch****, University of Toronto, Toronto, ON.*
9:45 - 10:15am	**Selected Abstracts**
9:45am	"Naturally Derived Microcarriers As Tissue-Specific 3-D Cell Culture Platforms." ***Anna Kornmuller****, Western University.*
9:55am	"T1ρ Mri Detection And Histological Evaluation Of Autologous Nucleus Pulposus Cell-Seeded Hydrogel Delivery To Isolated Human Intervertebral Discs In Dynamic Culture." ***Derek H. Rosenzweig****, McGill University, Montreal, QC.*
10:05am	"Blood And Lymphatic Microvascularization Of Self-Assembled Skin Substitutes Using Primary Human Endothelial Cells." ***Jennifer Bourland****, Centre de recherche en organogenese expérimentale de l'Universite Laval / LOEX, Quebec, QC.*
10:15 - 10:30am	**Coffee and Networking Break**
10:30 - 11:00am Invited talk	"Osteogenesis Imperfecta – What causes it? What to do about it?" ***Frank Rauch****, McGill University, Shriners Hospital for Children, Montreal, QC.*
11:00 - 11:30am	**Selected Abstracts**
11:00am	"Chiral Switching Of Calcium Carbonate Biomineral Induced By A Single Chiral Enantiomer Of Amino Acid." ***Wenge Jiang****, McGill University, Montreal, QC.*
11:10am	"An Injectable Chitosan Scaffold With Mechanical Properties Optimized For IVD Repair." ***Yasaman Alinejad****, École de technologie supérieure (ETS), Montréal, QC.*
11:20am	"Regulation Of Matrix Gla Protein Gene Expression By An Intronic Sequence." ***Jobran Mohammed Moshi****, McGill University, Montreal, QC.*
11:30 - 12:15pm Invited talk	"Nuclear receptors in cartilage and osteoarthritis." ***Frank Beier****, Western University*
12:15 - 12:45pm	**AWARDS PRESENTATION** **CONCLUDING REMARKS**
12:45 - 2:30pm	**LUNCH** **POSTER BREAKDOWN**
End of Conference	

Abstracts for Oral Presentations

SESSION I

BONE AND CARTILAGE DISEASE: MECHANISTIC AND REGULATORY ASPECTS

KEYNOTE LECTURE

Antonio NANCI, PhD
Faculty of Dentistry, Université de Montréal

Antonio Nanci is a full Professor at the Faculty of Dentistry and affiliated with the Department of Biochemistry and Molecular Medicine at Université de Montréal. He is the Director of the Department of Stomatology of the Faculty, of the Laboratory for the Study of Calcified Tissues and Biomaterials (LSCTB), and of the Electron Imaging Facility of the University. His research interests are the basic cell biological processes of normal and pathological calcified tissues, periodontal tissue regeneration, and the osseointegration of biomaterials. His work exploits animal and cell culture models, and integrates structural and cell and molecular approaches. Nanci holds a Canada Research Chair in Calcified Tissues, Biomaterials, and Structural Imaging.

Influence Of Surface Topography And Micromotion On Healing At The Bone-Implant Interface

While current dental and orthopedic implants are relatively effective, the problem is that longer living elders and younger patients will require multiple replacements in their lifetime. Because more interventions will take place later in life, compromised health/bone status and healing capacity will pose additional clinical challenges. A better understanding and control of the healing events at the bone-implant interface where cell fate decisions are made is mandatory to meet these challenges.

When implants are loaded, they are inevitably subjected to displacement relative to bone. Such micromotion generates stress/strain states that cause beneficial or detrimental interfacial tissue deformation. What constitutes safe and dangerous micromotion is still not well defined. More so, new generations of implants benefit from surface modifications but their influence on the mechanobiology at the bone-implant interface is also not known. We are particularly interested in nanotopography because it has been shown to differentially affect cell activity and to interact synergistically with mechanical loading.

This presentation will review our histological and molecular work on nanotopography and micromotion using a system that allows control over implant displacement and, in turn, interfacial tissue deformation. Our ultimate objective is to achieve sophisticated cellular activity through controlled biomechanical intervention and physicochemical cueing without resorting to bioactive agents, a prospect with wide-ranging implications in regenerative medicine. Supported by CIHR, CRC program, NIH and NSERC.

Title: Microrna-34a: Role In The Development Of Osteoarthritis During Obesity

Helal Endisha[1,2,3], Poulami Datta[1,2], Anirudh Sharma[1,2], Ghazaleh Tavallaee [1,2,3] and Mohit Kapoor [1,2,3].

1. *Arthritis Program, Toronto Western Hospital, University Health Network, Toronto, Ontario, Canada.*
2. *Division of Genetics and Development, Krembil Research Institute, University Health Network, Toronto, Ontario, Canada.*
3. *Department of Surgery and Department of laboratory Medicine and Pathobiology, University of Toronto, Ontario, Canada.*

OBJECTIVE: Mice fed a high-fat diet (HFD) exhibit an accelerated surgically-induced osteoarthritis (OA) phenotype compared to lean diet (LD) fed mice. MicroRNAs are endogenous short non-coding RNA segments that are negative regulators of gene expression. Recent studies have shown that miR-34a is elevated in obesity. 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 at 9 weeks of age (baseline) and at the end of a HFD or LD course. Human plasma was taken at preadmission from end-stage OA patients undergoing total knee replacement (TKR) surgery. Patients with no co-morbidities were segregated into normal weight (BMI= 18.5-29.9 kg/m2) and obese groups (BMI≥30 kg/m2). Chondrocytes and synovial fibroblasts (SF) were plated in replicates and transfected with 100nM miR-34a mimic followed by RNA isolation and qRT-PCR.

RESULTS: Plasma miR-34a levels were significantly up-regulated in HFD mice and correlated with percent body fat, body weight, and fasting blood glucose levels compared to baseline mice and LD controls. Human plasma miR-34a levels were up-regulated in obese end-stage OA patients compared to non-obese patients. In-situ hybridization shows HFD mouse knee joints express significantly higher levels of miR-34a than LD mouse knees and localized to cartilage and synovial membrane. Mir-34a mimic-treated chondrocytes showed significantly increased expression of MMP13 and reduced expression of SIRT1, Aggrecan, and autophagy markers, ATG5 and ULK1. Mir-34a-treated SFs show significantly increased Type I collagen (mRNA and protein), TNF-α, TGF-β, IL-6, ULK1, ATG5 and ATG3.

CONCLUSIONS: This study will be the first to elucidate a mechanistic role for miR-34a in the development of OA during obesity and its potential as a therapeutic target.

Title: Cartilage-Specific Ulk-1 Knockout Induces Inhibition Of Autophagy Pathway And Increased Cartilage Destruction With Secondary Inflammatory Effects On Synovium

Wu B[1,2], Abou RM[1,2], Rockel JS[1,2], Kapoor M[1,2,3].

1. *Arthritis Program, Toronto Western Hospital, University Health Network, Toronto, Ontario, Canada.*
2. *Division of Genetics and Development, Krembil Research Institute, University Health Network, Toronto, Ontario, Canada.*
3. *Department of Surgery and Department of laboratory Medicine and Pathobiology, University of Toronto, Ontario, Canada.*

Objectives: ULK-1 knockout in mouse has been shown to induce an accelerated osteoarthritis (OA) phenotype. It is pertinent to elucidate the role of ULK-1 in autophagy and catabolic cascades during OA pathogenesis. Further, secondary inflammatory effects of cartilage degeneration observed in synovium characterize total knee OA pathology for ULK-1 knockout.

Methods: Doxycycline-induced cartilage-specific ULK-1 knockout mice were generated using Cre-Lox mediated homologous recombination system and underwent DMM surgery to induce OA pathology. Hind limbs from mice treated with either doxycycline or PBS as control were isolated, fixed and sectioned for safranin-O/Fas green staining, immunohistochemistry, HE staining and trichrome staining.

Results: Safranin-O/Fas green staining indicated an accelerated OA phenotype in ULK-1 knockout mice by OARSI scoring and cellularity count. Immunohistochemistry against LC3-B indicated severe down regulation in autophagy while significantly increased expression of MMP-13 suggest upregulation in type II collagen depletion for ULK-1 knockout mice. Characterization of synovium through HE staining and trichrome staining revealed an increase in synovitis for ULK-1 knockout mice.

Conclusion: It is evident that ULK-1 has a potent effect in OA pathology through upregulation of the autophagy pathway. Downstream effects of autophagy inhibition appear to increase type II collagen depletion in articular cartilage ultimately exacerbating OA phenotype. Further, ULK-1 may have a protective roll against total joint destruction due, which is seen in severe synovitis of ULK-1 knockout mice.

Title: High-Fat Diet-Induced Acceleration Of Osteoarthritis Is Associated With Sustained Metabolite Signature Regulated By Leptin/Autotaxin Pathway

Poulami Datta[2], Yue Zhang[2], Alexa Parousis[2], Anirudh Sharma[2], Evgeny Rossomacha[2], Rajiv Gandhi[2], Brian Wu[2], Izabela Kacprzak[2], Nizar N. Mahomed[2], Rajiv Gandhi[2], Jason Rockel[2] and Mohit Kapoor[1,2,3].

1. *Arthritis Program, Toronto Western Hospital, University Health Network, Toronto, Ontario, Canada.*
2. *Division of Genetics and Development, Krembil Research Institute, University Health Network, Toronto, Ontario, Canada.*
3. *Department of Surgery and Department of laboratory Medicine and Pathobiology, University of Toronto, Ontario, Canada.*

Rationale and 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. However, high fat diet (HFD)-induced longitudinal changes to the metabolome and its contribution OA pathogenesis are poorly understood. We sought to identify longitudinal changes in metabolites resulting from different diet regimes and determine corresponding metabolic mechanisms contributing to OA pathogenesis.

Methods: 9-week old C57BL/6 mice were fed HFD or lean diet (LD) for 18 weeks followed by normal chow diet and evaluated longitudinally up to 12 months of age. Body composition, metabolic indices and comprehensive metabolite levels were measured. Mice were also subjected to surgically-induced OA at the end of diet. Knee joints were analyzed for OA pathogenesis. Primary human chondrocytes were treated with leptin and/or autotaxin inhibitor and the release/expression of selected metabolites or enzymes was determined.

Results: Compared to LD-fed mice, plasma leptin and physical parameters were significantly increased in HFD-fed mice and resolved after placing mice onto normal chow diet. At 9- and 12-months of age, knee joints of HFD-fed mice also had more severe spontaneous and surgically-induced OA. Interestingly, a signature of lysophosphatidyl choline (lysoPC) and phosphatidylcholine analogues was sustained up to at least 9-months of age in mice fed a HFD. In contrast to the plasma, knee joints from HFD-fed mice had longitudinally increased expression of leptin. In vitro, leptin increased total lysoPC release, and expression of MMP13, and autotaxin [(converts lysoPC to lysophosphatidic acid (LPA)]. Treatment with an autotaxin antagonist inhibited the expression MMP13, with or without leptin.

Conclusion: HFD maintains a distinct metabolic signature rich in lysophosphatidyl choline and phosphatidylcholine analogues and accelerates OA progression in both spontaneous and surgically induced OA. We continue to examine targets contributing to leptin-induced changes in the LysoPC/ATX/LPA axis in chondrocytes both in vitro and in vivo.

Title: Modulation Of Parathyroid Hormone Receptor 1 Signaling By Extracellular Nucleotides

Brandon H. Kim[1,2], Alexey Pereverzev[1], S. Jeffrey Dixon[1,2] and Peter Chidiac[1,2]

1. *Department of Physiology and Pharmacology, Schulich School of Medicine & Dentistry, Western University*
2. *Bone and Joint Institute, Western University*

Osteoporosis, a devastating bone disease, arises from an imbalance in the process of bone remodeling. PTH is an important regulator of bone remodeling that activates PTH1R, a G protein-coupled receptor (GPCR) that signals through Gs, Gq and β-arrestin. PTH and mechanical loading synergistically enhance bone formation, but the underlying mechanism remains elusive. Mechanical stimulation of osteoblasts causes release of ATP into the surrounding extracellular fluid. We have previously shown that extracellular nucleotides at micromolar concentrations activate high affinity P2 receptors, which amplify the calcium, but not cAMP response to PTH. Whether millimolar concentrations of extracellular ATP, sufficient to activate low affinity P2X7 receptors, further modulate PTH1R signaling remains to be elucidated. Using the osteoblast-like UMR-106 cell line, we monitored adenylyl cyclase activity in real-time using a bioluminescence reporter, and cytosolic calcium using a fluorescent probe. Additionally, we used CRE-reporter luciferase to monitor CREB activity to assess the function of signaling. For G protein-independent signaling, we transfected HEK293 cells with split fragment Emerald luciferase complementation assay plasmids, to monitor β-arrestin-1 recruitment to PTH1R. In the presence of millimolar concentration (but not micromolar) of ATP, the potency of PTH was increased as measured by its effects on cytosolic cAMP levels. Moreover, the enhancing effect of ATP was also observable with b-arrestin-1 recruitment, calcium release, and CREB activity. Importantly, cytidine 5'-monophosphate (CMP, a nucleotide that is not P2 agonist) mimicked enhancing effect of ATP, and the enhancing effect was not inhibited by P2 receptor antagonists, suggesting that P2 nucleotide receptors are not implicated. Taken together, these data suggest that two distinct mechanisms exist for nucleotide-induced potentiation of PTHR1 signaling. First, at micromolar concentrations, nucleotides amplify PTH-induced calcium release, through a mechanism dependent of P2 receptor activation. Second, at millimolar concentrations, nucleotides enhance PTH1R signaling independent of P2 receptors, through a heretofore-unrecognized allosteric interaction.

Title: Role Of Osteoblast Menin In Bone Metabolism: In-Vivo Studies Of Knockout Mice.

Jad Abi-Rafeh[1,2], Ildi Troka[1,2], Lucie Canaff[1,2] and Geoffrey N. Hendy[1,2]

1. *Department of Medicine, Division of Experimental Medicine, McGill University.*
2. *Metabolic Disorders and Complications Program, McGill University Health Center-Research Institute, Glen Site.*

Introduction: In humans, mutations in the MEN1 tumour suppressor gene cause the Multiple Endocrine Neoplasia Type 1 disorder. Menin, the product of the MEN1 gene, is predominantly a nuclear protein that also facilitates cell proliferation and differentiation control. Our previous in-vivo study illustrated the importance of menin for proper functioning of mature osteoblasts and maintenance of bone mass in adult mice. At present, we examine the role of menin at earlier stages in the osteoblast lineage.

Methods: This is implemented through the Cre-LoxP recombination system. Prx1-Cre;Men1f/f, Osx-Cre;Men1f/f and OC-Cre;Men1f/f mice represent knockout of the Men1 gene in the osteochondro progenitor, osteoblast progenitor as well as the mature osteoblast, respectively. Mice were sacrificed and analyzed at 6 months of age.

Results: Prx1-Cre;Men1f/f and Osx-Cre;Men1f/f mice were smaller than wild-type littermates. Bone mineral density and femur lengths were reduced in Prx1-Cre;Men1f/f and Osx-Cre;Men1f/f mice, but were unaffected in the OC-Cre;Men1f/f strain. By 3-dimensional micro-CT femur imaging, all 3 strains of knockout mice showed decreased trabecular bone volume, altered trabecular structure and decreased cortical bone thickness. Trabecular number was decreased, whereas trabecular spacing was increased in all cases. Femur biomechanics were compromised in the Prx1-Cre; Men1f/f mice as assessed by 3-Point Bending Test. Serum biochemistry profiles were largely unaffected, while ongoing histomorphometric analysis demonstrates an increase in osteoclast number and activity in these earlier menin knockout models. This is consistent with in-vitro findings supporting an increased osteoclastogenesis signaled by the menin knockout osteoblasts.

Conclusions: Osteoblast menin plays a crucial role in bone development in-vivo, and may serve as a potential gain-of-function therapeutic target for low bone mass disorders.

Title: Mechanically-Evoked Atp Release Is Regulated By Facilitated Membrane Resealing In Murine Osteoblasts

Nicholas Mikolajewicz[1,2] and Svetlana V. Komarova[1,2]

1. *Faculty of Dentistry, McGill University, Montreal, Canada*
2. *Shriners Hospital for Children, Montreal, Canada*

ATP release is one of the first events to occur in response to mechanical stimulation of mammalian cells. However, there is ongoing debate about the dominant pathways involved in the release of this ubiquitous signalling molecule. We mechanically-stimulated compact bone-derived osteoblasts through direct membrane deformation or turbulent fluid shear. Using an ATP bioluminescence assay, we demonstrated that ATP was released in response to mechanical stimuli. We next examined the contribution of vesicular exocytosis to mechanically-induced ATP release. Using confocal microscopy, we confirmed that acidophilic dye, quinacrine, and fluorescent ATP analog, MANT-ATP, co-localize within the same vesicles. Mechanical stimulation of single osteoblast evoked rapid exocytosis of quinacrine-positive vesicles. Activation of protein kinase C (PKC) with phorbol-12-myristate 13-acetate (PMA) significantly potentiated vesicular exocytosis, while a broad-spectrum PKC inhibitor, bisindolylmaleimide II (BIS), significantly reduced exocytosis. Unexpectedly, increase in vesicular release coincided with a significant decrease in ATP released, and vice versa. Since PKC-mediated vesicular release has been implicated in membrane resealing, we examined membrane integrity in mechanically stimulated cells. By applying a membrane impermeable dye, Trypan blue (TB), before and 5 min after the mechanical stimulation, we have found that the membrane integrity was compromised in cells immediately upon mechanical stimulation, however this effect was reversible within 5 min indicating active membrane resealing. PMA pre-treatment significantly reduced immediate TB uptake, while in cells treated with BIS, cells remained TB-permeable 5 min after mechanical stimulation. Our data supports a model in which (i) mechanical stimuli compromise the integrity of the cellular membrane, allowing ATP release into the extracellular space, (ii) PKC-dependent vesicular exocytosis is critical for membrane resealing and (iii) cytosolic ATP, rather then vesicular ATP, is the primary source of ATP released in response to mechanical stimulation.

SESSION II

CONNECTIVE TISSUE DISEASE CHARACTERISTICS: CLINICAL AND FUNDAMENTAL ASPECTS

INVITED LECTURE

Florina MOLDOVAN MD, PhD
Faculty of Dentistry, Université de Montréal

Dr Florina Moldovan (MD, PhD, Montreal, Canada) is a Full Professor at the Faculty of Dentistry at the Université de Montréal, and Researcher at the Sainte-Justine Hospital Research Center. She is an internationally recognized expert in molecular biology of bone, cartilage and genetics of pediatric scoliosis. Her research programme always ties together fundamental and clinical research. Her work has led to the development of a state-of-the-art research program centered on the role of the puberty and genes involved in etiology of spinal deformities in scoliosis. Dr. Moldovan's research discovered POC5 gene as a first causative gene of familial scoliosis. This work covers the science from all angles possible, i.e. genetic mapping and exome sequencing, cell biology, animal models, including knock down and gene overexpression in zebra fish. For this scientific contribution, in 2016 she received a MEDAL given by the Yves Cotrel Foundation from the Institut de France in 2016. Dr Moldovan's research work was also awarded the 2014 Grammer European Spine Journal Award for her major new insights in the study of scoliosis. At the international level, she is also contributing to the scoliosis research as an active Member of Eurospine Society, Member of North American Spine Society (NASS), Board Member of The International Research Society of Spinal Deformities (IRSSD) and of the International Consortium for Scoliosis Genetics (ICSG). She is also member of the Network for Oral and Bone Health Research (RSBO), Network of Applied Medical Genetics (RMGO) and Network for Canadian Oral Health Research (NCOHR) as well as several Canadian professional societies. Dr. Moldovan has published 58 papers and given over 80 presentations in international meetings. Dr Moldovan's Research interest is focused on etiology of scoliosis as well as growing spine, pain and inflammation, funded by the Yves Cotrel Foundation- institute de France, and The Canadian Institutes of Health Research (CIHR) and Scoliosis Research Society (SRS).

Elucidating the Role of POC5 in Idiopathic Scoliosis.

Idiopathic scoliosis is a three-dimensional spinal deformity with a prevalence of up to 3% in general population. In patients between 10 and 18 years of age, it is termed adolescent idiopathic scoliosis (AIS) which affects primarily young adolescent girls. Due to the phenotypic complexity and heterogeneity, the etiology of AIS remains unknown but several studies strongly suggest a multifactorial origin and contribution of several genetic factors. Genetic analyses have identified several candidate loci predisposing and/or associated with AIS, and finally, some rare gene variants contributing to the occurrence of AIS were found. We recently identified genetic variants in POC5 gene responsible for IS in families of French descent (from France and French-Canadian families) using whole exome sequencing, thus

unravelling a functional role of the POC5 gene in scoliosis (Patten et al, JCI 2015). The POC5 gene encodes for a centrosomal protein, interacting with centrin and inversin and is involved in cell division, polarity and motility. The pathogenicity of POC5 variants was examined in zebrafish studies and human osteoblasts collected form AIS patients during surgery. In vivo studies, in Zebrafish, the overexpression of human POC5 variants results in spinal deformity and rotation which is similar to the deformities that we usually observe in AIS patients. On the other hand, *in vitro*, in human cell lines, POC5 gene appears to have a significant role in centriole maturation and primary cilia formation that as a consequence would have a possible impact on neurosensory mechanotransduction and neurosensory control of posture. Interestingly, POC5 gene is strongly expressed in the zebrafish brain suggesting that idiopathic scoliosis may primarily result from a brain dysfunction. In the context of this highly heterogeneous disorder with possible multigenic inheritance for at least a subgroup of patients, identifying POC5 as one of the IS-causing is a major step towards deciphering the genetic causes and molecular pathways of AIS.

Title: Characterizing Degeneration Of Facet Joint Cartilage In Adolescent Idiopathic Scoliosis

Daniel Bisson [1,2], Polly Lama [1,2], Derek H. Rosenzweig [1], Fahad Abduljabbar [1], Jean A. Ouellet [1,2] and Lisbet Haglund [1,2]

1. *The Orthopaedics Research Laboratory, Department of Surgery, McGill University, Montreal, Qc*
2. *Shriner's Hospital for Children, Montreal, Qc*

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, mass spectrometry and arrays to assess proteoglycan content, cell density and secreted factors such as proteases and inflammatory cytokines. 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 compared to the control group. Interestingly, cell density within the cartilage varied significantly between the two facet joints of the same vertebra in AIS patients but was constant in healthy donors. Furthermore, inflammatory cytokines such as IL-6 and the proteases MMP1, MMP2, MMP3, Cathepsin D and HTRA1 were all elevated in scoliotic facet joints. These findings indicate that AIS patients suffer from early cartilage degeneration in the facet joints. Currently, we are working on 3D and finite element models of the scoliotic spine to calculate the abnormal biomechanical loading on the facet joints in order to assess the relation between the cartilage degeneration and altered biomechanics.

Title: Effect Of Tissue-Specific Extracellular Matrix On The Adipogenic And Chondrogenic Differentiation Of Human Adipose-Derived Stromal Cells

Danielle Heinbuch[1], Cody Brown[1], Dr. Lauren Flynn[1]

1. *University of Western Ontario*

The extracellular matrix (ECM) plays a critical role in mediating cell function. The hypothesis for this project was that tissue-specific ECM could be incorporated in culture models to promote the lineage-specific differentiation of human adipose-derived stem/stromal cells (ASCs).

METHODS: Cryo-milling methods were established to generate ECM particles from lyophilized human decellularized adipose tissue (DAT) and porcine decellularized cartilage tissue (DCT). The cells were cultured in the form of 3-D aggregates comprised of 2.5x105 ASCs ± 0.075 mg ECM (DAT or DCT) in adipogenic, chondrogenic, or proliferation (control) medium. Adipogenesis was analyzed at 14 days in terms of GPDH enzyme activity, perilipin immunohistochemistry (IHC), and RT-qPCR analysis of adipogenic gene expression, and chondrogenesis was assessed at 28 days by IHC analysis of collagen II/I/VI/X and RT-qPCR analysis of chondrogenic gene expression (n=3, N=3).

RESULTS: The addition of either type of ECM enhanced differentiation for both the adipogenic and chondrogenic lineages as compared to ASC-only aggregates, with higher levels of differentiation noted in the samples cultured in inductive medium. For the adipogenic lineage, GPDH enzyme activity was enhanced in the induced DAT+ASC samples as compared to all other groups. In addition, significantly higher levels of GPDH activity were observed in the non-induced DAT+ASC samples as compared to both ASC-alone conditions, suggesting that the DAT had an adipo-inductive effect. RT-qPCR results confirmed increased LPL and ADIPOQ expression in the DAT+ASC group as compared to DCT+ASC or ASCs-alone. For the chondrogenic lineage, IHC staining results suggested enhanced collagen II expression in the DCT+ASC samples. Gene expression studies supported that the DCT promoted ASC chondrogenesis, showing enhanced SOX9 and AGG expression in the DCT+ASC group.

SIGNIFICANCE: Overall, these findings support the rationale of using tissue-specific ECM as a cell-instructive platform to enhance lineage-specific stem cell differentiation for applications in soft connective tissue regeneration

Title: Characterization Of Proteins In The Specialized Basal Lamina Associated With The Tooth

Fouillen A[1,2], *Dos Santos J*[1], *Mary C*[2], *Moffatt P*[3], *Baron C*[2] *And Nanci A*[1,2]

2. *Laboratory for the Study of Calcified Tissues and Biomaterials, Department of Stomatology, Université de Montréal, Montréal, H3T 1J4, Québec, Canada*
3. *Department of Biochemistry and Molecular Medicine, Université de Montréal, Montréal, H3T 1J4, Québec, Canada*
4. *Shriners Hospital for Children, Montréal, H4A 0A9, Québec, Canada.*

A specialized basal lamina (sBL) mediates adhesion of maturation stage ameloblasts and junctional epithelial cells to the tooth surface. It is distinct because it does not contain collagens type IV and VII, is enriched in laminin-332, and includes three novel constituents called amelotin (AMTN), odontogenic ameloblast-associated (ODAM), and secretory calcium-binding phosphoprotein proline-glutamine rich 1 (SCPPPQ1). The objective of this study was to clarify the structural biology of the sBL. Fluorescence and immunogold labeling showed that they co-localize. Quantitative analysis of the relative position of gold particles on the sBL demonstrates that the distribution of ODAM is skewed towards the cell while that of AMTN and SCPPPQ1 tends towards the tooth surface. Bacterial two hybrid analysis and co-immunoprecipitation, gel filtration of purified proteins and transmission electron and atomic force microscopies highlight the propensity of AMTN, ODAM, and SCPPPQ1 to interact with and among themselves and to form supramolecular aggregates. Together, the three proteins assemble into a reticular network reminiscent of the structural organization of BLs. Proteolytic and hydroxyapatite profiles of the proteins were also analysed, and revealed distinctive behaviour. These data suggest that AMTN, ODAM and SCPPPQ1 participate in structuring an extracellular matrix with the distinctive capacity to attach to epithelial cells to mineralized surfaces. This unique feature is particularly relevant for the adhesion of gingival epithelial cells to the tooth surface, which forms a protective seal that is the first line of defense against bacterial invasion.

Title: Use Of Unfixed Histology To Visualize And Characterize Meniscus Inter-Tie Coil Structures In Intact Knees And Knees Following Surgical Procedures

Colleen Mathieu[1], Jessica Guzmán-Morales [1], Dongfang Qian [1], Anik Chevrier [1], Robert McCormack [2], Mark B. Hurtig [3], Caroline D. Holmann [1].

1. *Ecole Polytechnique de Montreal, Montreal, QC,*
2. *University of British Columbia, Vancouver, BC, 3-University of Guelph, Guelph ON, Canada*

INTRODUCTION Organized patterns of large collagen tie fibers and inter-tie fibers in the extracellular matrix help maintain meniscal integrity and stability. The purpose of this study was to use unfixed cryosections to characterize inter-tie coils, a structure that is lost during standard histoprocessing. We tested the hypothesis that coil structure is influenced by knee surgery.

METHODS Menisci from rabbits N=3 intact, N=3 post-intra-articular (IA)-injection with saline or IL-1b, N=5 at 6-weeks post-microdrilling, and N=1 sheep were obtained following ethics committee-approved protocols. Samples were either fixed in formalin or processed unfixed by rapid sucrose infiltration then cryoembedded. Cryosections were stained with Safranin-O/Fast Green and H&E. Coils were imaged by light and polarized light microscopy and coil diameter measured by quantitative histomorphometry. Sections were also incubated 30 minutes with or without Clostridium collagenase type I or hyaluronidase. Statistical differences were tested by repeated measures ANOVA.

RESULTS Collagen tie fibers were observed in both unfixed and fixed tissue sections, but inter-tie coils were only visible in unfixed sections. Coils were observed in regions of the meniscus with low or no GAG deposition and their diameter measured 8 to 9 μm for normal rabbit and sheep meniscal tissue. Loss of birefringence and coil morphology resulted from digestion with collagenase, but not hyaluronidase, suggesting that coils contain collagenous structures. Average coil diameter was comparable after knee surgery, however one outlier had 15.5 μm diameter coils in a knee exhibiting mild pain upon flexion and another had 5 μm coils in a meniscus with evidence of ectopic calcification.

CONCLUSION Data in this study motivate the recommendation that meniscus histoprocessing includes unfixed tissue to study inter-tie coils that are expected to have an important biomechanical function. Our data also suggest a previously unsuspected link between abnormal coil morphology and knee pain.

Title: Role Of Phex-Fgf23 Axis And γ-Carboxylation On Vascular Calcification Caused By Mgp Deficiency

Abhinav Parashar[1], Juliana Marulanda[1] and Monzur Murshed[1,2]

1. *Faculty of Dentistry, McGill University, Montreal, Quebec, Canada*
2. *Department of Medicine, McGill University, Montreal, Quebec, Canada*

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, is involved 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 PHEX-FGF23 axis on the vascular calcification phenotype in MGP-deficient mice.

2) To investigate the functional roles of the Gla residues in MGP.

Results: We crossed Mgp+/- mice with the ApoE-Fgf23 transgenic mice to generate Mgp-/-;ApoE-Fgf23 mice. As is the case with the PHEX-deficient mice, these mice show high levels of circulating FGF23 and hypophosphatemia. Interestingly, Alizarin red staining and histological analyses showed that unlike Mgp-/- mice, at 4 weeks of age, these mice do not show any sign of vascular calcification. In a separate experiment, we generated Mgp- /-;SM22Glamut mice, which do not express the endogenous MGP, but express in the VSMCs a mutant form lacking the conserved Gla residues. Surprisingly, these mice never developed vascular calcification.

Conclusions: PHEX-FGF23 axis is a major regulator of vascular calcification in Mgp-/- mice and the known Gla residues are dispensable for MGP's anti-mineralization function.

Funding Support: Canadian Institutes of Health Research (CIHR)

Title: Male Mice Are More Susceptible To Whole-Body Vibration Induced Joint Damage Than Age-Matched Female Mice

Geoffrey J. Kerr [1,4], Matthew R. McCann [1,4], Maham Bushra [2], Michael A. Pest [1,4], David W. Holdsworth [3,4], Frank Beier [1,4], S. Jeffrey Dixon [1,4], and Cheryle A. Séguin [1,4]

1. *Department of Physiology and Pharmacology; Schulich School of Medicine and Dentistry, The University of Western Ontario, London, Ontario, Canada, N6A 5C1*
2. *Department of Pathology and Laboratory Medicine; Schulich School of Medicine and Dentistry, The University of Western Ontario, London, Ontario, Canada, N6A 5C1*
3. *Department of Medical Biophysics; Schulich School of Medicine and Dentistry, The University of Western Ontario, London, Ontario, Canada, N6A 5C1*
4. *Bone and Joint Institute, The University of Western Ontario, London, Ontario, Canada, N6A 5C1*

Introduction: Whole-body vibration (WBV) platforms are used in the fitness industry and clinically as potential treatments for numerous musculoskeletal conditions based on their reported ability to increase bone mineral density and muscle strength. Despite widespread use, there is an alarming lack of understanding of the direct effects of WBV on joint health. Previous work by our lab demonstrated that repeated exposure to WBV, using protocols that model those used clinically, induces intervertebral disc degeneration and osteoarthritis-like damage in the knee of skeletally mature, male CD-1 mice. Based on previous research illustrating gender differences in the development of post-traumatic osteoarthritis, as well as differences in the reported effects of WBV based on sex, genetics and age, this study aimed to determine whether the effects of WBV on joint health would differ based on animal gender. **Hypothesis:** The effects of WBV on joint health will differ between age-matched male and female mice. Methods: Six-month-old male and female wild-type mice (CD-1) were exposed to vertical WBV for 30 min/day, 5 days/week, for 8 weeks using protocols that model those used clinically (45 Hz, 0.3 g peak acceleration). Following WBV, joint tissues were examined through histological, radiological, and molecular analysis.

Results: Histological analysis of the knee suggest that female mice are less susceptible to WBV induced joint damage than age-matched male counterparts. Following 8 weeks of repeated exposure to WBV, male mice showed extensive damage in the knee joint. In contrast, following exposure to the same parameters of WBV, female mice did not show signs of joint damage.

Conclusions: Female mice exposed to WBV showed considerably less damage to the knee joint than their male counterparts. In the context of the diverse human population currently using WBV platforms these findings highlight the need for comprehensive studies evaluating the safety of WBV platforms.

SESSION III

BONE AND CARTILAGE: GENETIC AND DEVELOPMENTAL ASPECTS

INVITED LECTURE

Rebekah S. DECKER PhD
Investigator
Genomics Institute of the Novartis Research Foundation

Dr. Rebekah S. Decker obtained her PhD in Veterinary Science at the University of Kentucky, where she utilized the axolotl salamander as a novel vertebrate model for articular cartilage maturation and repair. She was awarded an individual NIH Kirschstein Post-Doctoral Fellowship to study with Dr. Maurizio Pacifici in The Translational Research Program in Pediatric Orthopaedics at the Children's Hospital of Philadelphia. Dr. Decker's research has focused on developmental mechanisms guiding articular cartilage morphogenesis. She was recognized as an Outstanding Young Investigator by the Osteoarthritis Research Society International in 2013 and 2014. In late 2016, Dr. Decker started a new position as an Investigator with The Musculoskeletal Regenerative Medicine group at The Genomics Institute of the Novartis Research Foundation in La Jolla, CA.

Building articular cartilage: A new blueprint

Synovial joints display distinct tissues and diverse morphologies, but it has long remained unclear which progenitors produce these tissues and how they acquire their unique architecture. At birth, the articular cartilage is a thin tissue composed of small chondrocytes within an isotropic matrix. Over time, the tissue attains its functional postnatal structure and thickness characterized by chondrocyte columns, zone-specific cell volumes and an anisotropic matrix. Recently, novel mouse models have been developed to characterize progenitor cell origin and assess how genetically traced embryonic or adult progenitors participate in postnatal joint morphogenesis and respond to acute joint injury.
Using novel Gdf5CreERT2 (Gdf5-CE), Prg4-CE and Dkk3-CE mice mated to R26-Confetti or single-color reporters, we found that joint tissues are produced locally by progenitors born at different locations within the developing joints. Chondrocyte columns present in mature knee articular cartilage consisted of non-daughter, partially overlapping lineage cells. Taken together, these data indicate that articular cartilage mainly grows by cell volume expansion and local cell rearrangement rather than appositional growth as proposed previously. Lineage tracing of cells labeled at prenatal or juvenile stages showed that joint injury in adults provoked a massive and rapid increase in synovial Prg4+ and CD44+/P75+ cells, which appear exquisitely responsive to acute injury and may represent pioneers in joint tissue repair. The data and insights gained by these studies greatly advance current basic knowledge of joint biology and can pave the way toward the design of informed, directed and specific therapies to repair or regenerate articular cartilage and other tissues during chronic or acute joint conditions.

Title: Identifying Gli Transcriptional Targets In Chondrosarcoma Using Next Generation Sequencing

***Ali SA*[1]*, Niu B*[2]*, Cheah KS*[2] *and Alman BA*[3]

1. *University Health Network, Canada*
2. *University of Hong Kong, Hong Kong*
3. *Duke University, USA*

Objective: Excessive Hedgehog signaling in chondrocytes is sufficient to cause formation of enchondroma-like lesions in mice, which can progress to chondrosarcoma. To elucidate mechanisms through which activation of Hedgehog signaling contributes to cartilage tumor formation, we identified Gli1 and Gli2 target genes in primary human chondrosarcoma using next generation sequencing.

Methods: Chromatin immunoprecipitation (ChIP) sequencing and microarray (chip) were used to identify genes that were bound by the transcription factors Gli1 and/or Gli2, and that changed in expression with Hedgehog signaling modulation. In silico analyses were conducted to identify and characterize Gli1 and Gli2 binding regions, including de novo motif analysis, co-localization with additional transcription factors, distance to transcriptional start site, and conservation between human and mouse. Supervised and unsupervised analyses of biological pathways and processes represented among putative Gli transcriptional targets were conducted.

Results: Gli1, Gli2, and Gli1/Gli2 binding regions were identified using ChIP-sequencing data. These were classified based on the presence or absence of the Gli-consensus binding motif, and de novo motif analysis identified additional transcription factor binding motifs in each fraction, suggesting interaction or competition with Gli, within a 250bp distance. Microarray data from human chondrosarcoma were overlapped onto Gli binding regions and revealed 204 upregulated genes and 106 downregulated genes. Unsupervised GO term analysis showed significant upregulation of genes involved in blood vessel development and the MAPK signaling pathway. ChIP-on-chip data from mouse limb bud chondrocytes were overlapped onto these data and revealed 48 genes, including VEGFA, that were common targets of mouse Gli1 or Gli3 and human Gli1 or Gli2 within 250bp regions of evolutionarily conserved DNA.

Conclusion: Our results suggest that Hedgehog signaling directly impacts pathways involved in angiogenesis in human chondrosarcoma. Therapeutic strategies to target Hedgehog-regulated angiogenesis in enchondromas and chondrosarcomas may reduce metastatic tumor formation and growth.

Title: Undercarboxylated Osteocalcin And Metabolic Phenotype In The Mouse Model Of Osteogenesis Imperfecta

Iris Boraschi-Diaz[1,2], Josephine T. Tauer[1], Omar El Rifai[3], Delphine Guillemette[1,4], Geneviève Lefebvre[4], Frank Rauch1, Mathieu Ferron[3,5], Svetlana V. Komarova[1,2]

1. *Shriners Hospital for Children-Canada, Montreal, Quebec, Canada.*
2. *Faculty of Dentistry, McGill University, Montreal, Quebec, Canada*
3. *Unité de recherche en physiologie intégrative et moléculaire, Institut de Recherches Cliniques de Montréal, Montréal, Québec, Canada*
4. *Département de mathématiques, Université du Québec à Montréal, Montréal, Québec, Canada.*
5. *Départements de Médecine et de Biochimie et Médecine Moléculaire, Université de Montréal, Montréal, Québec, Canada*

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. Osteocalcin is now recognized as a bone-derived regulator of insulin secretion and sensitivity and glucose homeostasis. Since OI is associated with increased rates of bone formation and resorption, we hypothesized that the levels of undercarboxylated osteocalcin are increased in OI. The objective of this study was to determine changes in osteocalcin and to elucidate the metabolic phenotype in the Col1a1Jrt/+ mouse, a model of dominant OI caused by a Col1a1 mutation. Circulating levels of undercarboxylated osteocalcin were higher in 4-week old OI mice and normal by 8 weeks of age. Young OI animals exhibited a sex-dependent metabolic phenotype, including increased insulin levels in males, improved glucose tolerance in females, lower levels of non-fasted glucose and low adiposity in both sexes. The rates of O2 consumption and CO2 production, as well as energy expenditure assessed using indirect calorimetry were significantly increased in OI animals of both sexes, while respiratory exchange ratio was significantly higher in OI males only. While OI mice have significant physical impairment that may contribute to metabolic differences, we specifically accounted for movement and compared OI and WT animals during the periods of similar activity levels. Taken together, our data strongly suggest that OI animals have alterations in whole body energy metabolism that are consistent with the action of undercarboxylated osteocalcin.

Title: Biological Repair Of Osteoarthritic Cartilage Using Short Link N

Muhammad Albesher[1,2], Michael P Grant[1,2], Laura M Epure[1], John Antoniou[1,2], Fackson Mwale[1,2]

1. *Lady Davis Institute for Medical Research, Montreal, QC, Canada,*
2. *McGill University, Montreal, QC, Canada*

INTRODUCTION: Osteoarthritis (OA) is a chronic degenerative joint disorder. In OA, there is an imbalance in the anabolic and catabolic activities of chondrocytes, favoring cartilage catalysis. The principle inflammatory cytokine involved in cartilage degradation is interleukin 1beta (IL-1beta). In order to stimulate the repair of OA affected joints, the balance between the production of extracellular matrix proteins and their degradation needs to be altered. The most common pharmacologic therapeutic agents used for treating OA are primarily palliative. There is currently no medicinal treatment intended to cure or reverse the course of the disease. It has previously been demonstrated that Link N (DHLSDNYTLDHDRAIH), a 16 residue peptide derived from stromelysin cleavage of link protein, can stimulate the production of aggrecan and collagen in normal cartilage and human intervertebral discs. Recently, we have demonstrated a shorter active sequence of Link N, short Link N (sLink N, DHLSDNYT), with enhanced properties at repairing degenerative intervertebral discs. In the present study, we aimed to evaluate the potential of sLink N as a therapeutic in the repair of articular cartilage.

METHODS: Articular cartilage was isolated from four donors undergoing total knee replacement (range age 50 – 70 years). Cells were isolated and and expanded in PrimeGrowthTM culture medium (Wisent Bioproducts, Canada; Cat# 319-510-CL, -S1, and -S2) and treated with sLink N (0.5, 5, 50, 500 or 5000 ng/ml) or sLink N in combination with IL-1beta (1 ng/ml) for 24h. Human articular cartilage explants were prepared from the same donors, and included cartilage with subchondral bone, using the PrimeGrowth Media Isolation kit (Wisent Bioproducts, Canada) and cultured for 21 days in growth medium containing IL-1beta (1ng/ml) and sLink N (0.5, 5, 50, 500 or 5000 ng/ml).

RESULTS: Treatment of OA chondrocytes significantly increased the synthesis of proteoglycan and Col II. The EC50 dose-response of sLink N on proteoglycan synthesis was 67 ± 41 nM [65 ± 40 ng/ml]. Proteoglycan synthesis reached a maximum of 194 ± 30% with the highest dose (5000 ng/ml) above control. When chondrocytes were cultured in the presence of IL-1beta to mimic an inflammatory milieu, GAG synthesis was also elevated by sLink N above control. Treatment of OA cartilage explants with sLink N increased the content of aggrecan and Col II even in the presence of IL-1beta.

DISCUSSION: Our results suggest that sLink N is a non-toxic, growth factor-like supplement that can increase cartilage matrix protein synthesis, and a chondroprotective agent, by modulating the catabolic effects of IL-1beta. sLink N is the first small-peptide to demonstrate potential in cartilage repair of OA joints.

SESSION IV

CONNECTIVE TISSUE REPAIR AND FIBROSIS: CELLULAR AND MOLECULAR ASPECTS

Title: Toll-Like Receptor Activation Induces Human Intervertebral Disc Degeneration

Emerson Krock[1,2], Jean A. Ouellet[2,3], Lisbet Haglund[1-3]

1. *Orthopedic Research Lab and*
2. *McGill Scoliosis and Spine Group, Department of Surgery, McGill University;*
3. *Shriner's Hospital for Children, Montreal, Canada*

Introduction: Intervertebral disc degeneration is a leading etiology of chronic low back pain. Degeneration is characterized by extracellular matrix (ECM) degradation, and cytokine, protease and neurotrophin increases. However, the early stages of degeneration are poorly understood. Evidence indicates toll-like receptor 2 (TLR2) contributes to degeneration. TLR2 is activated by endogenous ligands found in discs, such as fragmented hyaluronic acid, aggrecan, and fibronectin. Nucleus pulposus (NP) cells of non-degenerating discs express TLR2 and activation increases cytokines, proteases and neurotrophins. Therefore we hypothesize TLR2 activation will induce disc degeneration.

Methods: Discs from human organ donors lacking signs of degeneration were used. Three discs per spine (n=7 spines) were isolated and injected with either PBS, Pam2CSK4 (TLR2 agonist) or 30kDa fibronectin fragments (FN-f, TLR2 or 4 agonist). Discs were cultured ex vivo for 28 days and media was changed every 3-4 days. On day 28 tissue protein was extracted using a 4M GuHCl buffer. Glycosaminoglycan (GAG) levels were analyzed by dimethylmethylene blue assay. MMP 3 and 13 were analyzed by ELISA and western blot. Conditioned media was analyzed by mass spectrometry and cytokine protein arrays.

Results: TLR2 agonist or FN-f injection decreased GAG content in the NP and increased GAG release into culture medium compared to PBS injected discs. MMP 3 and 13 secretion increased following TLR2 agonist or FN-f injection. MMP13 also increased in the NP of discs injected with TLR2 agonists. Agonists increased proinflammatory cytokine release, including TNFα, IL-1 and IFN-γ, compared to PBS. Mass spectrometry found discs injected with agonists secreted increased levels of proteases and ECM components compared to PBS.

Conclusions: TLR2 activation induces degenerative changes characterized by decreased GAG content and increased cytokine and protease production. Therefore, inhibition of TLR2 during early stages of disc degeneration could slow degeneration and prevent the development of chronic low back pain.

Title: Investigating The Role Of Pannexin 3 As A Regulator Of Intervertebral Disc Health

Meaghan E. Serjeant, Paxton M. Moon, Silvia Penuela, Dale W. Laird, Frank Beier and Cheryle A. Séguin

- *Department of Physiology and Pharmacology, Schulich School of Medicine and Dentistry, Bone and Joint Institute, University of Western Ontario*

Our ability to treat intervertebral disc (IVD) degeneration is hampered by an incomplete understanding of the processes that regulate IVD development, function and disease. Recent work by our group has shown that deletion of pannexin 3 (Panx3), a channel-forming glycoprotein, provides protection against surgically-induced osteoarthritis in mice. Although Panx3 expression is detected in the IVD, its function here remains unknown. The aim of this study is to determine the tissue-specific localization of Panx3 and to characterize its role in IVD tissues during age-related degeneration.

We investigated expression of Panx3 in IVD tissues. Microarray analysis of tissues from 2 month-old mice detected equivalent expression levels of Panx1 and Panx2 in the nucleus pulposus (NP) and annulus fibrosus (AF); in contrast Panx3 showed 29-fold increased expression in the AF. Enrichment of Panx3 in cells of the AF was confirmed by immunohistochemistry. Using the global Panx3 KO mouse model, we then investigated whether loss of Panx3 altered age-associated IVD degeneration. Lumbar spines from WT and KO mice were examined at 18 and 24 months-of-age and IVDs were assessed for signs of degeneration using histopathological criteria. The expression of anabolic and catabolic genes was quantified in thoracic IVDs using RT-qPCR. Histological evaluation showed no differences in IVD tissue morphology between WT and KO mice at either time point, however; a trend towards decreased degeneration was noted in 18 month-old KO mice. In keeping with this trend, a significant decrease in expression of markers of IVD degeneration was detected in KO mice at 18 months.

Our data suggest that deletion of Panx3 may delay the early stages of age-associated IVD degeneration; future studies will include mice at earlier timepoints. These studies will improve our understanding of the molecular mechanisms that regulate IVD function and disease, with the goal of identifying therapeutic targets for IVD degeneration.

Title: Functional Relevance Of Fibulin-4 Interactions With Latent Transforming Growth Factor Beta Binding Protein- 4 In The Context Of Fibronectin And Fibrillin-1

Heena Kumra[1*], Valentin Nelea[2*], Amelie Pagliuzza[1], Hana Hakami[1], Dieter P. Reinhardt[1,2]

1. *Faculty of Medicine and*
2. *Faculty of Dentistry, McGill University, Montreal, Canada*

*Co-first authors

Background: Elastogenesis is an intricate and well-orchestrated cellular mechanism which involves several extracellular matrix (ECM) proteins such as Fibulin-4 (FBLN4), Latent TGF-β binding protein-4 (LTBP4), Fibronectin (FN) and Fibrillin-1 (FBN1). These proteins play key roles in driving the assembly of tropoelastin into mature elastic fibers, which are essential for the elasticity of various soft tissues including skin and blood vessels.

Rationale: The aim of this study is to determine the structural and functional role of interaction of these ECM proteins in facilitating elastogenesis.

Methods and Results: Surface plasmon resonance spectroscopy (SPR) showed that recombinantly expressed LTBP4L (long isoform of LTBP4) and FBLN4 could each self-interact *in vitro*. Immunofluorescence demonstrated that both proteins assemble in cell culture. Deletion of FN using fibroblasts from *Fn* knockout mice disrupts the assembly of FBLN4 and LTBP4. To analyze if the dependency of FBLN4 and LTBP4 on FN is direct or is dependent on other ECM proteins, which require FN for their assembly, fibroblasts from *Fbn1* knockout mice were utilized. Knocking out *Fbn1* (FN assembly is still intact) disrupts the assembly of LTBP4 but not of FBLN4. This data shows direct dependency of FBLN4 on FN for its assembly, whereas LTBP4 requires FBN1, which in turn is dependent on FN for its assembly. Next, we observed by SPR that FBLN4 interacts with LTBP4. This interaction induced an unexpected conformational change in the structure of LTBP4, as revealed by atomic force microscopy. Furthermore, this conformational change triggered an increased binding of LTBP4 to FBN1 but a decreased binding to FN, as shown by solid phase assays. Immunofluorescence analyses revealed that the conformational change in LTBP4 induced by FBLN4 also affected LTBP4 assembly/deposition in cell culture.

Conclusions: These results highlight new interactions between the elastogenic proteins FBLN4 and LTBP4, their structural and functional consequences, and their dependency on FN and FBN1. These data provide a new paradigm of how elastic fibers assemble.

SESSION IV

BONE AND CARTILAGE DISEASE: TRANSLATIONAL AND CLINICAL ASPECTS

Title: Synovial Membrane Nerve Fiber Density Decreases With Osteoarthritis In Horses

Raymond PUJOL, Christiane GIRARD, Hélène RICHARD, Jason MCDOUGALL, Sheila LAVERTY

- *Comparative Orthopedic Research Laboratory, Department of Clinical Sciences, University of Montreal, St. Hyacinthe, Canada*

Osteoarthritis (OA) is a common cause of joint pain in both humans and horses. The tissue origin and mechanisms of pain generation in OA remain poorly understood. In recent years a correlation between pain and synovitis has been reported in human patients on MRI imaging studies. The horse is considered a model for both experimental and naturally occurring post-traumatic osteoarthritis.

We hypothesized that the innervation of the equine synovial membrane is altered in OA joints. The objectives of the study were to measure the nerve fiber density in normal equine joints and investigate the relationship between nerve fiber density, synovitis and OA.

Twenty-five equine metacarpo-phalangeal joints were collected post-mortem. The joints were dissected and the macroscopic lesions of the articular cartilage were scored. Synovial membrane samples (n = 50 samples) were harvested and fixed. Histological sections of the synovium were scored to provide a synovitis score. Immunohistochemical staining with S-100 protein, that identifies nerve fibers, and **α**-actin that stains blood vessels was also performed on site-matched specimens. The density of the nerve fibers in the synovium was correlated with the macroscopic OA score and synovitis score.

The nerve fiber density was higher ($p=0.006$) in the superficial layer ($\leq 200\mu m$) of the synovium when compared to the deeper layer in healthy equine joints. However, the synovial innervation significantly decreased ($p=0.009$) in the superficial layer with increased macroscopic OA score. An increased nerve fiber density in the deep layer was positively correlated ($p=0.06$) with vascular proliferation.

The decrease in nerve fiber density with increasing OA score suggests that neuropathic pain could have a role in joint pain symptoms in OA.

Title: Lactosylceramide Enhances Chondrocytic Differentiation

Lilit Antonyan[1], Corine Martineau[1], Rene St-Arnaud[1,2]

1. *Research Centre, Shriners Hospitals for Children*
2. *McGill University*

When studying bone healing in Cyp24a1-deficient mice, which cannot synthesize the vitamin D metabolite, 24,25(OH)2D, we have measured a significant impairment in callus formation during fracture repair. The callus formation defect can be corrected by exogenous administration of 24,25(OH)2D. From the callus of Cyp24a1-null mice, we cloned Fam57b2, encoding a transmembrane protein that specifically interacts with 24,25(OH)2D. Mice deficient for FAM57B2 in chondrocytes exhibit the same impaired callus formation during fracture repair than Cyp24a1-deficient mice, providing genetic evidence that FAM57B2 is an effector of 24,25(OH)2D-mediated signaling during fracture repair. In addition to transmembrane motifs, FAM57B2 contains a domain related to acyl-CoA-dependent ceramide synthase, suggesting potential enzymatic activity. Ceramide serves as a substrate to produce glycosphingolipids through glucosylceramide and the key branching intermediate, lactosylceramide (LacCer).

LacCer was shown to act as a signaling molecule that regulates several aspects of cellular function. We have measured 24,25(OH)2D-dependent production of LacCer by FAM57B2, suggesting allosteric regulation of the enzymatic activity by the vitamin D metabolite. We hypothesized that LacCer acts as a second messenger molecule in chondrocytes to optimize fracture repair. To determine the potential effect of LacCer on chondrocytes, we used ATDC5 cells cultured in media containing insulin, transferrin, and selenium (ITS) for differentiation. We treated differentiated cultures of ATDC5 cells with 10 uM of LacCer and measured cellular responses. Treatment with LacCer had no effect on adhesion, migration or apoptosis of the cells. When assessing differentiation using gene expression monitoring by RT-qPCR, we measured a reproducible two-fold enhancement of the expression of the differentiation marker Type X collagen over the levels induced by ITS treatment alone. A similar effect was measured when ATDC5 cells were stably transfected with an expression vector for Fam57b2. Our results show that LacCer enhances chondrocytic differentiation and future experiments will aim to characterize the cellular pathways involved.

Title: Novel Electromechanical Arthroscopic Device For The Objective And Quantitative Evaluation Of Cartilage Quality

Insaf Hadjab[1,2], Sotcheadt Sim[1,2], Martin Garon[2], Eric Quenneville[2], Michael D.Buschmann[1]

1. *Institute of Biomedical Engineering, Polytechnique Montreal*
2. *Biomomentum Inc.*

Purpose: Arthroscopy is a gold standard procedure to gain deeper and direct assessment of cartilage integrity when used in combination with macroscopic visual classification systems. Unfortunately, their outcomes remain qualitative and subjective. To overcome these limitations, a quantitative assessment device is needed. Cartilage's streaming potential is a compression-induced electrokinetic phenomenon. It results from mobile positive ions in the interstitial fluid moving relative to fixed negative charges on proteoglycans. This phenomenon, reflecting cartilage electromechanical properties, is a reliable indicator of its integrity. The purpose of this study was to develop a novel diagnostic version of the electromechanical arthroscopic device for objective and quantitative cartilage evaluation.

Methods: We obtained institutionally ethical approvals (RTI-Surgical, Polytechnique-Montreal and MR-Hospital) for protocols allowing the collection of 200 human knee articular surfaces from cadaveric donors and osteoarthritic patients. Samples were assessed with the electromechanical probe (Biomomentum) which measures a quantitative parameter reflecting streaming potentials. Samples were distributed along several studies, in collaboration with different research teams, to establish the relationships of electromechanical properties regarding either conventional (histology, mechanics and biochemistry) or clinical (μCT&MRI-gagCEST) techniques outcomes and to evaluate the sensitivity and specificity of this novel device. Finally, an electromechanical database was built and statistical modeling was performed to conceive an electromechanical grading system representative of cartilage integrity.

Results: Electromechanical properties correlated significantly with conventional and clinical outcomes. ROC-curves and effect size analyses revealed better sensitivity and specificity to distinguish between various levels of cartilage degeneration with the electromechanical probe than with any other conventional or clinical outcome. Lastly, the newly developed electromechanical grading system provided a more accurate and sensitive grading of cartilage compared to widely used visual arthroscopic scoring.

Conclusion: This project led to successful development of a novel objective and quantitative electromechanical grading system. Clinical applications requiring decision making or monitoring of cartilage integrity could benefit greatly from this device. Also, it could be useful in evaluating cartilage treatments and repair strategies.

INVITED SPEAKER

V. A. Shiva AYYADURAI, PhD
Chairman and CEO, CytoSolve, Inc.

Title: Mechanistic Modeling: The Pathway to Precision Medicine

There is a growing and critical need for integrating molecular systems science with computation to model complex disease processes for accelerating drug discovery, drug repurposing, validation of complementary and alternative medicine (CAM) therapies, and identification of efficacious multi-combination therapeutics, while ensuring a personalized and precise medicine. Such needs cannot be advanced without collaborative integration of knowledge across biological disciplines. This talk will share the recent successes, through multiple case studies, in the use of CytoSolve, a computational systems biology collaboratory, initially developed at M.I.T., that provides an integrative approach to address these critical needs.

Previous approaches, largely based on statistical techniques or "big data", have been unscalable and largely useless to scientists who seek to understand complex biological mechanisms. CytoSolve's successes have been published and cited in peer-reviewed journals such as Nature, CELL, IEEE, and others for its potential to develop multi-combination therapies. These successes including: FDA allowance for a multi-combination pancreatic cancer therapeutic; the Department of Defense (DoD) and the United States Pharmacopeia (USP) understanding of toxicity and adverse reaction multi-combination nutritional supplements; and, modeling of rare diseases in orphan drug domains such as Neuromyelitis Optica (NMO) and Hereditary Angioedema (HAE) have inspired renowned scientists and researchers at pharmaceutical companies, research centers at universities such as MD Anderson, Harvard, UHN, MGH and others to use CytoSolve to model complex biological phenomena and diseases to accelerate the development of multi-combination therapeutics. This talk will provide an introduction to a disruptive platform that will likely revolutionize development of therapeutics in the 21st century.

Banquet
May 19 (6:00pm - 9:00pm)
(Ticketed Event)

Venue
McGill La Citadel
(26th Floor Lounge)
410 Sherbrooke St W, Montreal, QC. H3A 1B3

Plenary Talk

"Mechanistic Modeling: The Pathway to Precision Medicine"
V. A. Shiva Ayyadurai, Chairman and CEO, CytoSolve, Inc.

Dr. V.A. Shiva Ayyadurai, the inventor of email and polymath, holds four degrees from MIT and is a world-renowned systems scientist. He is a Fulbright Scholar, Lemelson-MIT Awards Finalist, First Outstanding Scientist and Technologist of Indian Origin (STIO), Westinghouse Science Talent Honors Award recipient, and was nominated for the U.S. National Medal of Technology and Innovation. In 1982, the US government recognized Ayyadurai as the inventor of email by awarding him the first Copyright for "Email" at a time when Copyright was the only way to protect software inventions. His interest in human health also began early, when as a child, he observed his grandmother, a village farmer and healer, practice Siddha, India's oldest system of traditional medicine. This motivated his future study and research in systems biology at MIT, leading to his discovery of Systems Health®, a major breakthrough that provides an integrative framework linking eastern and western medicine. His latest invention CytoSolve®, emerging from his doctoral research at MIT, provides a revolutionary platform for modeling complex biological phenomena, to support the development multi-combination medicines without animal testing.

SESSION V

TISSUE REPAIR, REPLACE OR RE-ENGINEER

KEYNOTE LECTURE

Christopher McCULLOCH, B.Sc., D.D.S., Ph.D., F.R.C.D(C)
Professor, Director of the Matrix Dynamics Group, University of Toronto

Christopher McCulloch is the Canada Research Chair and Professor and Director of the Matrix Dynamics Group at the University of Toronto. He focusses on the biology of the extracellular matrix and how the matrix regulates cellular responses in wound healing, inflammatory and fibrotic conditions. Dr. McCulloch is a recognized leader in the field of matrix biology as indicated by continuous funding from the Canadian Institutes of Health Research and other federal agencies in Canada for the last 30 years and the publication of more than 280 peer-reviewed publications in high ranking journals. He has made seminal contributions to the field including the role of the extracellular matrix in in shaping cellular responses of fibroblasts to diabetes and to inflammatory mediators. Dr. McCulloch has also made important contributions in the structure and regulation of the cytoskeleton and how the cytoskeleton affects signaling processes in fibroblasts and contributes to transmission of biophysical signals that alter cellular signaling pathways.

Connective Tissue Signaling Through Cell Adhesions in Matrix Homeostasis and Fibrosis

Fibrosis is a high prevalence, progressive and intractable condition that culminates in end-stage organ failure. The increasing global incidence of fibrosis is associated with age and multiple chronic inflammatory diseases, such as diabetes, heart failure, kidney disease, periodontitis, and cancer. Fibrosis is characterized by excessive build-up of a densely collagenous extracellular matrix with altered mechanical and molecular properties. This pathological matrix comprises the scar tissue that obstructs organ function. Despite intensive investigation on how matrix remodeling is perturbed in fibrotic diseases, the control systems that mediate fibrosis are complex and not well defined. Notably, the adhesions of connective tissue cells to the underlying extracellular matrix comprise critical signaling networks. These networks are molecular targets for the dysregulated matrix signaling seen in fibrotic disease.

Here I will describe a new, cell adhesion-based signaling system that regulates collagen remodeling by phagocytosis and pericellular proteolysis. I will use the discovery of this signaling system to demonstrate the central role of cell adhesions in matrix homeostasis and fibrotic disease. This signaling system involves the Transient Receptor Potential Vanilloid-4 channel, which is recruited to adhesions upon cell attachment to the matrix. The normal function of these Ca^{2+} permeable channels is essential for generating cell extensions, which are required for collagen remodeling and prevention of fibrosis. I will show how Ca^{2+} influx through these channels regulates the interaction of the actin binding protein Flightless I with non-muscle myosin, proteins that enable the formation of cell extensions and matrix homeostasis. Drugs that block these channels inhibit the formation of cell extensions, block collagen remodeling and promote fibrosis.

Title: Naturally Derived Microcarriers As Tissue-Specific 3-D Cell Culture Platforms

Anna Kornmuller[1], Claire Yu [2], Lauren E. Flynn[3]

1. *Biomedical Engineering Graduate Program, Western University*
2. *Department of Chemical Engineering, Queen's University*
3. *Department of Chemical and Biochemical Engineering & Department of Anatomy and Cell Biology, Western University*

To improve upon conventional static two-dimensional culture methods, naturally derived microcarriers comprised exclusively of tissue-specific extracellular matrix (ECM) have the potential to be employed as substrates to support anchorage-dependent cells in three-dimensional (3-D) dynamic culturing systems. In this study, microcarriers derived from decellularized human adipose tissue (DAT), porcine dermis (DDT) and porcine left ventricle (DLV) were fabricated by electrospraying pure ECM suspensions (15, 25, and 35 mg/mL) into liquid nitrogen [1].The microcarriers were stable in long-term culture without chemical crosslinking or other additives. Measurement of Feret's diameter demonstrated that the hydrated microcarriers ranged between 400-550 μm in size. Ultrastructure was examined by optical and scanning electron microscopy, which indicated that the microcarriers were highly porous. IHC analysis was performed to assess the presence of key ECM components. Mechanical compression testing using a Cell Scale micro-mechanical testing system indicated that the microcarriers were soft and compliant, with a Young's modulus ranging between 0.02 – 0.4 kPa, depending on the ECM source and concentration. In vitro 3-D cell culture studies were performed by culturing the DAT microcarriers with human adipose-derived stem/stromal cells (ASCs), DDT microcarriers with human dermal fibroblasts, and DLV microcarriers with h9c2 cardiomyoblasts (25,000 cells/mg). The ASCs and fibroblasts were dynamically cultured for 1 week within a spinner culture system, and cell attachment and proliferation were confirmed by confocal microscopy and flow cytometry. Expansion of the h9c2 cells was noted only under static conditions. Cell viability was maintained post-injection through 18 - 22 G needles, although microcarrier deformation and cell detachment were noted with the 22 G needles. Overall, ECM-derived microcarriers are promising cell instructive substrates that can be used in 3-D culture systems and additionally have the potential to be clinically translatable as injectable cell delivery vehicles. References: [1] Yu. et al. Biomaterials. 120(2017). 66-80.

Title: T1ρ MRI Detection And Histological Evaluation Of Autologous Nucleus Pulposus Cell-Seeded Hydrogel Delivery To Isolated Human Intervertebral Discs In Dynamic Culture.

Derek H. Rosenzweig[1], Rayan Fairag[1], Axel P. Mathieu[2], Julien Tremblay Gravel[1], Janet Moir[1], David Eglin[3], Jean A. Ouellet[4] and Lisbet Haglund[1,4]

1. *The Orthopaedic Research Lab, McGill University, Montreal, C*
2. *Brain Imaging Centre, The Douglas Mental Health Institute, Montreal, QC*
3. *AO Research Institute Davos, Davos, Switzerland*
4. *McGill Scoliosis and Spine Research Group*

Intervertebral disc (IVD) degeneration has been directly linked to low back pain, a condition with tremendous socioeconomic impact that affects multitudes of people world-wide. IVDs are the largest avascular organs and therefore cannot effectively self-repair following initiation of degeneration. Current treatment options are either exercise and pain management or invasive surgical procedures to remove the painful degenerate disc. There is a current need for assessing novel treatment modalities, which can indicate clinical relevance. 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 to first assess IVD cell growth and matrix deposition within a thermo-reversible hydrogel under dynamic loading. Next, we assessed human disc repair whereby intact lumbar IVDs are harvested from organ donors, subjected to T1ρ MRI and cultured under physiological conditions in a bioreactor system. Isolated autologous nucleus pulposus cells suspended in this injectable hydrogel were implanted into IVDs, cultured for 5 weeks and then evaluated and quantified for tissue repair by both a novel T1ρ MRI sequence and histology. Post-treatment scans revealed increased T1ρ values for cell-seeded hydrogel-treated discs compared to pre-treatment scans. Histological analysis revealed that hydrogel alone can retain proteoglycan, but cell-seeded hydrogel retained increased proteoglycan and showed evidence of neo-tissue formation after 5 weeks of dynamic load. In combination with novel T1ρ MRI, the bioreactor provides an experimental platform for evaluation of tissue engineering and biological repair modalities for treatment of human IVD degeneration. Furthermore, the effects of physiological mechanical load on the injected, cell-seeded hydrogels can be determined. Such knowledge is important for pre-clinical evaluation of cell/hydrogel injections and patient advisement on physical activities following a biological repair procedure.

Title: Blood And Lymphatic Microvascularization Of Self-Assembled Skin Substitutes Using Primary Human Endothelial Cells

Jennifer Bourland, Todd Galbraith, Julie Fradette, François A. Auger

- *Centre de recherche en organogenese expérimentale de l'Universite Laval / LOEX, Quebec, Qc, Canada*
- *Division of Regenerative Medicine, CHU de Quebec – Universite Laval Research Center, Quebec, Qc, Canada*
- *Department of Surgery, Faculty of Medicine, Universite Laval, Quebec, Qc, Canada*

Bilamellar living skin substitutes are currently undergoing clinical trials for burn treatment and pathologies such as vascular ulcers. The substitute's vascularization is a key step allowing the graft's fast integration. The lymphatic vascularization is also recognized as a crucial characteristic allowing immune cell delivery and drainage to the graft site. Having previously engineered skin substitutes featuring blood capillaries, we hypothesized we could recapitulate simultaneous angiogenesis and lymphangiogenesis leading to skin equivalents possessing both blood and lymphatic capillary networks.

First, we developed a dermis with lymphatic capillaries by the self-assembly approach, using primary human dermal fibroblasts and purified human dermal lymphatic endothelial cells (LEC). The resulting tissues presented capillaries with features of lymphatic vessels: lumen, blind ends, and filopodia. Then, a tissue engineered skin was generated by adding human primary keratinocytes to a dermis containing both LEC and dermal blood endothelial cells (BEC). Tissue morphology and capillary development were assessed by histology, immunofluorescence, confocal microscopy and 3D imaging reconstruction (Imaris software). Analyses revealed two distinct capillary networks. One was PDPN+ CD31+ with wide vessels (diameter: 50 µm), typical of lymphatic capillaries. The second network was PDPN- and CD31+ with thin (diameter: 10 µm) and highly connected capillaries, features of blood capillaries. Cytokine secretion was determined by ELISA. Skin substitutes secreted VEGF-C, promoting lymphangiogenesis and LECs secreted CCL21, a chemokine involved in immune cell recruitment. Capillaries were embedded in human matrix composed mainly of collagen type I and fibronectin.

We developed the first skin substitute without any exogenous component featuring both blood and lymphatic capillaries. The addition of blood capillaries to skin substitutes was previously shown to improve graft revascularization, therefore lymphatic capillaries should help with wound drainage. This new skin substitute is a promising step towards an optimal treatment for patients suffering from burns or ulcers.

INVITED LECTURE

Frank RAUCH, MD
Shriners Hospital for Children and McGill University.

Frank Rauch, MD, is a Professor of Pediatrics and clinician-scientist at the Shriners Hospital for Children and at McGill University. He obtained his MD degree from the Technical University of Munich, and trained as a pediatrician at the Children's Hospital of Cologne University, Germany. His clinical activities and research program concentrate on improving bone health in children, with a special focus on genetic conditions leading to fractures and on the role of the muscle system in bone diseases. In his recent work, Dr. Rauch has identified new genetic causes of brittle bone disorders and has assessed the long-term effects of bisphosphonate treatment in children with osteogenesis imperfecta. He is also collaborating with Statistics Canada in a study that assesses muscle and bone health in Canadians. Dr. Rauch is currently serving as Editor-in-Chief of the Journal of Musculoskeletal and Neuronal Interactions. Dr. Rauch has authored or coauthored more than 200 original publications.

Osteogenesis Imperfecta – What Causes It? What To Do About It?

Osteogenesis imperfecta (OI) is usually caused by dominant mutations affecting one of the two genes that code for two collagen type I, but a recessive form of OI is present in 5-10% of individuals with a clinical diagnosis of OI. Most of the involved genes code for proteins that play a role in the processing of collagen type I protein, or interfere with osteoblast function. Specific phenotypes are caused by mutations in *SERPINF1* (recessive OI type VI), *P4HB* (Cole-Carpenter syndrome) and *SEC24D* ('Cole-Carpenter like'). For most of these gene defects the mechanisms linking mutation to phenotype remain to be elucidated. Regarding specific medical therapy of bone fragility in children, bisphosphonates are currently the main treatment option. Recent data indicate that children with moderate to severe OI have the ability to reshape most compressed vertebra, if treatment is started early enough and is continued throughout the growing period. However, bisphosphonate therapy does not have a major effect on the development of scoliosis and the incidence of long-bone fractures remains elevated. Even though children with moderate OI (OI type IV) typically achieve independent ambulation, this is rarely the case for children with severe OI (OI type III). Newer medications are being evaluated in an attempt to improve on the therapeutic efficacy of bisphosphonates but the available information about their action in OI is very limited at present.

Title Chiral Switching Of Calcium Carbonate Biomineral Induced By A Single Chiral Enantiomer Of Amino Acid

***Wenge Jiang*[1], *Michael S. Pacella*[2], *Hojatollah Vali*[3], *Jeffrey J. Gray*[4], *Marc D. McKee*[1, 3]**

1. *Faculty of Dentistry, McGill University, Montreal, QC, Canada*
2. *Department of Biomedical Engineering, Johns Hopkins University, Baltimore, MD, USA*
3. *Department of Anatomy and Cell Biology, Faculty of Medicine, McGill University, Montreal, QC, Canada*
4. *Department of Chemical and Biomolecular Engineering, Johns Hopkins University, Baltimore, MD, USA*

Chirality − a fundamental handedness phenomenon of asymmetry – is one of the most striking features of the living world where an object or molecule is not identical to its mirror image (and thus cannot be superimposed onto it). Chirality exists at all levels in biological world, ranging from individual molecules to the long helical tooth of the narwhal. For biomineralization, calcium carbonate-producing organisms record Earth's transition from an inorganic to a biological world, and chiral switching is common in many hardened structures of invertebrate terrestrial and marine organisms. This occurs at both the micro-level (coccolith skeletons) and macro-level (helical gastropod snail shells), and indeed pathological chiral calcium carbonate vaterite otoconia have been found in the human inner ear. Until now, mechanistic knowledge of how this chirality (and chiral switching) occurs in these hierarchically organized, calcium carbonate suprastructures has remained elusive. Here, we present a chiral switching mechanism for calcium carbonate vaterite suprastructures (called toroids) that involves only a single enantiomeric form of the acidic amino acid aspartic acid (Asp). This finding provides insight on fundamental biomineralization processes applicable to chiral architectures seen across different organisms in biology, and on how pathological chiral vaterite otoconia might form in the human ear. High-resolution scanning electron microscopy (SEM), transmission electron microscopy (TEM), focused-ion beam (FIB) sectioning, and RosettaSurface energy-minimization computational simulations revealed that the single enantiomer L-Asp induced chiral switching in vaterite toroids in a manner that can be separated into two formative stages involving the crystalline vaterite platelets that form the toroids: i) a platelet layer tilting stage towards an achiral vertical organization, and ii) a platelet layer rotation stage where successional chiral switching events occur at the surface of the vaterite toroids. In conclusion, this finding provides insight into how simple homochiral biomolecules can create hierarchically organized, chiral biomineralized suprastructures.

Title: An Injectable Chitosan Scaffold With Mechanical Properties Optimized For IVD Repair

Yasaman Alinejad

- *Department of Mechanical Engineering, École de technologie supérieure (ETS), Montréal, QC, Canada.*
- *Laboratory of Endovascular Biomaterials (LBeV), Centre de recherche du CHUM (CRCHUM), Montréal, QC, Canada.*
- *Lady Davis Institute for Medical Research, SMBD-Jewish General Hospital, McGill University, Montréal, QC, Canada.*

Introduction: Low back pain is one of the most prevalent debilitating diseases that affects approximately 1 in 50 Canadians. Although its etiology is often unclear, it is believed that intervertebral disc (IVD) degeneration plays a major role. There are currently no therapies to regenerate disc tissue; surgical disc excision and vertebral fusion are performed when conventional pain management therapies are ineffective. Although effective for short-term pain relief, spinal surgeries are not without problems: they are invasive, alter spinal mechanics leading to subsequent adjacent-level disc degeneration, and have early failure rates. Minimally invasive approaches that either prolong the need for surgery or repair the disc are being pursued. Chitosan (CH) thermosensitive hydrogels are particularly attractive as injectable scaffolds for IVD repair since CH cationic nature makes it tissue-adhesive and facilitates the entrapment of the highly anionic glycoaminoglycans (GAGs). Yet mechanical properties and cytocompatibility of such hydrogels are poor. In the present study we characterize four CH thermosensitive hydrogel formulations and determine which one is most suitable for disc repair and IVD cell viability.

Materials and methods: CH hydrogels were prepared by mixing an acidic chitosan solution (2% w/v) with different formulations of gelling agent. They remain a solution at room temperature and gel at 37°C. Mechanical properties (elasticity, viscosity, and shear) were characterized and compared with human nucleus pulposus (NP) tissue. The cytocompatibility of the gels were assessed on bovine NP cells by measuring cell viability (LIVE/DEAD® assay), metabolism (AlamarBlue assay), and glycosaminoglycan (GAG) synthesis (DMMB assay). Injectability was also tested using human cadaveric discs.

Results: Although two of the hydrogels exhibited mechanical properties similar to human NP tissue (Equilibrium modulus: 5.2±0.6 and 8.0±0.8 kPa; Complex shear modulus: ~12 kPa), only one had a pH and osmolarity suitable for IVD cells (~pH 7 and ~400 mOsmol/L, respectively). NP cells embedded in this gel were maintained at a viability of >85%, had the highest metabolic activity, and greatest GAG retention following a 14 day incubation period under standard culture conditions. Injectability in human degenerative discs demonstrated feasibility of use and filling of nuclear clefts.

Conclusions: We provide evidence for a novel thermosensitive hydrogel that can be used as a scaffold in disc repair and provides a suitable niche for IVD cell viability.

Title: Regulation Of Matrix Gla Protein Gene Expression By An Intronic Sequence

Jobran Moshi, Monzur Murshed

Introduction: Matrix Gla protein (MGP) is a potent inhibitor of extracellular matrix (ECM) mineralization. MGP-deficiency in humans leads to Keutel syndrome, a rare genetic disease, hallmarked by abnormal soft tissue calcification. How Mgp gene is regulated in various cell types is still not well-understood. In a recent study, SOX9, a chondrogenic transcription factor, has been shown to interact with several regions in the promoter as well as intronic sequences of Mgp gene. In the current study, we investigated the effect of a SOX9 binding intronic sequence on the Mgp promoter activity.

Methods: We amplified the rat intronic sequence earlier shown to bind SOX9. This sequence was inserted in front of the 2 kb mouse proximal promoter driving the firefly luciferase gene. This construct was used to transfect ATDC5 chondrogenic cells or primary vascular smooth muscle cells (VSMCs). The transfected cells were grown for 48 hrs and luciferase activity was measured.

Results: Transfection of ATDC5 cells by the pMgp-Luc construct induced luciferase activity in ATDC5 cells. Unexpectedly, when the SOX9 binding intronic sequence was added upstream of the promoter, there was a significant downregulation of the luciferase activity in the transfected cells. Co-transfection with a Sox9 expression vector failed to increase the luciferase activity. We next split the intronic sequence into 3 segments and cloned in front of the 2.0 kb Mgp proximal promoter driving the luciferase gene. Interestingly, we found that all 3 fragments induced the luciferase activity. We identified two CCCTC sequences within the intronic sequence, which are known to bind a DNA interacting protein CTCF with promoter silencing activity. Deletion of one CTCF binding site in the intronic sequence was sufficient to inhibit the repressor activity.

Conclusion: Our study shows the presence of a regulatory element in the intron 1 of Mgp gene. We identified the presence of two CCCTC binding sites within this intronic sequence, which has been shown to bind SOX9. Selective ablation of the upstream CCCTC sequence leads to an upregulation promoter activity suggesting that the intronic sequence confers important epigenetic regulation of the Mgp promoter.

INVITED LECTURE

Frank BEIER, PhD
Professor
Canada Research Chair in Musculoskeletal Health

Frank Beier is the Canada Research Chair in Musculoskeletal Research at the University of Western Ontario and a member of Western's Bone and Joint Institute. He is a Professor and Assistant Chair of the Department of Physiology and Pharmacology. His lab explores mechanisms controlling cartilage and joint biology, using genetically engineered mice in combination with surgical, dietary and activity manipulations. Dr. Beier has published over 100 peer-reviewed articles and gave 100 invited presentations. His work is supported by the Canadian Institutes of Health Research (from which he won a foundation award in the inaugural competition in 2015) and The Arthritis Society. He is a member of the Board of Directors of the Osteoarthritis Research Society International, the Faculty of 1000 and several editorial boards, including the Deputy Editor for *Osteoarthritis & Cartilage*. He is the chair of the 2017 Cartilage Gordon Conference and has just completed a four-year term on the SBSR study section at NIH.

Nuclear receptors in cartilage and osteoarthritis

Osteoarthritis (OA) is a devastating group of diseases affecting millions of Canadians, with no pharmacological treatments that address the underlying pathology. Thus, patients suffer from pain, immobility, and loss of independence for many years. OA is characterized by degeneration of articular cartilage and other joint tissues and can be initiated by several factors, including trauma, metabolic processes, and inflammation. One of the reasons for our lack of drugs to treat OA is the incomplete understanding of the cellular and molecular mechanisms driving the disease. Our lab has used genomic approaches in animal models of OA to identify such mechanisms. Amongst the molecular pathways identified were nuclear receptors, a family of ligand-controlled transcription factors. We have used genetically engineered mouse strains (e.g. cartilage-specific KO mice) and several models of OA (surgery, aging) to determine the roles of selected family members in OA. In particular, we have demonstrated that PPARdelta promotes post-traumatic OA, which was further validated using pharmacological approaches in a rat model. In contrast, LXR and PPARgamma appear to act in a protective manner in the joint. Our microarray analyses have identified target genes of these nuclear receptors in cartilage and suggest potential therapeutic targets. At the CCTC, I will present our recent work on the role and mechanisms of these proteins in cartilage homeostasis and joint disease.

CONFERENCE INFORMATION

Conference Venue:

McGill New Residence Hall

3625 Avenue du Parc, Montréal, QC, H2X 3P8

On-site Registration:

Cash and cheque will be accepted for onsite registration up to the start of the Conference.

$150 CAD for trainees/assistants and $275 CAD for researchers.

Wireless Network:

Login Information provided along with registration package.

For dual participants (SRGCs and CCTC), we request to keep the same login information.

Location of Sessions:

McGill New Residence Hall Ball Room - Prince Arthur B

Location of Poster Sessions:

McGill New Residence Hall Ball Room - Prince Arthur A

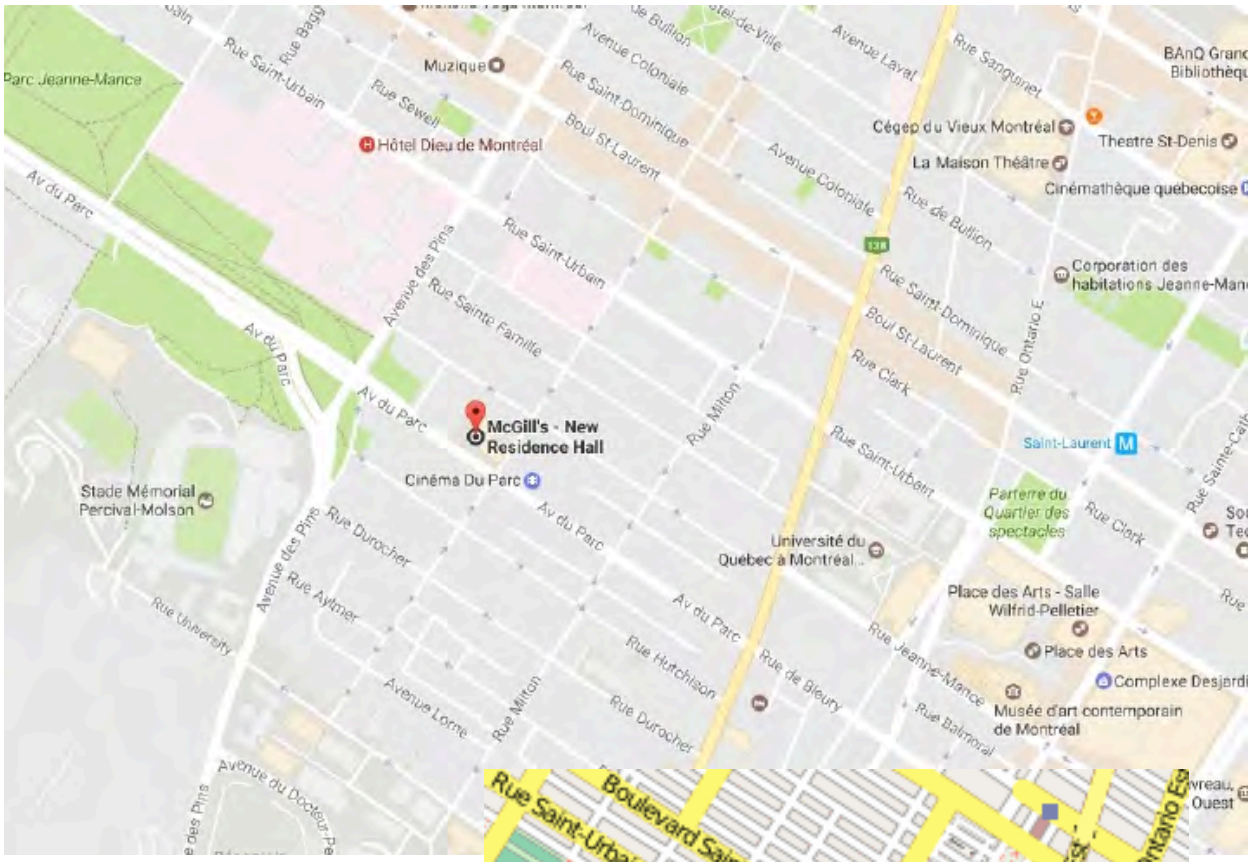
Meeting Rooms

NRH Salle Du Parc / NRH Salle Des Pins

Banquet Venue:

McGill La Citadel 26th Floor Lounge

410 Sherbrooke St W, Montreal, QC. H3A 1B3

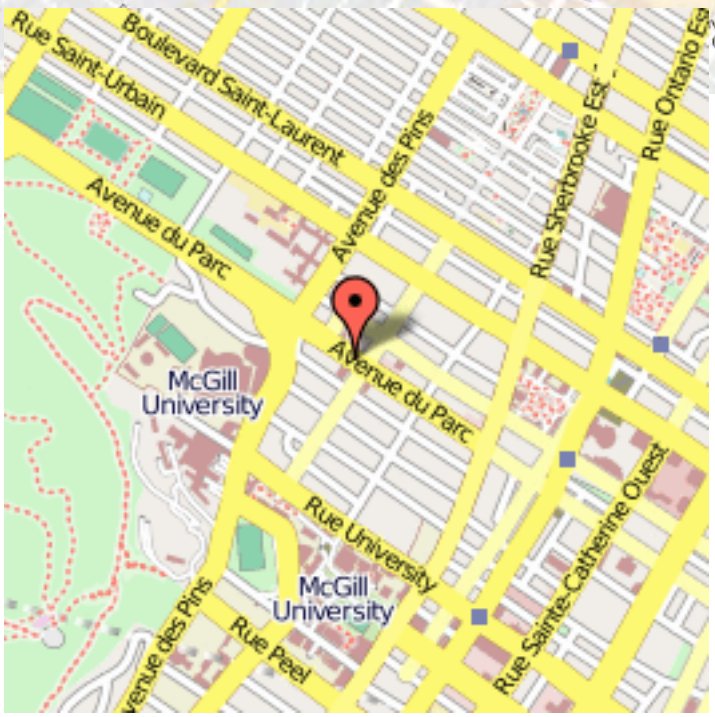


Venue Maps

Conference Venue:

McGill New Residence Hall

3625 Avenue du Parc, Montréal, QC, H2X 3P8



Website: https://www.mcgill.ca/accommodations/summer/nrh

Directions: https://www.google.ca/maps/dir/''/mcgill+new+residence+hall/

Banquet Venue

McGill La Citadel (26th Floor Lounge)

410 Sherbrooke St W, Montreal, QC. H3A 1B3

POSTER ABSTRACTS

THEME	PAGES
TISSUE ENGINEERING	5 - 13
CONNECTIVE TISSUE IN DISEASE: CLINICAL AND FUNDAMENTAL ASPECTS	14 - 20
CONNECTIVE TISSUE REMODELING EXTRACELLULAR MATRIX	21 - 27
BONE CARTILAGE AND TOOTH DEVELOPMENT	28 - 37
GENETICS OF CONNECTIVE TISSUE STEM CELLS IN TISSUE REGENERATION BIOMATERIALS	38 - 44

P-01: Validation of a New Strategy to Repair Jaw Bone Voids in The Presence of Anti-Resorptive Induced Osteonecrosis

KP Rangel Berridi 1, 2, N Makhoul 3, Z Jabbour 1, E Jalili Dil4, B Favis 4, C Hoemann 4, JE Henderson 1,2. *1. Bone Engineering Labs, Injury Repair & Recovery Program, Research Institute-McGill University Health Center. 2. Experimental Surgery. 3. Faculty of Dentistry, McGill University. 4. Department of Chemical Engineering, École Polythechnique, Université de Montréal.*

PURPOSE: Bisphosphonate-related osteonecrosis of the jaw (BRONJ) is the presence of necrotic bone following tooth extraction in cancer patients who have received high doses of bisphosphonates to inhibit bone metastases. Patients with BRONJ require surgical resection of dead bone, which leaves a large defect that, will not heal spontaneously. Biocompatible cements like Norian Putty are commonly used as void fillers but they have a high failure rate. The goal of this study is to use a rat model to determine if a novel, bioplastic (PCL/c) scaffold is superior to Norian Putty as void filler. METHODS: BRONJ will be induced in rats that have received cancer doses of zoledronate and dexamethasone and undergone mandibular tooth extraction. Surgical removal of dead tissue will generate large 3mm x 2mm x 3mm defects surrounded by healthy bone. Group 1 will receive Norian Putty in the left defect and PCL scaffold alone in the right defect. Group 2 will receive Norian Putty in the left and PCL/c scaffold in the right defect. After 6 weeks, Bone repair will be evaluated after 6 weeks using micro-CT, and histology to determine cellular activity.
RESULTS: Preliminary data on the capacity of the PCL/c scaffold to expedite cartilage repair in rabbit knee joints will be presented by Dr Hoemann's group at the 2017 ORS meeting. Bone repair was impaired after tooth extraction in rats with BRONJ (Jabbour et al 2014 Oral Oncol. v50). It is anticipated the PCL/c scaffold will be superior to Norian Putty in expediting repair after surgical removal of necrotic bone. CONCLUSIONS: There is currently no effective treatment for BRONJ, leaving patients with chronic pain and disability. A successful outcome of our work could identify PCL/c as an effective scaffold to expedite bone regeneration and effective repair of large bone voids.

P-02: Freeze-Dried Chitosan-PRP in a Rabbit Model of Rotator Cuff Repair

G Deprés-Tremblay1, A Chevrier2, M Snow3, S Rodeo MD4 and MD Buschmann1, 2 *1.Biomedical Engineering Institute; 2.Chemical Engineering Department, Polytechnique Montreal, Montreal, QC, Canada; 3.The Royal Orthopaedic Hospital, Birmingham, UK; 4.Orthopaedic Surgery, the Hospital for Special Surgery, New York, NY, USA*

The purpose of this study was to apply chitosan-PRP implants in conjunction with transosseous suturing in a rabbit model of rotator cuff tear and to assess healing histologically. A pilot study in 4 rabbits was first performed to assess feasibility, followed by a pivotal study in 13 rabbits to assess efficacy. Bilateral full-thickness tears were created in the supraspinatus tendons (SSP) of the rotator cuff of NZW rabbits. The tears were immediately repaired via a transosseous suturing technique. On the treated side, a chitosan-PRP hybrid mixture was additionally injected in the bony trough and at the repaired site. Pilot study; chitosan-PRP implants induced recruitment of polymorphonuclear cells (PMNs) to the tendon and to the muscle endomysial space from 1 day to 2 weeks post-surgery. Endochondral ossification and new bone formation were apparent at 2 weeks in the control shoulder only. At 8 weeks post-surgery, the SSP tendon insertion site in the control shoulder showed abnormal integration, with significant bone overgrowth into the tendon. In contrast, the superior part of the SSP enthesis in the treated shoulder had a structure that was similar to the intact normal shoulders. Pivotal study; one day post-implantation, the chitosan-PRP hybrid was found adhering to the SSP tendon surface, within the bony trough and in some areas of the SSP tendon/muscle unit. SSP tendon structure was altered in both treated and control shoulders at 7 days. Chitosan-PRP implants induced recruitment of PMNs and synthesis of a highly vascularized granulation tissue. In contrast, the granulation tissue in the suturing control shoulder was less vascularized and devoid of inflammatory cells. Histology is still ongoing for the shoulders collected at 8 weeks post-surgery. Chitosan-PRP implants in conjunction with transosseous suturing showed promising histological findings at the SSP insertion site in the pilot study compared to suturing alone.

P-03: Freeze-Dried Chitosan-PRP Implants Injected in to Meniscus Tears and Improve Repair in an Ovine Model

L Ghazi zadeh1, A Chevrier2, M Hurtig3, J Farr4, Rodeo S5, CD Hoemann1, 2 and MD Buschmann1, 2. *1.Biomedical Engineering Institute; 2.Chemical Engineering Department, Polytechnique Montreal, Montreal, QC, Canada ; 3.Department of clinical studies, University of Guelph, Guelph, ON, Canada; 4.OrthoIndy Knee Care Institute and Cartilage Restoration Center of Indiana, Greenwood, IN USA; 5.Orthopaedic Surgery, The Hospital for Special Surgery, New York, NY, USA*

Introduction: Meniscal tears are the most common knee injuries. Surgical treatment of symptomatic meniscal tears involves partial removal of torn meniscus. This alters knee biomechanics and kinematics of meniscus tissue as well as knee and develop radiographic evidence of osteoarthritis (OA) and degenerative arthritis. Lesions in the vascular region of the meniscus have high potential for repair by formation of a fibrin clot while healing is very limited in the avascular portion of the meniscus. Tears are further away from the periphery may benefit from different augmentation modalities. Trephination had some success in pre-clinical models and in clinical studies and facilitate incorporation of cells and growth factors to the tear into the substance of the meniscus to stimulate healing. lack of neo-vascularization and cell recruitment as well as channels collapse are tough to be the primary reasons for failure. The meniscus wrapping technique was developed in order to augment the rate of healing for complex tears. The starting hypothesis was that the collagen matrix used for wrapping would act as a bioreactor to guide cell ingrowth and improve suture stability. The arthroscopic method combines suturing techniques with the use of a collagen membrane to wrap the meniscus and the optional application of bone marrow aspirate or PRP concentrate under the membrane. We have recently developed freeze-dried chitosan (CS) formulations that can be solubilized in platelet-rich plasma (PRP) to form injectable implants that coagulate in situ. These implants induce vascularization and cell recruitment in vivo, both of which are desirable in the context of meniscus repair. In the current study, we combined the meniscus wrapping technique and the application of CS-PRP implants in a unilateral complex tear model in the sheep knee. Structural and functional outcomes were assessed at 6 weeks as a pilot study. The purpose of this study was to investigate whether healing of ovine meniscus tears can be augmented by applying chitosan-PRP implants and wrapping the meniscus with a collagen membrane. Methods: Polymer formulations containing 1% (w/v) chitosan (80% DDA and Mn 40 kDa), 1% (w/v) trehalose (as lyoprotectant) and 42.2 mM calcium chloride (as clot activator) were freeze dried and solubilized in autologous PRP before its application. Unilateral 10 mm longitudinal tears were created in the medial meniscus of six skeletally mature ewes and treated by suturing and injecting chitosan-PRP in the tear (n=2), wrapping the meniscus with a collagen membrane and injecting chitosan-PRP in the tear and under the wrap (n=2) and wrapping only (n=2) (Fig 1). Tissue repair was assessed through histology at 6 weeks. Results: Complete healing and seamless integration were observed in one chitosan-PRP treated tear (Fig 2 a&b), while there was partial healing in one tear treated with chitosan-PRP and wrapping (Fig 2 c&d). The repair tissue was highly cellular and well integrated to surrounding host meniscus (Fig 2 a to d), but structurally different than unoperated controls (Fig 2 g&h). There was no healing in the menisci treated with wrapping alone (Fig 2 e&f). Significant cell infiltration was observed at the outer portion of all treated menisci compared to unoperated controls (Fig 2 a, c & e vs g). Suture tracks were abundant in menisci treated with the wrapping technique (Fig 2 c&e). Discussion: Chitosan-PRP implants showed superior regenerative effect over wrapping the meniscus with a collagen membrane. Using the wrap in conjunction with chitosan-PRP implants did not further improve repair and the additional sutures needed to secure the wrap created significant damage to the menisci. This suggests that chitosan-PRP implants by themselves could be efficient in overcoming the current limitations of meniscus repair.

P-04: Development of a Culture System For Testing Tissue Engineering Approaches and Biological Repair of The Degenerate Intervertebral Disc

R Fairag, D Rosenzweig, J Moir, M Weber and L Haglund.
McGill Scoliosis and Spine Group, Orthopedic Research Laboratory, Department of Surgery, Faculty of Medicine, McGill University, Montreal, QC, Canada.

Introduction: Intervertebral disc (IVD) degeneration is a major cause of back pain. Cell based therapy can be promising in treating early stage degeneration, slow the degenerative process and enhance regeneration by implantation of nucleus pulposus or stem cells incorporated with suitable injectable scaffolds. The purpose of this study is to 1- Establish culture model to help in understanding pathophysiology of intervertebral disc degeneration and regeneration options. 2- Investigate the ability of HA-pNIPAM hydrogel to work as injectable carrier for cells delivery. 3- Assess the effect of mechanical compressive loading on cell differentiation, viability and matrix production. Methods and materials: To assess the ability of nucleus pulposus cells to survive three-dimensional loading culture and whether mesenchymal stem cells differentiate towards a disc-like phenotype and produce matrix, Cells were encapsulated within HA-pNIPAM injectable hydrogel and embedded within silicone/agarose constructs mimicking IVD structure. These constructs were cultured either without load or under dynamic compressive loading (10% of construct height) for three weeks. Cell viability was determined with LIVE/DEAD assay and DAPI staining, while histological manifestations were assessed using safranin-O staining, and DNA was quantified with HOECHST assay. Results: The silicone/agarose model can withstand 10% cyclic compressive loading over the three week culture period. The bioreactor system proved a consistent around 10% compression throughout whole culture period. Hydrogels seeded with human cells showed excellent cell viability after three weeks of culture. Histological analysis of constructs showed positive proteoglycan staining in both unloaded and loaded cultures. Conclusion: Silicon/agarose constructs could withstand compression within the boundaries of physiological load. The HA-pNIPAM hydrogel is viable composition to be used as an injectable medium for delivery of cells into degenerate discs. Nucleus pulposus cells survived loading and produced more matrix than unloaded constructs. This system can be used to evaluate various hydrogels, bioactive factors and cell types.

P-05: Human Adipose Stromal/Stem Cell-Derived Reconstructed Osseous Tissues As a Platform For Studying The Osteoanabolic Activity of Melatonin

WP Clafshenkel1,2, F Kawecki1,2, T Galbraith1, FA Auger1,2, J Fradette1,2
1. Centre de recherche en organogénèse expérimentale de l'Univ Laval/LOEX, CHU de Québec Research Center – Univ Laval, Division of Regenerative Medicine, QC, Canada 2. Dept of Surgery, Univ Laval, QC.

In vitro models of human bone tissues that represent the complex structural and functional characteristics of native bone are necessary for the rapid and efficient screening of new therapeutic compounds for diseases like osteoporosis. Recently, we have developed a biomimetic, cell-sheet based three-dimensional (3D) model of human reconstructed osseous tissues (hROTs) originating from human adipose-derived stem/stromal cells (hASCs) that recapitulates several important hallmarks of native bone tissue. The impact of melatonin on osteogenesis in hASCs remains understudied; thus, we hypothesize that this model system represents a reliable pharmacological platform for the investigation of the osteoanabolic activity of melatonin on the developing tissues. The direct effects of melatonin on cell sheet manipulability, early osteogenesis, matrix maturation, and mineralization were assessed. Treatment with melatonin or osteogenic induction did not appreciably affect the ability to manipulate cell sheets. Preliminary data suggest that hROTs supplemented with 100 µM melatonin demonstrated a significant increase in alkaline phosphatase (ALP) activity after 21 days of induction compared to non-treated (2-fold, $p < 0.001$) and 20 µM-treated (1.8-fold, $p < 0.01$) tissues. Likewise, 100 µM melatonin-treated tissues demonstrated a modest but significant increase in hydroxyapatite deposition after 14 days of induction (1.3-fold, $p < 0.05$). These results suggest that the osteoanabolic effects of melatonin on these parameters may be time and concentration dependent. By modeling native osseous tissues using self-assembled hROTs, we can study the impact of natural biological molecules like melatonin on the processes of osteogenesis in a 3D in vitro setting. Human ROTs may be valuable in future studies for identifying and studying osteogenic mechanisms and targets, and accelerating the development of new multi-functional drugs that potentially re-establish or slow the progression of bone loss.

P-06: Contractile Phenotype of Bovine Outer Annulus Fibrosus Cells Is Regulated By Actin Through Yap-Taz/MRTF Signalling Pathway

R Gawri1, 2, W Wu1, 2, J Parreno2 & R Kandel1, 2, 3

1. Institute of Biomaterials and Biomedical Engineering, Univ of Toronto; 2. Pathology and Laboratory Medicine and Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, ON; 3. Laboratory Medicine and Pathobiology, Univ of Toronto, Toronto, ON.

Introduction: Intervertebral discs (IVDs) are composed of nucleus pulposus surrounded by collagenous annulus fibrosus (AF). AF disseminates compressive forces by twisting of collagen fibers and AF cells attached to these fibers adapt by changing shape with help of contractile molecules such as actin. Mechanism(s) by which expression of these proteins is regulated has not been elucidated in AF, although MRTF signalling may be involved as shown in other cells. We hypothesize that oAF cells express more contractile proteins, such as vinculin and transgelin, than iAF and this is regulated through actin regulated signalling pathways, such as MRTF. Methods and Materials: IVDs were harvested from bovine tails and AF tissue was divided into outer (oAF) and inner (iAF) regions, cells were isolated and seeded separately. Selected oAF cells were cultured in presence or absence of either Latrunclin B or Cytochalasin D and analyzed for αSMA, transgelin and vinculin gene and protein expression. oAF cells nucleofected with MRTF siRNA were examined for gene and protein expression of contractile molecules. Results: Presence of αSMA, filamentous and globular actin was confirmed in both native oAF and iAF tissue and in oAF and iAF cells in culture by immunostaining. Gene and protein expression analysis showed that oAF cells in culture expressed more αSMA, transgelin and vinculin compared to iAF cells. Modulation of actin by depolymerizing agents altered their expression. MRTF knockdown, resulted in significant downregulation of gene and protein expression of transgelin, vinculin and αSMA. Discussion: We demonstrate that oAF cells express αSMA, transgelin and vinculin, molecules implicated in modulation cell shape and matrix contraction. Expression of these molecules is regulated, in part, by MRTF-A –actin signalling pathway. As oAF cells bear most of the tensile forces in IVD, it is important to understand mechanism(s) regulating these contractile molecules to identify sequence of events that trigger IVD degeneration cascade.

P-07: Surface-Treated Tubular Polymeric Template Allows For Modular 3D Vascular Tissue Engineering

JM Bourget1,2a*, T Galbraith1*, V Roy1,2, M Picard-Deland1,2, JF Morin3, R Gauvin1,2b & FA Auger1,2

1. Axe médecine régénératrice, Hôpital du Saint-Sacrement, Centre de recherche du CHU de Québec-Univ Laval and Centre de recherche en organogénèse expérimentale de l'Université Laval / LOEX, Québec, QC; 2.Dépt de chirurgie, Faculté de médecine, Univ Laval, Québec, QC; 3. Dépt de chimie, Faculté des sciences et génie, Univ Laval, 1045 avenue de la Médecine, Pavillon Alexandre-Vachon, Québec, QC. a. Current address: Énergie, matériaux et télécommunication, Institut national de recherche scientifique, Varennes, Qc and National Research Council, Boucherville, QC. b. Current address: Centre Québécois sur les Matériaux Fonctionnels (CQMF), Univ Laval, Québec, QC. *These authors contributed equally to the work

There is a strong clinical need to develop a small caliber tissue-engineered blood vessel (TEBV). Such a substitute can be engineered using the self-assembly approach in which cells produce their own extracellular matrix. However, this approach is currently limited to the production of flat sheets that need to be further rolled into the final desired shape, in the present case, a tubular construct. In this study, human fibroblasts were seeded directly on UVC-treated cylindrical polyethylene terephthalate glycol-modified (PETG) mandrels of 4.8 mm diameter. After 21 days of culture in medium containing ascorbic acid, the mesenchymal cells generated a tubular tissue that can be manipulated. Four of those tissues were superimposed together to form a tubular construct and were compared with tubular constructs of similar thicknesses produced using the standard sheet-rolling approach. Histological staining of transverse sections of stacked tubular constructs showed cohesion between concentric layers, without the presence of inner or outer edges. Superimposition of tubular tissues led to a construct with a two-fold increase in ultimate tensile strength. This study lays the foundation for a broad field of possible applications such as custom-made reconstructed tissues of specialized shapes using a surface treated 3D structure as a template.

P-08: The Key Role of Fibronectin in The Maturation of Tissue Engineered Blood Vessels

D Pezzoli1, **J Di Paolo**2, H Kumra2, G Candiani4, DP Reinhardt2,3, D Mantovani1

1. Laboratory for Biomaterials and Bioengineering, Dept of Mining, Metallurgical and Materials Engineering and CHU de Québec Research Centre, Université Laval, Quebec City, QC; 2. Faculty of Medicine; 3. Faculty of Dentistry, McGill University, Montreal, QC; 4. Dept of Chemistry, Materials and Chemical Engineering, Politecnico di Milano, Milan, Italy

Background and Rationale: In the western world, coronary artery disease is responsible for more than half of all cardiovascular disease-related deaths. One of the most pressing clinical problems of the field is the need for small diameter vascular grafts for coronary bypass surgery. Tissue engineered blood vessels (TEBVs) have been proposed as arterial substitutes but, despite the many advancements in the field, further work is required to address the biggest issue toward their clinical application, the lack of strength and elasticity caused by limited elastic fiber formation. Methods and Results: In this study, TEBVs were prepared using collagen gels cellularized with porcine aortic smooth muscle cells (SMCs) and supplemented with human plasma fibronectin (FN), a known master organizer of the extracellular matrix (ECM) that could promote cell adhesion and a synthetic phenotype in SMCs. The maturation of the constructs over time was investigated in terms of matrix contraction, mechanical properties (stress-relaxation tests), expression and deposition of ECM proteins (immunohistochemistry and quantitative PCR). Results showed a time-dependent increase in SMC-mediated gel contraction and mechanical properties and, in FN supplemented constructs, tensile elastic modulus was more than twice higher than in control gels ($p < 0.05$), reaching the relevant value of 0.12 ± 0.02 MPa after 7 days of maturation. In addition, supplementation with FN increased the production by SMCs and deposition in the construct of elastic fiber-related proteins such as fibrillin-1 and tropoelastin, and influenced the expression profile of several ECM-related genes. Conclusions: Altogether, this study demonstrates the pivotal role of FN in directing the maturation and remodeling of collagen gel-based scaffolds by SMCs toward the production of physiological-like, elastic fiber-containing TEBVs with superior mechanical properties and its use should be taken into account in tissue engineering approaches to promote the cell-guided generation of mature tissues with an organized ECM.

<u>P-09:</u> Tissue-Engineered Skin Model Derived From Neurofibromatosis Type 1 Patients to Study Tumor Genesis and to Predict Response to Therapy.

V Roy1,4, É Marques1,4, L Touzel-Deschênes1,4, P Kannu2, N Dupré3,4, HT Khuong1,4 & F Gros-Louis1,4
1. Dept of Surgery, Faculty of medicine, Laval Univ; 2. Dept of paediatrics, Faculty of medicine, Univ of Toronto; 3. Dept of neurological sciences, Faculty of medicine, Laval Univ; 4. Regenerative medicine, Centre de Recherche du CHU de Québec-Laval Univ, LOEX/HEJ, CHU de Québec-Laval Univ

Background: Neurofibromatosis type 1 (NF1) is an autosomal dominant multisystemic disorder caused by aberrations in the neurofibromin gene. The population incidence is approximately 1 per 3000. Typically, patients develop multiple cutaneous tumours that grown from axon of peripheral nerve, called neurofibromas. These benign tumours are generally composed of Schwann cells (SC) and fibroblasts, but others cells type can also be found. Highly variable clinical manifestations between NF1 patients are observed. Actually, there is no specific treatment for this stigmatizing disease. Objective: The purpose of this study is to develop a tissue engineered human skin model derived from NF1 patients to characterized and understand the formation of neurofibromas. Methods: The auto-assembly model was used to generate tissue-engineered skin (TES) in vitro with fibroblasts and keratinocytes isolated from NF1 patients (n=3). We used spheroid suspension culture to generated neurofibroma-like tumours; one composed of immortalized SC and another with an equal number of SC and fibroblasts. Then, spheroids were added on the dermis 10 days before keratinocytes seeding. Results: We first determined the best conditions for the formation of spheroids. Densification area was significantly increased already at day 3 and continued until day 10. Spheroids growth was significantly faster than control cells. Immunofluorescence revealed that spheroids/neurofibromas-like, seeded with NF1-TES, are in a proliferative state. Furthermore, non-apparent activation of apoptosis within spheroid is detected. Neurofibroma-like tumours composed of SC seem to affect the proper formation of the epidermis. Conclusion: Our NF1 skin model could become a unique tool to better characterize the mechanism of action of a new drug on NF1 tumor shape and growth as well as to assess tumorigenic properties of each of the tested NF1 gene mutation, and ultimately provide better tools to develop new therapies for patients through development of precision/personalized medicine strategies.

P-10: Comparative Study of Cell Source On The Functional Characteristics of Human Reconstructed Connective Tissues Engineered Under Serum-Free Conditions.

M. Safoine1, V Trottier1, K Aubin1, MA Plourde Campagna2, J Ruel2 & J Fradette1

1. Centre de recherche en organogénèse expérimentale / LOEX, Division of Regenerative Medicine, CHU de Québec Research Center, Dept of Surgery, Faculty of Medicine, Univ Laval, Québec, QC; 2. Bureau de design, Mechanical Engineering Department, Univ Laval, Québec, QC.

INTRODUCTION: Tissue engineering allows the production of human substitutes intended to be grafted. Several cell sources are considered for connective tissue reconstruction, with dermal fibroblasts (DFs) remaining the most used. In recent years, adipose-derived stem cells (ASCs) have gained major interest due to their minimal donor site morbidity and their secretome exhibiting a major therapeutic potential. The aim of this study was to investigate the effect of cell source on stromal reconstruction. We hypothesized that ASCs allow the production of human reconstructed connective tissues (rCTs) exhibiting enhanced functional properties compared to DFs. METHODS: DFs and ASCs were used to engineer human connective cell-sheets which were superposed to form DFs-hrCTs and ASCs-hrCTs according to the self-assembly method. A commercially available GMP grade serum-free medium (SFM) was used for the entire tissue production limiting animal derivatives. The tissue structure was analyzed using Masson's trichrome staining and immunolabelings. Mechanical properties were evaluated using uniaxial tensile tests (Instron E1000) and secretory profiles were determined by ELISA to assess their biological functionality. RESULTS: Both tissues displayed similar histological appearance and a diversified matrix composition [fibronectin, collagens type I and III, glycoproteins (tenascin C) and proteoglycans (decorin, perlecan)]. ASC-rCTs showed an enhanced matrix formation being 2.9x thicker than DFs-rCTs and a slightly but non-statistically significant increased contraction ($p = 0.0944$). Interestingly, the use of SFM compared to standard fetal bovine serum-containing medium increased ASCs-rCTs thickness by 4.4x and diminished contraction by 1.6x. Mechanical tests revealed comparable ultimate failure strength for both tissue types. Finally, ASCs-rCTs secreted 7x more angiopoietin-1 and PAI-1 than DFs-rCTs. CONCLUSION: ASCs are a cellular type of choice for stromal reconstruction comparable and even superior to DFs especially regarding their secretion of pro-angiogenic molecules. Those reconstructed tissues have extensive surgical applications as graft substrates and can also provide stromal support for skin reconstruction.

P-11: Injectable, Anisotropic Dense Fibrin Hydrogels For Cell Delivery

G Griffanti and SN Nazhat

Dept of Mining and Materials Engineering, Faculty of Engineering, McGill University

Bone tissue engineering (BTE) aims to design biocompatible and biodegradable scaffolds that have structural and functional equivalence to the native extracellular matrix (ECM). Therefore, the recreation of the appropriate tissue micro-architecture is of significance as it determines its mechanical properties and provides the proper environment for cellular activity. Fibrin hydrogels have largely been used as surgical glues and hemostatic agents as they are easy to obtain directly from the patient's blood. They also support stem cell proliferation and differentiation, and can be promising as scaffolds for BTE. However, the low mechanical stiffness of fibrin hydrogels and their extensive cell-induced contraction limit their use in BTE. Increasing the hydrogel concentration could be a route to overcome these drawbacks. Recently, gel aspiration-ejection (GAE) has been developed to produce injectable, anisotropic, cell seeded, collagen gels with controllable fibrillar density and alignment. In this study, GAE was applied to generate injectable fibrin hydrogel for cell delivery in BTE. GAE generated injectable fibrin gels of controllable fibril density and anisotropy comprising of impacting bundles of aligned fibrils, which were correlated with scaffold modulus and toughness. Moreover, the alignment, proliferation and osteoblastic differentiation of seeded mesenchymal stromal cells were controlled through gel density and anisotropy. In sum, the structural and mechanical properties of fibrin hydrogels can be modulated to mimic the native ECM of bone and support osteoblastic outcomes through GAE, thus providing potential for BTE.

P-12: PrimeGrowth™ IVD Cell: a Novel Optimized Medium For The Culturing of IVD Cells

MP Grant1,2, LM Epure2, Y Alsaran1,2, J Antoniou1,2 and F Mwale1,2

1. Dept of Surgery, McGill University; & 2. Orthopaedic Research Laboratory, Lady Davis Institute for Medical Research, SMBD-Jewish General Hospital, Montreal, QC.

INTRODUCTION: Investigating mechanisms, potential biologics, and scaffolds in the regeneration of the intervertebral disc (IVD) requires the use of model systems. Cell culturing of IVD cells is often employed as the first step in the development process. However, the native IVD cell environment is unique in terms of nutrient availability, metabolic exchange, O2 tension, and osmolality. Notwithstanding, IVD cells are often cultured in standard DMEM medium subjecting the cells to a new environment that may alter responses to treatments. To this end, we have formulated a medium, PrimeGrowthTM IVD Cell, in collaboration with Wisent Bioproducts (Montreal, Quebec) optimized for IVD cells. METHODS: Bovine nucleus pulposus (NP) and annulus fibrosus (AF) cells were cultured in PrimeGrowthTM IVD Cell, DMEM, Alpha MEM, and Ham's F12 media supplemented with 10% FBS and antibiotics. Cells were prepared for 3D culturing in alginate beads at a density of 2*10^6 cells/mL for 12 days. A modified GAG assay was performed on the beads to determine proteoglycan content and the hydroxyproline assay was performed to determine collagen content. Gene expression of matrix proteins type I and II collagen and aggrecan were determined by qPCR. 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 growth in the beads at the end of the incubation period was determined using Quant-iT dsDNA Assay Kit (Thermo Fisher Scientific). RESULTS: PrimeGrowthTM IVD Cell medium demonstrated consistency and significantly higher synthesis of Col II and aggrecan in both NP and AF cells. Although IVD cell viability and growth were unaltered for all media tested, certain culture media significantly increased the expression levels of Col I. DISCUSSION: Devising a medium unique for the culturing of IVD cells that better mimics their physiological environment may not only improve the translation of therapeutics but could better standardize results across laboratories. PrimeGrowth™ IVD Cell is the first ready-to-use media formulated to support the growth and maintenance of IVD cells with greater reliability, consistency and improved control.

P-13: Development of a Tissue-Engineered Spinal Cord Model to Recapitulate Amyotrophic Lateral Sclerosis

A Louit1,2, MJ Beaudet2 et F Berthod1,2.

1.Dept de chirurgie, Fac de médecine, Univ Laval; 2. LOEX, Centre de recherche du CHU de Québec-Univ Laval, Hôpital de l'Enfant-Jésus.

Background: Amyotrophic lateral sclerosis (ALS) is an incurable neurodegenerative disease triggering motor neurons (MN) degeneration, and causing patient death due to respiratory failure within 3 to 5 years of diagnosis. In some patients, a mutation in the Superoxide Dismutase 1 (SOD1) gene, that induces protein misfolding and aggregate formation in MN, has been identified as a cause of ALS. Recent evidence has shown that the combination of MN, astrocytes, microglia and myoblasts, may constitute a metabolic unit and that non-neuronal cells could contribute to the ALS development. Objectives: Our purpose is to extract MN, astrocytes and microglia from spinal cord of transgenic mouse embryos reproducing the disease phenotype, to maintain these cells in long term culture and to develop a 3D spinal cord model recapitulating ALS. Methods: MN, astrocytes, and microglia have been extracted from transgenic SOD1G93A mice embryos overexpressing the mutant human SOD1 protein, or wild type SOD1WT mice overexpressing the normal SOD1 human protein. These cells have been purified and seeded on 3D collagen sponges.
Results: The cell extraction method has been optimized to obtain a high yield (> 1 million per embryo) of 90% pure MN. The 3D model showed the close cell-cell interaction between astrocytes and MN. We also noticed that when SOD1G93A MN were cultured in presence of mutant astrocytes, mutant microglia or both, there was a drastic reduction in TUJ1-positive neurites, compared to controls made of SOD1WT astrocytes and microglia. Conclusion: Diseased MN are able to organize into nerve fibers in presence of normal glial cells, but this organization is greatly affected by diseased glial cells. In vitro ALS modeling should provide a better understanding of the disease mechanism, and could serve as a screening platform for future drugs.

P-14: The Role of Inflammation in Adipose-Derived Stromal Cell-Mediated Soft Tissue Regeneration Within Decellularized Adipose Tissue Scaffolds

KP Robb1, KM Parkins2,3, JA Ronald2,3,4, GA Dekaban2,6, & LE Flynn 3,7.

1. Biomedical Engineering Graduate Program, Western University, London, ON; 2. Robarts Res Inst, Western Univ; 3. Dept of Medical Biophysics, Western Univ; 4. Lawson Health Res Inst, London, ON; 5. Dept of Microbiology & Immunology, Western Univ; 6. Dept of Anatomy & Cell Biology, Schulich School of Medicine & Dentistry, Western Univ; 7. Dept of Chemical & Biochemical Engineering, Western Univ.

Introduction: Decellularized adipose tissue (DAT) represents a promising adipogenic bioscaffold for applications in soft tissue augmentation and reconstruction. Previous work demonstrated that seeding DAT scaffolds with allogeneic adipose-derived stem/stromal cells (ASCs) enhanced adipogenesis in an immunocompetent rat model [1]. Moreover, immunohistochemical staining suggested that ASC seeding promoted a pro-regenerative M2 macrophage phenotype that may have contributed to constructive remodelling of the scaffold into host-derived fat. The objective of the current study is to investigate the interactions between ASCs and macrophages during in vivo adipose tissue regeneration using transgenic reporter mouse strains to track and characterize the ASC and myeloid cell populations. Methods: ASCs were isolated from transgenic dsRed mice, and their immunophenotype and multilineage differentiation capacity were validated. DAT scaffolds were prepared from discarded human fat as previously described. ASC-seeded (2E6 cells/7 mg scaffold) and unseeded DAT scaffolds were implanted into MacGreen mice in which cells of the myeloid lineage, including macrophages, express EGFP. In vivo optical imaging was used to track the dsRed+ ASCs and EGFP+ cells within the mice from 2 h - 8 weeks, complemented by histological and immunohistochemical analyses in a separate subset of mice. Results: Optical imaging confirmed donor ASC retention within the DAT up to 8 weeks post-implantation. Myeloid cell infiltration increased over time in both implant groups, with a significantly higher EGFP+ signal detected in seeded implants after 4 weeks. Staining results confirmed that ASC seeding promoted scaffold remodelling, with enhanced cell infiltration, angiogenesis, and adipogenesis observed at 4 and 8 weeks. Discussion: Allogeneic ASC seeding promotes myeloid cell infiltration, as well as scaffold remodelling and tissue regeneration in DAT bioscaffolds. In ongoing work, macrophage phenotype is being characterized to investigate the role of different subpopulations over time in the regenerative process.

P-15: Impact of Non-Steroidal Anti-Inflammatory Drugs On Bone Repair

J Ramirez-GarciaLuna1 2, Y Al-Saran1 2, T Wong13, J Henderson1,2, P Martineau1,2
1.Bone Engineering Labs, Injury, Repair & Recovery Program, Research Institute-McGill University Health Centre. 2. Experimental Surgery, Faculty of Medicine, McGill University. 3.Biotechnology Program, UBC.

PURPOSE: Fracture repair starts with a localized inflammatory response during which immune and vascular precursor cells are attracted to the defect and bone marrow derived mesenchymal stem cells differentiate to repair the tissue. Non-steroidal anti-inflammatory (NSAIDs) drugs administered for pain relief following fracture also modify the local inflammatory response, although their mechanism of action in this respect is not known. The goal of this research is to use a mouse model of bone repair to identify the mechanism whereby administration of NSAIDs in the early phase of repair modifies the healing process. METHODS: Bone repair in skeletally mature Bl6 mice was quantified in 2mm cortical defects drilled in the femur. Mice received either a 5 mg/kg NSAID pellet (N=9) or a placebo pellet (N=10) implanted subcutaneously for continuous delivery for 14 days PO. The quantity and quality of bone and re-vascularization of the defect were quantified using micro CT. Comparisons were made by ANOVA at the 95% confidence level. RESULTS: In a standardized region spanning the defect, NSAID treated mice had significantly less bone than control mice treated with placebo, with fewer trabeculae that were less well connected and more porous. In contrast to the significant reduction in bone quantity and quality there were no detectable differences in the number, volume or connectivity of blood vessels in the NSAID treated mice. CONCLUSION: Systemic treatment with NSAIDs during the inflammatory phase of bone repair impairs healing by inhibiting bone formation and/or turnover rather than affecting the re-vascularization of the repair tissue. The use of NSAIDs for pain management in patients, like the elderly, who are already at risk of complications in fracture healing, should be re-considered.
ACKNOWLEDGEMENTS: JRGL is supported by a CONACYT scholarship and the Bone Engineering Labs are supported in part by FRQS-RSBO and the Jo Miller Orthopaedic Fund.

P-16: 3D Droplet Scaffolding For Osteocyte Mechanical Unloading in a Rotating Wall Vessel

R Fournier and R E. Harrison
University of Toronto, Scarborough, ON.

Osteocytes play a central role in astronaut bone loss and other disuse-related bone pathologies. However, research on this cell type is limited as they are deeply embedded in the dense, calcified matrix of bone tissue. Osteocytes are therefore difficult to purify and impossible to see under light microscopy. We addressed this limitation by developing 3D hydrogel droplets and culturing MLO-Y4 osteocyte-like cells within them to produce an environment more closely resembling osteocyte organization in vivo. The droplets were compatible with the Rotary Cell Culture System (Synthecon, Inc., USA) which allowed us to observe and analyze the response of MLO-Y4 cells to simulated microgravity. Droplets were fabricated by combining the cell suspension with a diluted and neutralized solution of type I rat tail collagen (ibidi GmbH, Germany) and pipetting 2.5uL onto a hydrophobic surface. In our preliminary work, we have successfully imaged MLO-Y4 cells in these droplets by confocal microscopy and scanning electron microscopy. This enabled visualization of changes to both the intracellular and intercellular 3D architecture of cells in simulated microgravity which will be compared to static 1g and mechanically loaded controls. Furthermore, we have shown that mRNA and protein can be quickly and easily extracted from cells encapsulated in gel droplets for downstream analysis of gene and protein expression. Our method greatly improves current models used to study osteocyte mechanical unloading as we incorporate three-dimensionality which allows MLO-Y4 osteocytes to extend their dendrites, the membrane branches where most mechanosensing occurs, in all directions. This may improve sensitivity to mechanical forces and the physiological relevance of the model. Upon further validation of our method, we intend to utilize next generation mRNA sequencing to identify novel genes affected in simulated microgravity. These genes will be studied to elucidate the mechanisms by which osteocytes are responsible for astronaut bone loss.

P-17: Development and Validation of an Algorithm to Predict The Clinical Outcome of Wound Healing Using Thermographic Scans

J Ramirez-GarciaLuna1,2, MA Martínez-Jiménez2, VJ Lesage-Soria2, ES Kolosovas-Machuca3, JM Shiguettomi-Medina4, G Mariel-Cárdenas3, FJ Gonzáles3, F Alcocer2

1.Experimental Surgery, Faculty of Medicine, McGill University, Canada; 2.Dept of Surgery, Faculty of Medicine, Universidad Autonoma de San Luis Potosi, Mexico; 3.Coordination for the Innovation and Application of Science and Technology, Universidad Autonoma de San Luis Potosi, Mexico; 4.Dept of Children's Orthopaedics, Aarhus University, Denmark.

Purpose: To develop a model to predict the treatment of patients with burn injuries based on thermographic imaging of the wound. Treatment was categorized as conservative if the wound healed spontaneously, skin graft if it healed after receiving a graft; or amputation.

Methods: Infrared thermography was performed in 40 burned patients (52% partial thickness and 48% full thickness) within the first 3 days after the injury using a FLIR T400 infrared camera. We obtained the mean temperature of the wound, of the adjacent healthy skin, and the difference between them (temperature difference, TD). The wounds received standard care and their outcome was registered. Statistical analysis was performed using ANOVA to compare TD between groups and identify potentially confounding factors. Multivariate generalized lineal models were used to adjust confounding factors for the outcome. Finally, through a classification and regression training algorithm, a prediction model was developed using all variables associated to the outcome. A value $p < 0.05$ was considered statistically significant. Results: 18 (45%) patients underwent conservative treatment, 10 (25%) received skin grafts, and 12 (30%) required an amputation. TD in patients who received conservative treatment was 1.7±0.9 degrees, 3.2±0.9 in patients who received skin grafts, and 7.6±1.8 in patients who underwent amputations ($p < 0.001$). After multivariate adjustment of potential confounding factors, only TD predicted the outcome. The classification and regression training algorithm developed has an accuracy of 81.61% for diagnostic classification. The algorithm misclassifies treatment outcome in 10.5% of the patients to conservative treatment, 10.5% to skin graft, and 0% to amputation. Conclusion: Infrared thermography can be used in the initial assessment of a burned patient to predict its treatment outcome, as TD is a function of the vascularization and degree of viable tissue in the wound. This non-invasive imaging modality can enhance patient care and avoid unnecessary surgical procedures.

P-18: Microfibrillar Associated Protein Type 4 (MFAP4) Associates With Aortic Dissection in Marfan Syndrome

Wanga S, Yin X, Skroblin P, Franken R, Koolbergen DR, Groenink M, Zwinderman AH, de Vries CJ, Mulder BJ, Reinhardt D, Mayr M, de Waard V

McGill Univ, Montreal, QC.

Aim: Marfan syndrome is a disorder with mutations in the fibrillin-1 gene, leading to elastic fiber degradation and increased TGF-beta signaling. The life-threatening feature of Marfan is aneurysm formation with a risk of fatal aortic dissections. In a proteomics screen, we identified MFAP4, an elastic fiber protein, to be increased in the Marfan aorta. We aim to study the role of MFAP4 in Marfan aortic disease. Methods and results: MFAP4 co-localizes in the aorta with elastin and collagen fibers. In vitro experiments show that MFAP4 expression is upregulated by TGF-beta, which could explain the increased MFAP4 protein levels in the Marfan aorta. Plasma MFAP4 levels correlate with aortic root diameter in Marfan patients (r 0.30, p 0.01). High plasma MFAP4 associates with poor dissection-free survival, with all type B dissections occurring in the upper tertile (Figure 1). The aortic distensibility, as measure for aortic stiffness and damage, was calculated throughout the aorta. The aortic distensibility in the descending thoracic aorta, where type B dissections occur, is lower in Marfan patients with high plasma MFAP4. Conclusion: High plasma MFAP4 predicts type B aortic dissections.

P-19: Contribution of The Posterior Joint Capsule to Reversibility of Knee Flexion Contractures.

H Zhou1,2, G Trudel, MD1, HK Uhthoff, MD1 and O Laneuville, PhD1,2.
1.Bone and Joint Research Laboratory, Faculty of Medicine, Univ of Ottawa, 451 Smyth Road, Ottawa, ON; 2. Dept of Biology, Faculty of Science, Univ of Ottawa, 30 Marie Curie, Ottawa, ON.

A joint contracture is defined as a limitation in the passive range of motion (ROM) of a joint. For the knee joint, full extension aligns the femur and tibia to 180 degrees, but when a contracture is present, gait is abnormal and the patient mobility is reduced. Previous research has identified the posterior knee capsule as one culprit causing immobility-induced knee flexion contractures with little potential for spontaneous reversibility. However, much of the mechanisms regarding spontaneous reversibility and molecular pathways involved remains unknown. This study aimed to provide quantitative measures of the posterior capsule synovial layer length in knee flexion contractures after various durations of unassisted recovery. For this, 250 male Sprague-Dawley rats had one knee surgically immobilized in flexion with a Delrin® plate at a 135° angle for one of six durations: 1, 2, 4, 8, 16, or 32 weeks. The contralateral knee constituted as the control. Immobilization was lifted by removing the plate and the rats were allowed free activity for one, two, and four times the duration of immobilization. Following remobilization, the posterior capsule length increased at all time points, but experimental capsules remained shorter than the contralateral. The separation of the femoral and tibial posterior capsule revealed that changes in posterior capsule length were largely due to changes in the femoral capsule. This research contributes to the efforts at understanding and designing treatments to improve the reversibility of joint contractures and supports the rationale for targeting the posterior knee capsule.

P-20: Blocking Toll-Like Receptor 4 Activation Decreases Disc Degeneration and Pain in a Mouse Model

E Krock1,2, M Millecamps3,4, LS Stone2-4 and L Haglund1,3,5
1.Orthopaedic Research Laboratory; 2.McGill Scoliosis and Spine Research Group, Faculty of Medicine; 3.Alan Edwards Centre for Research on Pain; 4.Faculty of Dentistry, McGill Univ; 5.The Shriners Hospital for Children, Montreal, QC.

Introduction: Intervertebral disc degeneration is a leading cause of chronic low back pain, but no disease-modifying drugs exist. During degeneration cytokines, neurotrophins and proteases increase and the extracellular matric (ECM) is proteolytically degraded and fragmented, ultimately resulting in pain. In discs, toll-like receptor (TLR) activation increases cytokines, neurotrophins and proteases. TLRs are activated by endogenous alarmins, such as fragmented ECM found in degenerating discs. With increasing evidence suggesting a role for TLRs, we hypothesized TLR4 inhibition will reduce disc degeneration and pain in SPARC-null mice. Aging SPARC-null mice have progressive disc degeneration and behavioral signs of back pain compared to wild-type mice.
Methods: 7-month old wild-type and SPARC-null mice were injected i.p. with TAK-242, a TLR4 specific inhibitor or vehicle, 3 times/week for 8 weeks. Behavior was assessed weekly. Radiating pain was measured by hind paw acetone evoked behavior. Axial pain was measured by grip strength. After 8 weeks lumbar discs were excised and cultured for 48 hours. Conditioned culture medium was assessed by protein array. Lumbar spinal cords were stained for the pain-related markers CGRP (pain neurotransmitter) and GFAP (astrocytes). Results: SPARC-null mice display increased axial and radiating pain compared to wild-type mice, which TLR4 inhibition decreases after 6 and 8 weeks of treatment. Compared to wild-type, lumbar discs from SPARC-null mice secrete more proinflammatory cytokines including IL-1β and TNFα, which TLR4 inhibition decreases. CGRP and astrocyte staining increase in SPARC-null mice compared to wild-type, which TLR4 inhibition decreases. Conclusions: Continuous TLR4 inhibition reduces back pain behavior and pain signaling, likely by acting on discs to reduce proinflammatory cytokine secretion. Furthermore, TLR4 inhibition decreases CGRP, indicating there is decreased pain signaling from the periphery (i.e. degenerating discs). These results indicate TLRs are potential therapeutic targets to slow disc degeneration and reduce chronic low back pain.

P-21: The Effect of Tungsten On The Intervertebral Disc: an in Vivo Study

MP Grant1,2, A Bolt1,2, Y Alsaran1,2, LM Epure2, A Papanastasiou2, J Antoniou1,2, KK Mann1,2 & F Mwale1,2

1. McGill Univ, Montreal, QC; 2. SMBD-Jewish General Hospital, Lady Davis Institute for Medical Research, Montreal, QC

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. Although the toxicity of tungsten is not well described, recent reports have demonstrated carbide-cobalt nanoparticles, a tungsten-based alloy commonly used commercially in the manufacturing of industrial goods, induced cell death and genotoxicity in a human liver and renal cell line. 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 the intervertebral disc. Methods: Five week old C576BL/6 mice were given regular tap water (Control) or water supplemented with 15 ppm sodium tungstate for 1 month. Discs were dissected and processed for proteoglycan and collagen content. Portions of the lower spine were fixed and processed for histology. Bovine nucleus pulposus (bNP) and annulus fibrosus (bAF) cells were prepared for 3D culturing in alginate beads. 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. Bovine IVD organ cultures were also prepared and cultured for 1 month in 15 ppm sodium tungstate using the PrimeGrowthTM organ culture system. Results: Tungsten dose-dependently decreased the synthesis of proteoglycan in in vivo discs (n = 8). The effect was also observed in bNP cells (n = 4). Although collagen content was increased in both discs and cells, this was the result of upregulation of Col I and not Col II, as determined by gene expression and immunohistochemistry (n = 8). Disc height in tungsten treated mice was significantly lower than control; however, cell viability was not affected. Discussion: We provide evidence that tungsten affects matrix protein synthesis in the IVD, possibly enhancing disc fibrosis. Tungsten toxicity may play a role in degenerate disc disease.

<u>P-22:</u> Cytokine Treatments Reveal Novel Gene Expression Signatures in Primary Human Cells Derived From Rotator Cuff Disease.

L Robinson1,2,4, **AM Pena-Diaz**1,2, GS Athwal1,2,3, KJ Faber1,2,3 & DB O'Gorman1,2,3,4

1. Molecular Cell Biology Laboratory, Roth McFarlane Hand & Upper Limb Centre; 2. Lawson Health Research Institute, London, ON; 3. Depts of Surgery; 4. Biochemistry, University of Western Ontario, London, ON.

Rotator cuff disease (RCD) is characterized by spontaneous tearing of rotator cuff tendons and affects 25-50% of the elderly. Tendinosis, or disease-associated loss of tendon strength and integrity, is a common problem for RCD patients and places significant limitations on post-operative success during surgical cuff repair. It is thought that insufficient tissue repair processes may contribute to the etiology of pre- and post-operative tendinosis, although pathophysiology is poorly understood. We hypothesized that tendon cells from diseased supraspinatus tendons exhibit maladjusted signaling responses to early- and late-stage inflammatory cytokines during tissue repair, and that cytokine-induced changes in gene expression are modulated by oxygen levels in culture. Primary human cells derived from torn supraspinatus tendons (RCD) or intact subscapularis tendons designated "aged rotator cuff (ARC)" and "normal rotator cuff (NRC)", were cultured on collagen lattices in atmospheric (21%) or physiological (2%) oxygen. The cultures were treated with cytokine regimens comprising TNFα, IL-1ß, IFNγ (C1) and/or TGFß1, IL-4, IL-10 (C2) to mimic the early and late inflammation stages of tissue repair respectively. qPCR of tissue repair-associated genes was performed. Oxygen levels did not significantly affect the expression of tissue-repair associated genes although cultures in 2% oxygen exhibited modest reductions independent of cytokine treatment. RCD cells showed a significant different response compared to ARC and/or NRC cells: upregulation of the myofibroblast-associated gene ACTA2 with C2 treatments and downregulation of the tenocyte marker SCX with C1 and C2 treatment combination. Additionally, the extracellular matrix- related gene WISP1 was upregulated only in RCD cells treated sequentially with C1+C2 compared to control, but not in ARC and NRC cells. These findings suggest that RCD cells exhibit distinct molecular signatures in response to cytokines during tissue repair compared to healthy tenocytes. Insights into cytokine-induced changes in gene expression may help to inform post-operative prescriptive practices.

P-23: Ca2+ and PO4 Regulate Matrix Protein Synthesis in Intervertebral Disc Cells.

Y Alsaran1,2, MP Grant1,2, LM Epure2, A Papanastasiou2, J Antoniou1,2, F Mwale1, 2
1. Div of Surgery, McGill Univ, Montreal, QC; 2. Lady Davis Institute for Medical Res, McGill Univ, Montreal, QC.

INTRODUCTION: Degenerative disc disease (DDD) is a common cause of lower back pain. Calcification of the intervertebral disc (IVD) has been correlated with DDD, and is especially prevalent in scoliotic discs. The appearance of calcium deposits has been shown to increase with age, and is the product of free calcium (Ca2+) and phosphate (PO4). Our preliminary data suggest that ionic calcium and PO4 content are increased from [2.0 – 8.0 mM] and [1.0 – 4.0 mM], from mild to severe degenerative in human IVDs, respectively. The roles of these individual ions on IVD function and degeneration is unknown. In this study, we evaluated the effects of Ca2+, PO4, and their combination on IVD nucleus pulposus (NP) and annulus fibrosis (AF) cells. METHODS: Bovine NP and AF cells were prepared for 3D culturing in alginate beads. Beads were cultured in medium containing increasing Ca2+ [1.0, 3.0, 6.0 mM], PO4 [1.0, 2.0, 4.0 mM], or their combination and cultured for 12 days. The DMMB and hydroxyproline assays were performed on the beads to determine proteoglycan and collagen content. Gene expression for matrix proteins was determined by qPCR. 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 growth in beads at the end of the incubation period was determined using Quant-iT dsDNA Assay Kit (Thermo Fisher Scientific). RESULTS: Using similar Ca2+ concentrations found in degenerate discs [1.8 – 6.0 mM], Ca2+ dose-dependently decreased matrix protein synthesis of aggrecan and Col II in NP and AF cells (n = 4). Although modest effects were observed on matrix protein synthesis with increasing PO4, it significantly affected cell growth. The combination of P04 and Ca2+ synergistically decreased aggrecan and Col II (n = 4). Interestingly, direct activation of the calcium-sensing receptor (CaSR) with cinacalcet and PO4 had similar effects (n = 3). DISCUSSION: Our results suggest that changes in the local concentrations of calcium and P04 are not benign, and that the combination of these ions affects IVD cell growth and matrix protein synthesis. Inhibiting CaSR may provide a novel therapeutic in disc degeneration.

P-24: The Role of Toll Like Receptor 2 Dimers in Isolated Human Disc Cells

JB Currie & L Haglund.
McGill Univ, Montreal, QC.

Low back pain (LBP) is the leading cause of disability worldwide and the main cause of chronic LBP is intervertebral disc (IVD) degeneration. A hallmark of disc degeneration is the breakdown of the extracellular matrix, creating fragmented proteins. These fragmented proteins can be recognized by toll-like receptors (TLRs), cell surface receptors that were originally characterized in innate immunity. TLRs were recently shown to play a role in disc degeneration – our lab has shown that activation of TLR2 in human disc cells leads to an increase in inflammatory (IL1b) and nociceptive (NGF) factors and others have found that TLR2 is upregulated in disc degeneration. In order for TLR2 to become activated, it must dimerize with either TLR1, TLR2, or TLR6. However, the dimers involved in the regulation of inflammatory cytokines, neurotrophins, and proteases associated with disc degeneration are unknown and will be investigated in this study. Non-degenerate discs were obtained from the lumbar spines of Transplant Quebec organ donors and IVD cells were isolated. We evaluated TLR2 dimers by using specific TLR2/TLRx agonists, PGN (TLR2/2), Pam2CSK4 (TLR2/6) and Pam3CSK4 (TLR2/1). After challenging cells with either PGN, Pam2CSK4, or Pam3CSK4, RNA was collected and RT-qPCR was used to analyze IL-1β and NGF gene expression. When compared to untreated cells, cells challenged with PGN and Pam2CSK4 had increased gene expression of IL1b and NGF, while cells challenged with Pam3CSK4 did not. Following these results, we challenged cells with either Pam2CSK4 of IL1b and visualized TLR2 and TLR6 protein expression using immunocytochemistry. Preliminary results show that non-degenerate IVD cells treated Pam2CSK4 or IL1b express more TLR6 when compared to untreated cells. Preliminary results are similar for TLR2. Increased understanding of TLR dimers and their role in disc degeneration may provide a link between matrix breakdown and progressive inflammation that can be targeted therapeutically.

<u>P-25:</u> Tomographic and Histological Analysis of Ectopic Calcification in Diffuse Idiopathic Skeletal Hyperostosis (DISH)

DE Fournier, RJ Beach, CS Bailey, KE Willmore, CJD. Norley, DW Holdsworth, SJ Dixon & CA Séguin1,2
1. Anat and Cell Bio, Schulich School of Medicine & Dentistry, Bone and Joint Inst, The Univ of Western ON; 2. Physio and Pharma, Schulich School of Medicine & Dentistry, Bone and Joint Inst, The Univ of Western ON.

Diffuse idiopathic skeletal hyperostosis (DISH) is a non-inflammatory spondyloarthropathy characterized by calcification of the spinal ligaments and intervertebral disc (IVD) tissues. The Arthritis Society lists DISH as the second most common form of arthritis, with an estimated prevalence of 15-25% in North Americans over the age of 50. Despite its prevalence, the pathology of DISH is poorly understood and there are no disease-modifying treatments. The present study aims to characterize the pathological features associated with ectopic spine calcification. A cohort of 19 embalmed cadavers (13 male, 6 female; mean age 81 years, range 65-95) were dissected from the HEART Lab at Western University. Vertebral columns (cervical-thoracic) were scanned by μCT (150 μm isotropic resolution). A variety of image outputs were generated to: i) diagnose DISH using the current clinical standard; ii) identify features of ectopic calcification in DISH; and iii) assess the ability of various imaging modalities to detect these features. Histology and physical analyses were used to elucidate the tissue-specific changes associated with DISH. Eight (42%) specimens (6 male, 2 female; mean age 84 years) met the diagnostic criteria for DISH. Analysis of these spines identified remarkable heterogeneity in the presentation of ectopic calcification (mineral density and morphology) falling into 3 broad categories: i) continuous bands resembling cortical bone; ii) overt prominences of cortical and trabecular bone associated with IVDs; or a mixture of i) and ii). Analysis of calcified material by EDX showed high levels of calcium and phosphorous in a ratio of 1.5, slightly less than the stoichiometric ratio for hydroxyapatite. Histological examination of DISH specimens revealed histopathological features of both mature trabecular bone (outermost tissue) and irregular amorphous material (within the annulus fibrosis). Findings from this study will contribute to defining classifications of DISH as well as pathological changes associated with spinal ectopic calcification.

P-26: Transglutaminase Activity Regulates Differentiation, Migration and Fusion of Osteoclasts via Affecting Actin Dynamics and RhoA Activity

Sun H1, Kaartinen MT1,2

1. Faculty of Dentistry, McGill University, Montreal, QC, Canada; 2. Faculty of Medicine, Division of Experimental Medicine, McGill University, Montreal, QC, Canada

Osteoclasts are multinucleated macrophage lineage cells capable of resorbing mineralized bone. Increased osteoclast activity causes bone loss, i.e., osteopenia. Transglutaminases (TG) are a family of structurally and functionally related enzymes that catalyze the Ca2+-dependent crosslinking of proteins through introducing an isopeptide bond between a lysine and a glutamine residue. TG family is comprised of TG1-TG7 and Factor XIII-A (FXIII-A). TG2 and FXIII-A are both expressed in monocyte/macrophage lineage cells; however, their expression in osteoclasts and their potential role during osteoclastogenesis and osteoclast resorption have not so far been explored. To address the role of TGs in osteoclasts, we used murine bone marrow-derived macrophages (BMMs) which were differentiated into osteoclasts with M-CSF and RANKL. We report here that both macrophages and osteoclasts express mRNA of TG1, TG2 and FXIII-A. Immunofluoresence microscopic analysis showed all the three enzymes co-localized with podosomes in osteoclasts. To examine the role of TG activity in osteoclastogenesis, BMMs were treated during the osteoclastogenesis with NC9 - an irreversible TG inhibitor. Osteoclast size was decreased dramatically with low concentrations of NC9 and osteoclast differentiation was blocked completely with higher concentrations. When NC9 was added to the osteoclast precursors at different stages, it inhibited the differentiation, migration and fusion of pre-osteoclasts. Consistently, resorption pit assay showed that osteoclast resorption activity was inhibited by NC9 treatment. In addition, osteoclast podosome belt formation was found to decrease when treated with NC9 suggesting that TG activity regulated actin dynamics in osteoclast precursors. Finally, the levels of RhoA, regulator of actin dynamics and podosome belt/sealing zone formation, was found significantly elevated in NC9 group compared to control group and the inhibitory effect of NC9 on osteoclastogenesis was reversed by RhoA inhibitor. Taken together, our data suggests that TG activity regulates osteoclastogenesis via affecting cytoskeletal & actin dynamics and RhoA activity.

P-27: The Population Analysis of Space-Travellers After 55 Years Of Space Travel

T Corlett1, M Stavnichuk2, HP Luan3, L Lee3, SV Komarova4

1.McGill Univ, Montreal, Quebec, Arts Faculty Program Industrial Relations; 2. Dept. of Biochemistry; 3.Dept. of Anatomy & Cell biology; 4.Dept. of Dentistry, Shriners Children's Hospital.

Since the first instance of human space travel in 1961, many space missions have been completed, demonstrating the ability of humans to successfully function in the extra-terrestrial environment. Nevertheless a number of microgravity-related health issues, including bone loss, remain. We analyzed the population of all space-travelers with an emphasis on data suitability for bone-related studies. State-funded space agencies were consulted as primary sources, and third-party websites were used as secondary sources. To date, 551 humans (491 males/60 females) have travelled to space. The age of the average space-traveller increased from 34±4 in the 1960s to 45±4 in the 2010s. The proportion of astronauts accomplishing multiple flights, as well as the duration and frequency of EVA, have increased. Thus, space travelers represent a diverse population suitable for comparative analysis. However, the reported populations in bone-related papers vary from 1 to 38 people and no large-scale statistical analysis of the changes in bone in microgravity has been published. The small size of the population necessitates careful handling of space travelers' personal medical records. To examine the degree of data confidentiality, we attempted to identify space-travelers from the data disclosed in the paper. In the manuscripts published prior to 2000 it was often possible to uniquely identify space travelers based on presented data (such as age, mission name and duration). However, in recent studies we did not achieve better than 1 out of 10 identification, indicating that more rigorous ethical protocols are now successfully employed. We suggest the importance of state-independent oversight in order to centralize data processing, to ensure the privacy of former and active space travelers, and to supply the research institutes and the public alike with complete, rigorously verified data and analysis.

P-28: Cellular MicroRNA Regulation By Fibrillin-1 and Fibronectin Is Mediated Through Integrins

R Zhang1, K Zeyer1, H Kumra1, DP Reinhardt1,2
1. Faculty of Medicine & 2. Faculty of Dentistry, McGill Univ, Montreal, QC.

Background and Aim: Fibrillin-1 interaction with cells via RGD-dependent integrins is essential for tissue integrity. Defects of fibrillin-1 has been linked to diseases that affect the skeletal, cardiovascular, and ocular systems, including Marfan syndrome, Weil-Marchesani syndrome, geleophysic and acromicric dysplasia. Fibronectin is an important regulator of the assembly of fibrillin-1 containing microfibrils. Fibrillin-1 contains one evolutionarily conserved integrin binding Arg-Gly-Asp (RGD) sequence in its fourth TB domain, and fibronectin possess a RGD sequence in the 10th type III domain. MicroRNAs (miRNAs) are small non-coding RNA molecules with critical functions in post-transcriptional regulation of gene expression. This study addresses how cell interaction of fibrillin-1 and fibronectin regulates gene expression through miRNAs.
Experiments and Results: Human skin fibroblasts (HSFs) attached differently to plates coated with the RGD wild-type fragments of fibrillin-1 and fibronectin in comparison to the RGA-containing mutants. The interaction of HSFs with the RGD sequence of both, fibrillin 1 and fibronectin, showed increased proliferation. Surprisingly, a microarray analysis displayed differential expression of many miRNAs and mRNAs after 24 h of HSFs grown on the fibrillin-1 wild-type and RGA fragments. Differential miRNA expression occurred as early as 2 hours of cell interaction. Pathway analysis indicated that the differentially expressed miRNAs act together in regulating cell adhesion, migration and growth factors. Among the differentially expressed miRNAs, miR-1208 expression was inhibited when HSFs were seeded on fibrillin-1 and fibronectin wild-type fragments. Moreover, miR-1208 showed higher expression in the HSFs interacting with wild-type fibrillin-1 compared to fibronectin. Inhibition of miR-1208 partially rescued the proliferation rate and the cell morphology of HSFs seeded on RGA-mutant fibrillin-1 and fibronectin. Among the predicted targets of miR-1208, extracellular signal-regulated kinase (Erk) was shown to be negatively correlated with miR-1208 expression. Overexpression and knockdown of miR-1208 inhibited and promoted Erk signaling, respectively. miR-1208 was further found to be necessary in fibroblast to myofibroblast differentiation. In summary, our analyses systematically investigated the miRNA profile change triggered by HSFs' interaction with the RGD cell-binding sequence of fibrillin-1 and fibronectin, and reveal miRNA involvement in proliferation and differentiation. These results shed new lights on the outside-in signaling of matrix proteins by regulating miRNAs.

P-29: Osteopenia in Marfan Syndrome: Role of Osteocytes

EA Zimmermann1*, **K Tiedemann**1*, C Julien1, DP Reinhardt2, S Komarova1,2 & B Willie1,2
*1. Shriners Hospital for Children, Montreal, QC; 2. McGill University, Montreal, QC. * Co-first authors*

Introduction: Bones have the capacity for self-repair and adaptation to changing mechanical loads. Osteocyte bone cells embedded in the bone matrix are thought to play a major role in sensing mechanical signals and coordinating bone formation and resorption. Thus, an altered osteocyte network morphology may directly affect bone's mechanoresponsiveness, with subsequent imbalances in bone formation and resorption contributing to low bone mass (osteopenia). Marfan syndrome is a genetic disorder exhibiting osteopenia due to a genetic defect in fibrillin-1; however, the role of osteocytes in Marfan syndrome has not been investigated to date.
Hypothesis: Osteocytes contribute to the osteopenic phenotype of Marfan syndrome because of their abnormal lacuno-canalicular network, density and behavior. Methods: We used the Fbn1C1039G/+ mouse model of Marfan syndrome, in which heterozygous mice have a missense mutation in fibrillin-1. Using the femur and tibia of 8-week-old Fbn1C1039G/+ and wild type mice, we characterized bone morphology, osteocyte lacunar density and mineralization with μCT; whole bone mechanical properties with three-point bending; and evaluated osteoclast and osteocyte marker genes using real-time qPCR. Results: Consistent with the Marfan phenotype, μCT and real-time qPCR evaluations show that Fbn1C1039G/+ mice are indeed osteopenic, suffer from long bone overgrowth and have a higher RANKL/OPG ratio compared to wild type. Furthermore, mechanical tests show trends towards lower elastic modulus and post-yield displacement in Fbn1C1039G/+ compared to wild type, which is consistent with lower mineralization in Fbn1C1039G/+ mice. Osteocyte lacunar densities measured with μCT were similar, but real-time qPCR showed lower Wnt1 and Lef1 expression in Fbn1C1039G/+ mice compared to wild type. Conclusion: Fbn1C1039G/+ mice exhibit osteopenia and long bone overgrowth consistent with the Marfan syndrome phenotype. Abnormal osteocyte signaling may contribute to altered mechanical properties observed in Fbn1C1039G/+ mice. Future work will investigate whether osteocytes' mechanosensing ability can be exploited to reduce osteopenia in Marfan syndrome.

P-30: Pulmonary Arginase-1+ Macrophage Accumulation Is Elevated in OSM-Induced Lung Inflammation

A Dubey, F Botelho, E Ayaub, L Izakelian, S Wong & CD Richards
McMaster Univ Immunology Research Centre, Dept of Pathology and Molecular Medicine, Hamilton, ON.

INTRODUCTION: Arginase-1 (Arg1) expression in mouse lung has largely been associated with progression of pathological wound healing or fibrotic processes. We have previously demonstrated that overexpression of Oncostatin M (OSM) induces pulmonary Arg1 expression and Arg1+ macrophage accumulation in murine models. Similarly, overexpression of OSM increases pulmonary IL-33 expression, which is a Th2-associated cytokine and alarmin. Here we further characterize the increased Arg1+ cell population in vivo and investigate potential effects of OSM-induced IL-33 expression on Arg1 enzymatic activity in vitro. METHODS: Adenovirus encoding OSM or IL-6 (AdOSM/AdIL-6) was administered to C57Bl/6 mice and were culled at day 7. Arg1 protein or mRNA levels in lung homogenates were assessed by immunohistochemistry, immunoblots and RT-PCR. Additionally, Arg1+ cell populations in the lung was identified using flow cytometry. Bone marrow derived macrophages (BMDM) were generated following 7 days in culture with MCSF and stimulated with IL-33, alone or in combination with M1/M2 cytokine cocktails. Arg1 enzymatic activity in vitro was measured by urea levels. RESULTS: Mice treated with AdOSM showed enhanced Arg1 protein and mRNA levels in whole lung homogenates, in comparison to AdDel70 or AdIL-6. Furthermore, flow cytometry data demonstrated enhanced levels of Lin+/F4-80+/Arg1+ cells in the lungs of AdOSM treated mice, in contrast to IL-6 overexpression. However, Arg1 protein expression induced by AdOSM was attenuated in IL-6-/- mice, indicating a requirement for IL-6 signalling by OSM in vivo. Additionally, preliminary results in vitro suggest that IL-33 (5ng/mL or 20ng/mL at 24 hours) enhances Arg1 enzymatic activity in BMDMs through direct stimulation of these cells. CONCLUSIONS: Thus, OSM functions to induce Arg1+ macrophage accumulation in models of airway inflammation in C57Bl/6 mice, which is mediated through its elevation of IL-6 in vivo. Additionally, preliminary data suggests that IL-33 up-regulation by OSM may also contribute to M2-like macrophage polarization in mouse lung.

P-31: Bone Loss in Space Travellers: Systematic Review and Meta-Analysis

M Stavnichuk, HP Luan, T Corlett, L Lee, M Morris, SV Komarova.
McGill Univ, Montreal, Quebec, Canada

Bone loss in astronauts is identified as a major challenge for long-duration space exploration. With the goal to examine the spatial and temporal aspects of microgravity-induced bone loss, the electronic databases Medline, Embase, PubMed, BIOSIS Previews, and Web of Science were searched for studies presenting numerical values for measurements of bone health in humans who traveled to space. We identified 29 studies containing bone density estimates using X-ray radiography, ultrasound, dual-energy X-ray absorptiometry, quantitative computed tomography or magnetic resonance imaging before and immediately after the space flight that lasted from 4 to 438 days. Measurements were available for 46 individuals and groups of 4 to 32 space travellers. While it was difficult to ensure that the same data were not included in different groups, we estimated that 124 of 551 astronauts who travelled to space before June 2016 were represented. We combined the data on percent change in bone density after the space flight of any duration in different regions of the skeleton. Weighted average changes in bone density in the region of head, shoulders and cervical vertebrae was 2.3+/-0.5% (n = 34); in the region of forearms, thoracic vertebrae and ribs: -1.2+/- 2.0% (n = 73); in the region of lumbar vertebrae and pelvic bones: -8.4+/- 3.0% (n = 119); and in the region of lower extremities: -5.3+/-3.2% (n = 124). Next we examined the changes in the heal bone in 46 space travellers as a function of time they spent in microgravity (4 to184 days). Bone changes were very heterogeneous among individuals, averaging -3.6+/-5.2% with extremes at -19.8% and +12%. While a weak negative correlation between time spent in microgravity and bone change was observed (R=-0.22), it was not statistically significant. Thus, bone loss due to microgravity is very heterogeneous and characterized by distinct spatial and temporal changes.

P-32: Fibrillin-1 Directly Regulates Adipocyte Differentiation and Expansion

TJ McKee1,3, K Tiedemann1,3, DP Reinhardt1,2, SV Komarova1,2,3.
1. Faculty of Dentistry, McGill University, Montreal; 2. Dept of Anatomy & Cell Biology, Faculty of Medicine, McGill University; 3. Shriners Hospitals for Children - Canada, Montreal

Marfan syndrome (MFS) is a heritable disease caused by mutations in an extracellular matrix protein fibrillin-1, which affects the cardiovascular, ocular, and skeletal systems. Fibrillin-1 expression was shown to correlate with obesity and increased adipocyte size in women while MFS is associated with lipodystrophy. Our goal was to examine the effect of fibrillin-1 on adipocyte differentiation and expansion. Mouse bone marrow-derived mesenchymal cells were cultured for 12 days with an adipogenic cocktail (insulin, dexamethasone, isobutylmethylxanthine, and indomethacin) with or without recombinantly produced fibrillin-1 N-terminal (F1N) and C-terminal (F1C) halves. Adipocytes were identified with the lipophilic dye Oil Red O, and adipocyte count, size, and lipid droplet count were evaluated. Both F1N and F1C significantly reduced adipocyte counts. While maximum inhibition of adipogenesis was achieved when fibrillin-1 halves were present for the duration of the experiment, significant decrease in adipocyte numbers was also evident F1N and F1C were present during the first 3 days of differentiation. The size of individual adipocytes was significantly increased in the presence of fibrillin-1 fragments, especially when F1N and F1C were present during days 4-12, the expansion phase of adipogenesis. F1N and F1C decreased the expression of adipose markers, peroxisome proliferator activator receptor-γ (Pparg) and adiponectin (Adipoq), but not that of lipoprotein lipase (Lpl). Finally, we characterized the adipose phenotype of the C1039G murine model for MFS with a mutation in the gene coding for fibrillin-1. C1039G mice tended to be lighter than their wild type counterparts, however had more interscapular brown fat and inguinal white fat in proportion to their body weight compared to wild type littermates. Our data suggest that fibrillin-1 can directly regulate adipocyte differentiation and expansion. Our results have the potential to translate into improved therapeutic strategies for correcting obesity and the significant societal burden it imposes.

P-33: Further Characterization of the Laminin Receptor 37/67LR in Colorectal Cancer Cells

G Cloutier, T Khalfaoui & JF Beaulieu.
Laboratory of Intestinal Physiopathology, Dept of Anatomy & Cell Biology, Faculty of Medicine and Health Sciences, Univ de Sherbrooke, Sherbrooke, QC.

The 37/67 laminin receptor (37/67LR) was the first non-integrin cell surface receptor for laminin to be discovered in the 1980's. Initially, the human gene for 37/67LR encoded one protein of 37 kDa named RPSA (ribosomal protein SA) associated with the small ribosomal subunit. In human cells, RPSA allows the formation of the polyribosome complex and plays a key role in the initiation of translation. The mechanism by which the ribosomal protein becomes the 37/67LR membrane receptor is still unclear. It is presumed that the process involves post-translational modifications combined with homo or hetero-dimerization with non-associated ribosome proteins. It has been shown that 37/67LR regulates adhesion and proliferation of normal human intestinal epithelial cells. Interestingly, overexpression of 37/67LR is correlated with aggressiveness and a poor prognosis in a wide variety of cancers. The aim of this study was to confirm the overexpression of the 37/67LR in the membrane of colorectal cancer cells. Our results show that a partial knockdown of 37/67LR expression with siRNA used at a dose that does not impact the ribosomal protein and translation process, affects cell adhesion to laminins which suggests a significant reduction in the expression of 37/67LR at the membrane. To confirm the presence of 37/67LR at the membrane we isolated the membrane from the ribosomal fraction. To do this we used a modified brush border extraction protocol to separate ribosome-containing fractions from the membranes and combined it with ultracentrifugation and detergent treatment to isolate the membrane associated 37/67LR. Further studies will be needed to characterize the membrane-associated form of 37/67LR and to test its ability to act as a receptor in colorectal cancer cells.

P-34: Fragmentation of SLRPS in Facet Joint Articular Cartilage From Patients With Adolescent Idiopathic Scoliosis.

P Lama1,2, D Bisson1, DH Rosenzweig2, P Roughley1, JA Ouellet2, L Haglund1,2
1. Shriners Hospitals for Children, Montreal, McGill Univ QC; 2. Montreal General Hospital, McGill Univ, Montreal, QC.

Introduction: Adolescent idiopathic scoliosis (AIS) is a triplanar deformity of the thoracolumbar spine which affects vertebrae, intervertebral discs and apophyseal (facet) joints. Degenerated articular cartilage has been shown to express increased levels of cytokines and matrix-degrading enzymes. We hypothesize that such changes within scoliotic facet cartilage leads to fragmentation of SLRP`s (Small Leucine Rich Proteoglycans) and fibronectin. Materials and Methods: Surgically removed scoliotic facets (n=20) were collected from patients aged 12-19 years. Articular facet cartilage was digested in 4 M Guanidium hydrochloride for 72 hours, precipitated in ethanol, and treated with keratanase and chondroitinase ABC for 12 hours. These samples were then analyzed with western blots using antibodies to biglycan, decorin, lumican, chondroadherin and fibronectin (1:1000). 12 um thin cartilage sections were stained with safranin-O and Fast Green for histology. Results: Fragmentation of SLRP's and fibronectin was greatest in tissues from patients with severe deformities. Fragmentation was most prominent at 36 Kda for biglycan, lumican and decorin, while fibronectin and chondroadherin fragments appeared between 35 to 22 Kda. Histology showed chondrocyte accumulation and formation of small, medium and large size cell clusters, and proteoglycan loss from the superficial zone of the tissue. Conclusions: Accumulation of SLRP's and fibronectin fragments in the cartilage matrix may activate TLR or other cellular receptors. Increased fragmentation of SLRP`s and fibronectin may also serve as potential biomarkers for cartilage degradation in AIS.

P-35: FBLN4 and LTBP4 Cell Receptor Interactions

H Hakami1, A Pagliuzza1, J Djokic1, K Lee1, C Syng Lee1, DP Reinhardt1,2
1.Faculty of Medicine and 2. Faculty of Dentistry, McGill Univ, Montreal, QC.

Fibulin-4 (FBLN4) and latent transforming growth factor-β binding protein-4 (LTBP4) are important elastogenesis accessory proteins. Mutations in FBLN4 and LTBP4 cause autosomal recessive cutis laxa type 1B and type 1C, respectively, in human. FBLN4-/- and LTBP4-/- mice are characterized by high perinatal/neonatal mortality and severely affected elastic fibers, suggesting their essential functions in survival and elastogenesis. 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 fibroblasts bind strongly to LTBP4. We further demonstrate the functionality of FBLN4 multimerization in cell binding. FBLN4 multimers, but not monomers, interact with cells. 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. However, 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, but not LTBP4, is entirely abolished. Our results exclude heparan-sulfate proteoglycans as LTBP4 cell binding receptors. These data establish a new paradigm for FBLN4 and LTBP4 role in elastogenesis. The determination of the FBLN4 and LTBP4 cell receptor(s) identity will reveal their significance in elastic fiber formation and homeostasis.

P-36: Dystroglycan: An Extracellular Matrix Receptor Worth Discovering

A Sallenbach-Morrissette, J Auclair & JF Beaulieu.
Dept of Anatomy & Cellular Biology, Faculty of Medicine and Health Sciences, Univ. de Sherbrooke

Dystroglycan is a protein encoded by the gene DAG1 (Dystrophin Associated Glycoprotein 1). It was first discovered in the muscle as part of the Dystrophin-associated protein complex. After post-translational modification, the protein dystroglycan is cleaved into two subunits; alpha-dystroglycan which is extracellular and beta-dystroglycan which is transmembrane. Dystroglycan links the extracellular matrix to the actin cytoskeleton. A mutation in this protein or an abnormal glycosylation of alpha-dystroglycan can lead to different types of muscular dystrophy such as Limb-Girdle or Duchenne Muscular Dystrophy. Even if the role of dystroglycan is well-known in the muscle, little is known about it in the intestinal epithelium. To start, we confirmed the localisation of the protein in the intestinal epithelium. In the early stages of development (9-12 weeks), dystroglycan is localised at the base of the proliferative epithelial cells and tends to concentrate in the lower half of the crypt at older stages (20 weeks) and in the adult epithelium. We also verified the expression of dystroglycan in two cellular models; it was well-expressed in HIEC (Human Intestinal Epithelial Crypt) cells, an undifferentiated and proliferative intestinal cell line. It was also detected in undifferentiated Caco-2/15 (enterocyte-like) cells. This suggests that the protein dystroglycan is important to maintaining the undifferentiated/proliferative state in the intestinal epithelium. To validate this hypothesis, we used an shRNA to silence the expression of dystroglycan. We then studied the effect of the downregulation of dystroglycan on proliferation, migration and differentiation of the cells. With the preliminary data, we can see a decrease in proliferation which was confirmed by a reduction in BrdU incorporation and a reduced migration in the cells downregulated for dystroglycan. No effect was observed on Caco-2/15 cell differentiation. These preliminary data suggest that dystroglycan is important for the maintenance of homeostasis in the crypts of the intestinal epithelium.

P-37: Role of TLR2 in Facet Joint Cartilage Degeneration in Adolescent Idiopathic Scoliosis

D Bisson1,2, P Lama1,2, DH Rosenzweig1, JA Ouellet1,2 and L Haglund1,2
1.The Orthopaedics Research Laboratory, Dept of Surgery, McGill Univ, Montreal, Qc; 2. Shriner's Hospital for Children, Montreal, QC.

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 extra-cellular matrix components (alarmins), TLR2 receptors in disc cells initiate the production of inflammatory cytokines and proteases. In this study, we investigate the potential role of TLR2 receptor activation in facet joints from AIS patients as a cause of early degeneration. Cartilage explants from consenting donors were isolated from the subchondral bone and cultured in the presence and absence of a TLR2 agonist (Pam2CSK4) in chondrocyte media, which was analyzed by mass spectrometry and ELISA for degenerative marker secretion. After the culture period, the cartilage was cryosectionned and stained with SafraninO – Fast green dyes to reveal proteoglycan content. Presence of alarmins to activate TLR2 in the cartilage was assessed using a reporter cell line expressing TLR2 (hTLR2-HEKblue) with a colorimetric assay. In the presence of the TLR2 agonist, gene and protein expression analysis showed elevated levels of 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. Finally, explant cultured media successfully activated TLR2 receptors in the reporter cell line. In conclusion, our data show that TLR2 activation lead to a degenerative pathway with the secretion of proteases, inflammatory cytokines and pain-related factors and ultimately proteoglycan loss in the affected cartilage. The presence of alarmins in scoliotic samples suggests TLR2 may have a role in early degeneration of cartilage seen in AIS.

P-38: CCN3 Is Reciprocally Regulated to CCN1 and CCN2 in Human Dermal Fibroblasts.

A Peidl and A Leask.
Dept of Physiol. and Pharmacology, Schulich School of Medicine and Dentistry; Western University, London, ON.

The CCN family of matricellular signaling proteins are emerging as potential therapeutic targets for multiple diseases involving excessive connective tissue deposition, such as the fibrotic disease systemic sclerosis (SSc). CCN2, a proadhesive member of the CCN family, is overexpressed by SSc fibroblasts, resulting in elevated CCN2 in blister fluid. CCN2 is also essential for two mouse models of SSc fibrosis. It has recently been shown that CCN3 is reciprocally regulated to CCN2 expression and may represent a potential endogenous inhibitor of the profibrotic activity of CCN2. Hence, investigating pathways that govern the reciprocal nature of CCN proteins may give insight into their role in fibrotic disease. The activity of specialized fibroblasts known as myofibroblasts are essential for fibrosis. Myofibroblast differentiation is TGFβ-induced and dependent on cell adhesion to the extracellular matrix. Herein, we have used real-time polymerase chain-reaction to evaluate CCN1, 2, and 3 mRNA levels in human dermal fibroblasts in vitro, +/- inhibitors of adhesive signaling. Specifically, inhibitors of focal adhesion kinase (FAK) (PF573228), TGFβ-activated kinase-1 (TAK1) ((5Z)-7-Oxozeaenol), mitogen-activated protein kinase kinase (MEK) (U0126), and yes-associated protein-1 (YAP1) (Verteporfin) were used to assess which pathways are important for CCN expression. Results indicate that baseline CCN3 mRNA levels in dermal fibroblasts decrease in response to TGFβ1, while CCN1 and CCN2 mRNA levels increase, consistent with observations that these proteins function reciprocally. Furthermore, results suggest that TGFβ1-induction of CCN1 and CCN2 is dependent on FAK, TAK1, MEK and YAP1 pathways; conversely, results suggest that CCN3 mRNA expression is independent of FAK, TAK1, MEK and YAP1 signaling in dermal fibroblasts. Gaining a better understanding of mechanisms underlying CCN protein activity is crucial, and may open the door for future therapies. Restoring a correct CCN2:CCN3 ratio (e.g. adding CCN3 or CCN3-derived peptides) may result in novel therapy for SSc fibrosis.

P-39: Inpp4b Regulates Osteoclastogenesis Through The PKCβ/GSK-3β/Nfatc1 Cascade

L Saad, M Pata, J Vacher.
Clinical Research Institute of Montreal, Montreal, QC.

Bone remodeling results from the action of the bone forming osteoblasts and bone resorbing osteoclasts. Disruption of this tightly controlled process can lead to major pathologies such as osteoporosis and osteopetrosis. The murine homolog of the Inositol polyphosphate 4-phosphatase type II (Inpp4b) has been shown to be a negative regulator of osteoclastogenesis via the Nfatc1 signaling pathway. Systemic loss of Inpp4b in the mouse led to enhanced osteoclast differentiation and decreased bone mass resulting in osteoporosis. However, the molecular mechanism through which Inpp4b can regulate the Nfatc1 signaling pathway and osteoclast differentiation remains unclear. 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 as revealed by the 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 negative regulator of Nfatc1 transcriptional activity was inactivated by phosphorylation in the same cells. In addition, pharmacological inhibition of PKCβ decreased GSK-3β phosphorylation and osteoclast formation. Taken together these results suggest that Inpp4b acts as a negative regulator of osteoclastogenesis by controlling the PKCβ/GSK-3β/Nfatc1 signaling pathway.

P-40: Micromotion Dramatically Changes The Gene Expression Profile of The Bone Healing Response Around Implants

R de Barros e Lima Bueno1, AP Dias1, KJ Ponce1, R Wazen1, JA Helms2, JB Brunski2, A Nanci1.
1. University of Montreal, Montreal, QC, Canada; 2. Stanford University, Stanford, CA, USA

INTRODUCTION: When bone implants are loaded, they are inevitably subjected to displacement relative to bone. Such micromotion generates stress/strain states that cause beneficial or detrimental interfacial tissue deformation. The objective of this study is to better understand the mechanobiology of bone healing at the tissue-implant interface and, more specifically, how loading alters the interfacial healing response at the gene level. METHODS: Screw shaped titanium implants 1.7 mm in diameter were placed in 2.0 mm holes in rat tibiae to create a model of gap-healing at an interface. Implants were held stable by a bone plate 1 that allows control over axial displacement and loading. Two regimens were applied, (a) one daily loading session of 60 cycle with an axial force 1.5N/cycle, and (b) two daily sessions. Such a force was estimated to provide an initial displacement of 150 μm, and finite element analysis indicates that it causes strains in excess of 30% at localized regions of the bone-implant interface. Controls consisted of unloaded implants. After 7 days, the implants with surrounding interfacial tissue were harvested using a trephine with an inner diameter of 3.75 mm, and processed for total RNA extraction and purification, followed by Microarray analysis using GeneChip® Rat Gene 2.0 ST Array (Affymetrix, Santa Clara, CA, USA). Analyses of microarray data were performed using Affymetrix® Expression Console™ Software for gene level normalization and signal summarization, and then Affymetrix Transcriptome Analysis Console (TAC) Software for exploration and differential gene expression analysis. Using the PANTHER Classification System (http://www.pantherdb.org/), a 'gene ontology' and molecular classification of the genes/ expressed sequence tags (ESTs) that were up- or downregulated $\geq$ 2-fold was performed for (i) Micromotion 1x/day vs. Unloaded, (ii) Micromotion 2x/day vs. Unloaded and (iii) Micromotion 2x/day vs. Micromotion 1x/day. Comparisons were performed using parametric test for independent data (ANOVA) and the level of significance was $p < 0.05$. All experimental protocols and animal handling described above were approved by the Comité de déontologie de l'expérimentation sur les animaux of Université de Montréal. RESULTS SECTION: Analysis of the microarray results revealed different gene expression profiles during osseointegration between Micromotion 1x/day vs. Unloaded, Micromotion 2x/day vs. Unloaded and Micromotion 2x/day vs. Micromotion 1x/day. (Table 1). Relatively high percentages of unknown genes with no hit for protein classification were noted in all analyses. Noticeably, the Micromotion 1x/day when compared with Unloaded, showed an upregulation in expression of genes associated with IL-1, 2, 3 and type II interferon (IFNG) signaling pathways and downregulation in expression of genes associated with Wnt, TGF-beta and focal adhesion signaling pathways. The Micromotion 2x when compared with Unloaded, showed all genes as unknown and/or unclassified. The Micromotion 2x/day when compared with Micromotion 1x/day, showed upregulation in expression of genes associated with MAPK signaling pathway and downregulation in expression of genes associated with Type II interferon (IFNG), IL-1 and Prostaglandin signaling pathways. DISCUSSION: While some major gene pathways such as the inflammatory and immune response are affected, the high percentage of unknown genes suggests that there are unsuspected players in the process of osseointegration that need to be taken into consideration. Because by day 7 bone healing is already well-established in our model, the downregulation of some Wnt signaling-associated genes in the Micromotion 1x/day group as compared to Unloaded implants may be a reflection that the loading regimen may have had a beneficial effect on bone formation. SIGNIFICANCE: We have shown that simply loading or changing frequency associated with micromotion can induce major changes in the gene expression profile at the immediate proximity of the bone-implant interface. Basing the outcome of an implant on stable healing may therefore not provide an accurate evaluation of success or failure under load.

P-41: Mathematical Model Connecting Physical Chemistry of Hydroxyapatite Formation to Biological Processes of Bone Matrix Maturation

B Ostapienko1,2, SV Komarova1,2

1.Dept of Biomedical Engineering, Fac of Medicine and Fac of Dentistry, McGill Univ; 2.Shriners Hospital for Children-Canada, Montreal, QC

Abnormal mineralization of bone matrix, as observed in osteogenesis imperfecta and vitamin D deficiency, results in severe clinical problems including bone deformities and fractures. To better understand the physicochemical processes occurring during bone mineralization, we have developed a mathematical model that examines hydroxyapatite mineral formation on collagen matrices. We described the reactions occurring between Ca2+, PO43- and OH- ions in the aqueous phase and hydroxyapatite precipitate as single solid phase mineral and accounted for mass balance and electro-neutrality requirements. The hydroxyapatite crystal growth formula included Gibbs free energy cost, temperature, ion concentration and activity based on an ionic strength, and a kinetic factor describing the probability of the hydroxyapatite precipitation based on Ca2+, PO43- and OH- concentrations. The kinetics of collagen matrix maturation, turnover of mineralization inhibitors and formation of nucleators was adopted from the previous model by Komarova et al., Front Cell Dev Biol, 2015. Within physiological ranges for ion concentrations, the model predicted dynamic changes in matrix maturation and hydroxyapatite formation consistent with healthy bone mineralization. When concentration of Ca2, PO43 or OH was varied individually, we observed a biphasic effect – hydroxyapatite formation increased with increasing ion concentration at lower concentrations ranges, but after a certain point the relationship reversed. While physiological concentrations of Ca2 and PO43 were at ranges where mineral formation was directly proportional to ion concentrations; physiological levels of OH- ions corresponded to ranges where mineral formation was inversely proportional to ion conc. Consequently, increase in [OH] caused decrease in mineral formation. The developed model predicts chemical mechanisms by which hydroxyapatite mineral forms on collagen matrices, along with hyper- or hypo calcemia and phosphatemia. Moreover, the model allows direct analysis of the role of environmental parameters, such as temperature and acid-base homeostasis in bone mineralization.

P-42: Characterization of Osteoclastogenic Effects of Cancer – Derived Factors

Z Sabirova1,2, G Sadvakassova1,2, M Juhas1,2, K Tiedemann1,2, SV Komarova1,2.

1.Shriners Hospital for Children-Canada, Montreal, QC; 2.Faculty of Dentistry, McGill University, Montreal, QC

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 (CDP), which has three isoforms exhibiting a high degree of homology. CDP1, CDP2, and CDP3 were recombinantly produced in a bacterial system. Antibodies to purified CDP1 and CDP2 were specific, while antibodies to CDP3 cross-reacted with CDP1. Using immunoblotting, we demonstrated that CDP2 was present in MDA-MB-231 CM in high amount. Next, we tested the effect of each CDP isoform on osteoclast differentiation. All three isoforms induced osteoclast formation from late osteoclast precursors in a concentration dependent manner. Using mass spectrometry, we confirmed that CDP2 was found in all samples of the MDA-MB-231 CM, while CDP3 was found only in some samples and CDP1 was not present in any sample. Finally, we demonstrated that CDP2 was present in CM from cancer cell lines such as MDA-MB-231, K562 myelogenous leukemia and 4T1 mouse breast cancer, but not in NSF fibroblasts CM. Taking together, our data indicate that CDP2 is released by breast cancer cells and can induce octeoclastogenesis from late osteoclast precursors in a RANKL-independent manner.

P-43: Role of Osteoblast Menin in Bone Metabolism: Ex Vivo Studies of Knockout Mice

I Troka1,2, J Abi-Rafeh1,2, L Canaff1,2, GN Hendy1,2.
1.Dept of Medicine, Division of Experimental Medicine, McGill University; 2.Metabolic Disorders and Complications Program, McGill University Health Center-Research Institute, Glen Site.

Introduction: In humans, mutations in the MEN1 tumour suppressor gene cause the Multiple Endocrine Neoplasia Type 1 disorder. Menin, the product of the MEN1 gene, is predominantly a nuclear protein that also facilitates cell proliferation and differentiation control. Our previous in-vivo study illustrated the importance of menin for proper functioning of mature osteoblasts and maintenance of bone mass in adult mice. In the present study, we examined the in vivo role of menin at earlier stages of the osteoblast lineage through conditional knockout of the Men1 gene. Methods: This was implemented through the Cre-LoxP recombination system and Prx1-Cre; Men1f/f and Osx-Cre; Men1f/f mice represent knockout of the Men1 gene in the mesenchymal stem cell and the preosteoblast, respectively. Ex vivo studies are performed on primary calvarial osteoblasts and bone marrow mesenchymal stem cells isolated from both wild-type and knockout mice of our two strain. Results: Our results demonstrate impaired trabecular and cortical bone formation in the earlier menin knockout mice models. Mineralization and differentiation of the primary calvarial osteoblasts in the knockout mice were deficient relative to those of wild-type mice as assessed by Alizarin red and von Kossa staining. Gene expression profiling of RNA extracted from the primary calvarial osteoblast in Osx-Cre; Men1f/f mice revealed reduced osteoblast markers, increased proliferation markers and increased RANKL/OPG ratio that would favor osteoclastogenesis in the knockout animals. This is consistent with ongoing in vivo histomorphometric analysis that demonstrates an increase in osteoclast number and activity in the Prx1-Cre; Men1f/f and Osx-Cre; Men1f/f animals. Conclusions: Osteoblast menin plays a crucial role in the development as well as maintenance of bone mass, and may serve as a potential gain-of-function therapeutic target for low bone mass disorders, such as osteoporosis.

P-44: Improving Bone Geometry By Increasing Muscle Mass – New Treatment Option For Osteogenesis Imperfecta?

Tauer JT, Rauch F
Shriners Hospital of Canada, Montreal QC

Objective: Osteogenesis imperfecta (OI) is primarily characterized by bone fragility but is also associated with lower muscle mass and function. As muscle mass and bone mass are closely linked, an intervention that increases muscle mass should also increase bone mass. Here we investigated the effect of a novel activin receptor IIB ligand trap, ACE-2494, on skeletal muscle mass and bone properties in a mouse model of severe dominant OI, the Col1a1Jrt/+ mouse. Methods: ACE-2494 (3 mg or 10 mg per kg body mass) or vehicle was injected subcutaneously twice per week for 4 weeks into male OI and wild-type (WT) mice, starting at 8 weeks of age.

Results: At baseline, OI mice had 20% lower body mass than control littermates. This difference persisted during the intervention as OI and WT exhibited a similar dose-dependent increase in body mass during ACE-2494 treatment. ACE-2494 injections led to a dose-dependent gain in muscle mass in OI and WT cohorts. Quadriceps weights were increased by about 35% and 50%, gastrocnemicus by about 32% and 55%, and tibialis anterior by about 55% and 70% dose-dependently in WT and OI. Soleus weights were increased by 20% and 26% and EDL weights by 26% and 73% dose-dependently in WT. ACE-2494 in OI mice, increased soleus and EDL mass to a similar extent in both dose groups (by 70% and 60%, respectively). ACE-2494 had no effect on heart muscle mass or liver mass. There was also no effect on either femoral length or trabecular bone volume in the distal femoral metaphysis. However, ACE-2494 treatment resulted in an increased mid-diaphyseal periosteal diameter in OI mice only, leading to an improved polar moment of inertia. Conclusion: ACE-2494 increases muscle mass and seems to improve diaphyseal bone geometry in a model of severe OI.

P-45: Usp53, a Novel Target Gene of The PTH-Activated αNAC Transcriptional Coregulator

H Hariri1,2, W Addison1,2, M Pellicelli1,2 & R St-Arnaud1,2

1. Research Centre, Shriners Hospital for Children - Canada, Montreal, QC; 2. McGill Univ, Montreal, QC.

Upon PTH treatment of osteoblasts, cAMP accumulates to activate PKA, which in turn phosphorylates Nascent-polypeptide-associated complex and coregulator alpha (αNAC) on residue Ser99. This leads to nuclear translocation of the αNAC protein. Little is known about potential transcriptional targets of the PTH-activated, PKA-phosphorylated αNAC protein in osteoblasts. In order to identify such targets, we performed ChIP-seq against αNAC and RNA-seq experiments in PTH-treated MC3T3-E1 osteoblastic cells. Genes showing differential responses in both data sets were further validated using conventional ChIP and RT-qPCR. These experiments identified Usp53 as a potential αNAC target gene. Conventional ChIP assays in PTH-treated MC3T3-E1 cells confirmed a 4-fold enrichment of αNAC binding at the Usp53 promoter region. PTH treatment also increased the mRNA expression of Usp53 by 3-fold. We then established shRNA-mediated Naca (αNAC) knockdown in MC3T3-E1 cells. Interestingly, PTH-driven transcriptional induction of Usp53 was completely blunted following αNAC knockdown. Usp53 promoter fragments were subsequently cloned upstream of a luciferase reporter vector. In transiently transfected MC3T3-E1 cells, PTH treatment stimulated transcription from the Usp53 promoter and αNAC knockdown abrogated the response. In order to understand the role of USP53 in osteoblastic differentiation, we established shRNA- mediated Usp53 knockdown in ST2 stromal cells and MC3T3-E1 cells. Usp53 knockdown enhanced the differentiation of ST2 into osteoblasts and inhibited their differentiation into adipocytes. Increased differentiation was also observed in Usp53 knockdown MC3T3-E1 cells. Usp53 knockdown up-regulated the expression level of osteoblastic differentiation markers such as: Bglap (Osteocalcin) and Alpl (Alkaline phosphatase) and down-regulated expression of regulators of adipogenesis such as Pparg and Cebpa. In vivo osteogenesis assay performed in immunocompromised mice using Usp53-knockdown bone marrow stromal cells (BMSCs) has also showed an increase in osteoblast number (OSX immunostaining) and a decrease in adipocyte counts (perilipin immunodetection). These experiments identify Usp53 as a novel target of αNAC downstream of PTH signal transduction. Future studies will address the mechanism through which USP53 affects mesenchymal cell lineage-making decisions and differentiation.

P-46: Cell Autonomous Role of Phosphatase Orphan 1 in Skeletal Tissue Development

A Enagrat1, Z Khavandgar1, J Li2, JL Millan3, C Farquharson4 & M Murshed1,2,5.

1. Faculty of Dentistry; 2. Dept of Medicine, McGill Univ, Montreal; 3. Sanford Children's Health Research Center, Sanford-Burnham Medical Research Institute, La Jolla, CA; 4. The Roslin Institute and Royal (Dick) School of Veterinary Studies, Univ of Edinburgh, UK; 5. Shriners Hospital for Children, McGill Univ, Montreal, QC

Introduction. Phosphatase orphan 1 (PHOSPHO1), is a member of the haloacid dehalogenase (HAD) superfamily of Mg+2-dependent hydrolases, which shows high affinity to phosphocholine, and phosphoethanolamine. This intracellular enzyme is expressed highly in the mineralized tissues. Global deficiency of PHOSPHO1 in phospho1-/-mice leads to skeletal abnormalities, osteomalacia, scoliosis, and spontaneous fractures. Objective. To investigate whether osteoblast-specific restoration of Phospho1 expression in Phospho1-/- mice will prevent the skeletal abnormalities and improve the mechanical properties of their bones. Methods. We constructed a Col1a1-Phospho1 transgene in which a 2.3 kb proximal Col1a1 promoter drives the osteoblast-specific transgene expression. A total of 5 transgenic lines were generated. Transgene expression was confirmed by semi-quantitative PCR. Phosph01-/-;Col1a1-Phospho1 mice were generated to examine the bone mineralization status, bone volume over tissue volume and osteoblast and osteoclast numbers by histomorphometry. Finally, bone mechanical properties were examined by 3-point bending tests. Results. Semi-quantitative PCR analyses showed that out of five founders, only one expresses Phospho1 specifically in osteoblasts. Histological analyses showed normal osteoid volume in Phospho1-/--;Col1a1-Phospho1 mice. This reduction of osteoid volume improves the biomechanical properties of Phospho1-/-;Col1a1-Phospho1 mice as shown by 3-point bending test. Overall, there was an improvement of skeletal deformities as all the bone histomorphometric parameters became comparable to that of wild type mice. Conclusion. PHOSPHO1 regulates bone mineralization locally and osteoblast-specific restoration of Phospho1 is sufficient to prevent skeletal anomalies caused by PHOSPHO1 deficiency.

P-47: Prevascularization of Cell-secreted Bone-like Tissue

F Kawecki1,2, WP Clafshenkel1,2, T Galbraith1, M Fortin1,3, J Fradette1,2, FA Auger1,2.
1. Centre de recherche en organogénèse expérimentale de l'Université Laval / LOEX, Division of Regenerative Medicine, CHU de Québec Research Center - Université Laval, QC; 2. Dept of Surgery, Faculty of Medicine, Univ Laval, QC; 3. Dept of Oral and Maxillofacial Surgery, Faculty of Dentistry, Univ Laval, Québec, QC.

Several therapeutic approaches are exploited in maxillofacial reconstruction to achieve bone repair, such as using prosthetics, biomaterials, and autografts. Some tissue engineering strategies target the production of graftable bone substitutes using the patient's own cells. For example, adipose tissue is an abundant source of highly multipotent cells that can be harvested with a minimally-invasive lipoaspiration procedure. Moreover, prevascularized grafts have demonstrated better survival and quicker host inosculation which improve bone healing. We hypothesized that cell-based bone-like tissue devoid of exogenous biomaterials could be prevascularized in vitro during osteogenic differentiation. Using a self-assembly technique, osteogenically differentiating human adipose-derived stromal/stem cells (hASCs) were co-cultured with GFP-labeled human umbilical vein endothelial cells (HUVECs) to produce a 3D prevascularized (PV) bone-like tissue made of a cell-secreted extracellular matrix. In vitro studies compared hASCs alone to hASCs co-cultured with HUVECs (days 21-35) in osteogenic or non-osteogenic conditions using morphological analyses. Results showed that calcium content in the osteogenically-induced PV tissue was 9.5-fold greater than in the non-induced PV tissue ($n=3$, $p<0.024$), but no differences were observed between induced PV tissues and controls without endothelial cells. Additionally, a quantitative analysis revealed that the presence of hydroxyapatite in osteogenically-induced PV substitutes was 3.5-fold higher than non-induced substitutes ($n=3$, $p<0.013$). Then, ELISA analysis showed 11-fold more osteocalcin protein presence in osteogenically-induced PV matrix compared to the non-induced PV matrix ($n=3$, $p<0.0001$). Both 2D characterization using ImageJ software and terminal 3D reconstruction by Imaris® software supported a denser and more connected capillary network for induced PV tissues compared to non-induced PV control. We have demonstrated our ability to combine the osteogenic differentiation of hASCs and HUVEC-derived capillary network formation in a unique model of self-assembled prevascularized bone-like tissue. These methods have the potential to produce a tissue-engineered construct with better survival that may be useful for maxillofacial repair.

P-48: Therapeutic Potential of Osteoclast Inhibitory Fibrillin-1 Fragments

M Lakshmi1,3, K Tiedemann1,2, S Komarova1,2, DP Reinhardt2,3
1.Shriners Hospital for Children; 2. Fac of Dentistry, McGill Univ; 3. Fac of Medicine, McGill Univ, Montreal, QC.

Marfan syndrome due to mutations in fibrillin-1 gene is the most common type-I fibrillinopathy characterized by severe skeletal complications, including osteopenia, long bone overgrowth, and kyphosis. However, how fibrillin-1 mutations lead to the skeletal problems is poorly understood. The sub-fragment of fibrillin-1 rF31 (32 kDa) were recently identified as strong inhibitor of osteoclastogenesis in vitro and in vivo in healthy animals. To identify the most potent osteoclast inhibitory sub-fragments of fibrillin-1, we produced recombinant half fragments of rF31 and four different fibrillin-1 fragments spanning rF31 in the HEK293 system. The purified proteins were tested for their effect on osteoclastogenesis using primary osteoclasts. We observed reduced number and size of the differentiated osteoclasts. Next, we plan to examine if fibrillin-1 fragments exhibit sufficient anti-resorptive activity in a Marfan syndrome mouse model Fbn1mgR/mgR. To understand the baseline bone parameters as well as identify the therapeutic window in this mouse model, we analyzed bones of Fbn1mgR/mgR 4, 8, 12 and 15 weeks after birth. Fbn1mgR/mgR mice have increased length of long bones and increased body lengths at all time points compared to wild type littermates (WT). Using DEXA we found a trend of decreased BMD in young Fbn1mgR/mgR mice compared to WT, which became significant at 15 weeks of age. Primary osteoclast cultures were derived and differentiated from the bone marrow cells. We found no significant difference in osteoclast number between the WT and the Fbn1mgR/mgR mice. The expression of RANKL and OPG was increased in Fbn1mgR/mgR mice of both sexes, however RANKL/OPG ratio was affected differently – it was higher in Fbn1mgR/mgR males compared to wild type, but lower in females. In conclusion, we have identified smaller fragments of rF23 that exhibit osteoclast-inhibitory activity. Bone phenotype development in Fbn1mgR/mgR mice suggest that treatment can be started at 4 week old animals.

P-49: Ablation of Osteopontin in Osteomalacic Hyp Mice Partially Rescues The Deficient Mineralization Without Correcting Hypophosphatemia

B Hoac1, T Boukpessi1,2,3, DJ Buss1, C Chaussain2,3, M Murshed1,4, MD Mckee1,5

1. Faculty of Dentistry, McGill Univ, Montreal, QC 2. EA 2496, Laboratory Orofacial Pathologies, Imaging and Biotherapies, School of Dentistry University Paris Descartes Sorbonne Paris Cité, Paris, France; 3. AP-HP Dept of Odontology, Charles Foix and Bretonneau Hospitals, and Reference Center for Rare Diseases of Calcium and Phosphorus Metabolism, Paris, France; 4. Dept of Medicine, Faculty of Medicine, McGill Univ, Montreal, QC; 5. Dept of Anatomy and Cell Biology, Faculty of Medicine, McGill Univ, Montreal, QC.

Hereditary mutations in the PHEX gene (phosphate-regulating gene with homologies to endopeptidases on the X chromosome) cause the osteomalacic disease X-linked hypophosphatemia (XLH). PHEX is predominately expressed by osteocytes, osteoblasts and odontoblasts, and inactivating mutations lead to renal phosphate wasting, severe hypomineralization of bones and teeth, and the accumulation of mineralization-inhibiting peptides and proteins – including osteopontin (OPN). We have previously identified full-length OPN to be a physiologically relevant substrate for PHEX, and found increased OPN and OPN fragments in certain extracts from human XLH and Hyp (mouse model for XLH) bone. These findings suggest that the accumulation of OPN (and its fragments) may contribute to the mineralization defects observed in XLH/Hyp bone. To investigate the effect of OPN ablation in Hyp mice, Hyp;Opn-/- mice were generated and histomorphometric analysis showed significantly increased total mineralized bone volume, as compared to the Hyp mouse. XLH/Hyp bone has characteristic hypomineralized periosteocytic lesions that persist despite stable correction of serum phosphate. Recently, we showed that XLH human bone had a localized abundance of OPN in the pericellular hypomineralized area around osteocytes – an observation confirmed in this study in Hyp mice. To investigate the role of OPN in osteocyte perilacunar mineralization, we measured osteocyte lacunar area in Hyp;Opn-/- bone, but no significant improvement was observed. of particular note, serum phosphate levels were even lower in Hyp;Opn-/- mice than in Hyp mice, suggesting that this increased hypophosphatemia may hinder correction of the hypomineralized phenotype in Hyp mice in the absence of OPN. Further studies are required to determine if both serum phosphate normalization and ablation of OPN can improve osteocyte lacunae mineralization. In conclusion, this study shows that OPN accumulation contributes to the osteomalacic bone observed in XLH/Hyp, and removal of mineralization-inhibiting OPN, in addition to current phosphate therapies, may further correct these mineralization defects.

<u>P-50:</u> Phospholipid Interaction of V-ATPase A Subunit Isoforms As a Novel Therapeutic Target to Prevent Bone Loss.

A Chu1, G Saffi2, R Botelho2, M Glibowicka3, CM Deber3,4, Y Yao1 & **MF Manolson**1.
1.Faculty of Dentistry, Univ of Toronto, Toronto; 2. Dept of Chemistry and Biology, Ryerson Univ, Toronto; 3. Division of Molecular Structure and Function, Research Institute, Hospital for Sick Children, Toronto; 4. Dept of Biochemistry, Faculty of Medicine, Univ. of Toronto, Toronto.

INTRODUCTION: Bone loss results from excessive osteoclastic bone resorption. In osteoclasts (OCs), proton pumping V-ATPases are targeted to the plasma membrane to pump acid out as required to dissolve bone. V-ATPases have 14 subunits, the largest of which is the 100 kDa a subunit, which has four isoforms in mammalian cells, a1-4. Among the four a isoforms, a3 is expressed on the OC plasma membrane and a3 mutations result in osteopetrosis. Data suggests that interaction of a3 with the membrane signalling lipid PI(3,5)P2 regulates V-ATPase membrane recruitment. HYPOTHESIS: The hydrophilic N-terminal domain of V-ATPases a subunits contain a lipid binding domain, and in a3, this lipid binding domain is specific to phospholipids enriched in the plasma membrane of OCs. METHOD: Using a4 as a model, in vitro lipid specificity was determined by a protein-lipid overlay assay with phosphoinositides (PIP) arrays. In vivo membrane retention was assessed by cellular fractionation with GFP fusions in HEK293 cells. RESULTS: Homology model of a4NT revealed a conserved binding motif K219XnK234IKK237. In vitro overlay assay showed that WT a4 bound to PI(4)P while the double mutation K234A/K237A abolished binding. PolyPIPosomes pulldown assay also showed increased association to PI(4)P liposome in wildtype than in mutant. Circular dichroism spectra of WT and mutant were comparable suggesting that mutations disrupted lipid binding without altering protein structure. In HEK cells, wildtype a4NT co-purified with microsomal membrane while K234A/K237A mutant binding was reduced suggesting that the hydrophilic protein binds to lipids in vivo through the lipid binding domain. CONCLUSION: The N-terminal domain of V-ATPase a subunit contains lipid binding domains that could be involved in V-ATPases retention at different cellular locations.

P-51: Deletion of Menin Early in The Osteoblast Lineage Affects Bone Geometry and Mechanics in Adult Mice

M Asgari1, A Moussa1, J Abi-Rafeh2, GN Hendy2 and D Pasini1
1.Dept of Mechanical Engineering, McGill University; 2.RI-McGill Univ Health Center Glen Site, Montreal, QC

In vivo studies have demonstrated the significance of menin, the product of the multiple endocrine neoplasia type 1 (Men1) tumor suppressor gene, for proper functioning of the mature osteoblast and maintenance of bone mass in adult mice (J Biol Chem 13; 3910-24, 2015). In the present study, we used a combined approach of theory, simulations, and experimentation to provide prediction and comparison of the elastic responses of femora from Prx1-Cre; Men1f/f knockout (KO) and Men1f/f wild type (WT) mice, in response to physiological loading. Male mice were sacrificed and analyzed at nine months of age. Major geometrical changes were observed in the femora of KO mice; for example, KO mice femora were shorter than those of WT mice. Linear isotropic elasticity was used to find the local stiffness matrix of the femur at each point. Theory of thin-walled beams was then used to extract the explicit torsional, axial, and bending stiffness terms, and dimensionless shape factors were applied to account for geometric differences in cross-sections. Atomic Force Microscopy was used to find the local elastic modulus at each cross-section point. Micro-CT data were also used to find the shape transformers at each cross-section. The local elastic moduli, and the local shape transformers (e.g., ovalization factor, and bending and torsional stiffness factors) together establish a tool to compare the values of the components of the local stiffness matrix between the two femora. Furthermore, the micro-CT images were used to reconstruct 3D solid models of the femora, which were numerically studied via finite element analysis simulations. A number of benchmark problems were then assessed on the reconstructed 3D models, and the relevant metrics were evaluated and compared. Results from our computational approach and 3-point bending tests indicate that the flexural rigidity of the KO bones is lower than that of the WT bones.

P-52: Personalized Surgical Spine Reconstruction Post Tumor Resection

B Barimani, D Rosenzweig, L Haglund, M Weber
RI-MUHC, McGill University

INTRODUCTION: Up to 80% of patients with primary tumors of the breast, prostate or lung will develop spine metastases. Spine metastases cause pain, functional deficit and fractures leading to vertebral instability, spinal cord and nerve-root compression. With advancements in medical, radiation and surgical oncology, these patients are living longer thereby increasing the disease burden. Surgical bone resection of metastatic tumors lead to large bone defects, instability and poor bone repair. Current treatment involves antiresorptives like Zoledronate (a bisphosphonate), whilst techniques combining drugs and innovative grafts to help solve these issues in spine tumor patients is being explored. METHODS: Preparation of nanobeads: 10mg of nanobeads were placed in a 5ml vial, suspended in 1ml PBS, sonicated for 30 minutes, solution split into 2 vials and 500ul PBS added to each vial. Vial 1: 20ul chitosan added, vial 2: 20ul 0.11M HCl added. 1nm of fluorescently labelled zoledronate (Fam-Zol) added to each vial, incubated with agitation overnight at room temperature. Beads were washed x3 with 600ul PBS. Beads re-suspended in 500ul PBS and incubated at room temperature. Time points at 24 hour intervals, half volume removed and stored at -20°C. Same volume replaced for next time interval reading for total of 6 days. RESULTS: First 24 hours, chitosan coated nanobeads had slower release rate of the Fam-Zol (62.4% rate of non-chitosan). Over 146 hours, chitosan coated nanobeads retained more and had a slower release rate of Fam-Zol. Between 120-146 hours, chitosan coated nanobeads released Fam-Zol at 1.15x the rate of non-chitosan nanobeads. CONCLUSIONS: Bioactive chitosan coated nanobeads retain Fam-Zol for 146 hours (6 days) and release is gradual and stepwise. These nanobeads can be integrated into bone paste to develop a bioactive bone graft following bone tumor resection to deliver localized bisphosphonate drug facilitating bone stability and healing.

P-53: Mechanically Stimulated Aligned Dense Collagen Gels For Tendon Tissue Engineering

H Park & SN Nazhat
Dept of Mining and Materials Engineering, McGill University

Tendon tissue engineering aims to form constructs that are biomimetic, fulfilling both mechanical and structural roles of the native tissue. As the most prominent protein in tendons, collagen type I is an appropriate material in terms of its biocompatibility, but highly-hydrated collagen gels are of low mechanical properties and random fibrillar orientation. Densification of the gels have been proposed through plastic compression (PC)1 and gel aspiration-ejection (GAE)2, which show improved mechanical stability. Furthermore, GAE generates injectable dense collagen gels of increased levels of alignment. Mechanical loading is an accepted method of aligning collagen fibrils, increasing mechanical properties and initiating remodelling; therefore, this study investigated the effect of different mechanical loading protocols on the structural and mechanical properties of dense collagen (DC) hydrogels developed for tendon tissue engineering. Three different mechanical stimulation protocols were investigated up to 144 cycles by applying 20% strain: a block cycle consisting of four phases i.e. 5 minutes each of strain, hold, release and hold; a stress-release cycle consisting of 5 minutes strain and 5 minutes release; and a sinusoidal cycle at 0.00167 Hz. As-made DC gel mechanical properties were correlated with fibrillar densities and anisotropy, which were impacted by GAE needle size. Pre-alignment during GAE was also indicated as a cause for increased apparent modulus. Mechanical stimulation under the block cycle protocol resulted in statistically different gel ultimate tensile strength and apparent modulus values when compared to other protocols. Previously, mechanically loading of PC generated DC gels of random fibrillar orientation resulted in mechanical strengthening and structural reorganisation, increasing fibril diameters3. This study suggests that the block cycle loading protocol of GAE generated DC gels promoted remodelling of the gels in terms of alignment, impacting the mechanical properties. Future studies will investigate the effect mechanical loading on cell seeded DC gels for tendon tissue engineering.

1. BROWN, R. A et al. 2005. Advanced Functional Materials, 15, 1762-1770. 2. KAMRANPOUR, N. O., et al 2016. Biofabrication, 8, 015018. 3. CHEEMA, U., et al 2007. Engineering Functional Collagen Scaffolds: Advanced Functional Materials, 17, 2426-2431.

P-54: Ex Vivo Interactions of Skeletal and Immune Cells Using Contractile Collagen Microdroplets

M Abou Rjeili1, 2, S Mok5, J Ramirez-Garcia-Luna1, 3, C Moraes5, JE Henderson1, 4, PA Martineau1, 4
1.Bone Eng Labs, Injury Repair & Recovery Program, Res Inst-McGill Univ Health Centre; 2 Exp Medicine; 3.Exp Surgery and 4.Division of Orthopaedics, Faculty of Medicine, McGill Univ 5.Dept of Chemical Engineering

Purpose: Mast cells (MC) localize to fracture callus in the early inflammatory phase of bone repair. We previously showed impaired long bone healing in MC-null KitW-sh mice, also deficient in other cells that affect bone repair including osteoblasts and osteoclasts. Recent work using Cpa3Cre/+mice lacking MC reveals defects in bone healing associated with impaired vascularization & alterations in the balance between catabolic M1 and anabolic M2 macrophages in repaired tissue. The goal of this study is to use 3D co-culture to determine how MC influence vascular endothelial cells (VEC) and mesenchymal stem cells (MSC) in bone repair micro-environment. Methods: MC are differentiated from precursors in the suspension cells isolated from WT and Cpa3Cre/+mice bone marrow and grown in the presence of SCF and IL3. FACS analysis and RNA collection were performed at weekly intervals. VEC will be isolated from mouse aorta and MSC from bone marrow. An aqueous two-phase system of contractile collagen microdroplets will be printed with VEC or MSC and mature MC seeded in suspension around the droplet. MC will then be activated using compound 48/80 and the collagen droplets harvested at timed intervals for analyses. Results: 90% of cultured cells are mature MC after three weeks of culture, confirmed by PCR analysis of RNA harvested at weekly intervals. PCR analysis also showed increased mast cells genes expression in WT MC but not in MC from Cpa3Cre/+mice. In the presence of MC we anticipate VEC will form a network of vessels and MSC will differentiate into osteoblasts, evidenced by remodeling of the collagen droplet. Conclusion:3D collagen microdroplets mimic the in vivo micro-environment in which MC interact with VEC and MSC during bone repair. The technology provides a valuable tool to investigate molecular pathways by which MC influence re-vascularization and repair of cortical bone defects.

P-55: Cardiomyocyte Shape Control Improves Contractility Tests in Drug Screening Applications

T Shen, GT Kim, Y Shafieyan, and B Hinz
Lab of Tissue Repair and Regene, Inst of Bioengineering and Biotech and Faculty of Dentistry, Univ of Toronto

Background: Unexpected cardiovascular side effects of drugs put patients at risk. The drug development industry requires human cardiomyocyte (CM)-based preclinical assays with high throughput drug screening (HTS) capability but current HTS cannot directly assess CM contraction force as a central function. We have developed a novel HTS device that can measure changes in CM contractile force and frequency on silicone-based culture substrates. The device has been successfully benchmarked against existing technologies using a panel of drugs. However, the heterogeneity of CM populations introduces data variability. Hypothesis: Geometrically confining CMs into their 'physiological' shape on compliant silicone substrate improves force production and beating frequency by guiding CM sarcomere organization and avoiding cell aggregate formation. Objective: To standardize CM behavior for automated analysis in drug screens by patterning adhesion islands on the silicone surface of our contraction measurement device. Materials and Methods: To achieve CM shape patterning we developed microcontact printing with fluorescently tagged proteins to soft recipient surfaces. 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 analysis routines were developed to automate data analysis. Results: Distinct protein and cell patterns were achieved on silicone substrates with improved microcontact printing. We identified patterns that supported homogenous CM beating with high amplitudes. Data analysis was successfully automated to reduce analysis time. Significance/Impact: Cell shape confinement enhances our HTS-capable device by supporting homogenous beating and increasing force of CM contractions. Together with the heart-soft biomimetic culture surface, CM shape and position control further increases the efficacy of our device to evaluating cardiovascular side-effects in preclinical HTS tests.

P-56: Analysis of The Interplay of Lysosomal/ Autophagosomal Pathways in Osteoclasts Towards Correction of Osteopetrosis

C. Bauer1, M. Pata2, J. Vacher1,2

1.Clinical Research Institute of Montreal, Department of Medicine, Division of Experimental Medicine, McGill University; 2.Dept of Medicine, University of Montreal, Montreal, QC

Osteopetrosis is a disease characterized by the failure of osteoclasts (OCs) to resorb bone. As a consequence, bone mass is increased and bone marrow space is reduced; leading to premature death. The grey-lethal (gl/gl), Ostm1 null mouse model mimics severe human forms of osteopetrosis, and although the exact function of Ostm1 in osteoclasts is not fully understood, it appears to influence OC ruffled border (RB) formation. The RB is a platform for proton/protease excretion as well as bone material uptake and is formed through fusion of secretory lysosomes with the bone-apposed plasma membrane. Interestingly, our results implicate Ostm1 in autophagocytic clearance via the lysosomal pathway in neurons. Thus, it is imperative to assess Ostm1 role within the OC and the interplay of autophagy/lysosomes in OC RB formation. To assess these facets, we designed a transgene to stimulate the lysosomal/autophagy pathways in mature OCs. The expression of the Tfeb transcription factor, a master regulator of the autophagocytic/lysosomal pathways, was targeted to mature OCs with the Capthepsin K (Ctsk) promoter. To determine whether Ostm1's function is critical in the cellular clearance of autophagosomes, the expression of key autophagosomal/lysosomal trafficking and flux molecular regulators have been assessed in transgenic and non-transgenic wild-type (wt) mice. In vitro analyses of mature OC samples, cultured from transgenic wt mice, indicate that there is a significant upregulation of autophagy and lysosomal biogenesis in transgenic mice. These data demonstrate that Tfeb upregulation can stimulate autophagy/lysosomal pathways in OCs, thereby furthering our understanding of RB function. Concomitantly, assessing correction of OC maturation/activation and the osteopetrotic phenotype is ongoing in Tfeb gl/gl transgenic mouse OCs. Consequently, these studies will add to our understanding of the Ostm1's role in the lysosomal/autophagocytic pathways.

P-57: Real-Time Microscopy of Osteoblastic Cells Reveals Calcium Responses to Fluid Shear But Not Vibrational Mechanical Stimuli

D Lorusso1,2,3, HN Nikolov3, RJ Beach2, SM Sims1,2, SJ Dixon1,2, DW Holdsworth1,2,3,4,5

1. Bone and Joint Institute; 2. Dept of Physiology & Pharmacology; 3. Imaging Research Laboratories, Robarts Research Institute; 4. Dept of Medical Biophysics; 4. Dept of Surgery Schulich School of Medicine & Dentistry The University of Western Ontario, London, ON.

Mechanotransduction is the process by which cells sense – and respond to – the local mechanical environment. This ability to react to loads and forces is a critical component of mammalian 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 acceleration due to vibration. Our goal is to observe the immediate responses of cells to high-frequency vibrations. Here, we describe the development, validation, and utilization of a system for vibrating live cell cultures that is compatible with real-time optical microscopy and photometry. The motion-control system was mounted on an inverted microscope and the moving parts were suspended on linear air bushings, and actuated by a voice-coil. Accelerations were measured via an on-board calibrated accelerometer. To ensure vibrations were transferred effectively to the cell culture dish, motion waveforms were imaged with a high-speed camera at 1200 FPS. MC3T3-E1 osteoblast-like cells were then seeded onto compatible glass-bottom dishes. Cells were treated with fluorescent calcium dye fura-2 and exposed to vibration during photometry. Fluid shear stress (Lorusso Biomedical Microdevices 2016) and ATP were used as controls and applied simultaneous to vibration. During operation between 20 – 500 Hz and 0.1 – 1 g, sinusoidal motion of waveforms were observed from both optical and accelerometer-derived measurements, with displacements ranging from the nanometer to millimeter range. Cultured osteoblast-like cells were vibrated 0.3 g at 45 Hz during photometry and remained adherent and viable. No change in cytosolic calcium were observed in response to vibration. Fluid shear and ATP positive controls both caused calcium transients. We have developed, fabricated, and tested a motion-control system capable of – for the first time – delivering physiologically relevant vibrations to live-cells during real-time microscopy and photometry.

P-58: Characterizing The Distribution of Inorganic Polyphosphate During Mineralization of Articular Cartilage

A Zelinka1,2 and R Kandel1,2,3

1. Dept of Lab Medicine and Pathobiology, University of Toronto; 2. Lunenfeld-Tanenbaum Res Institute, Mount Sinai Hospital, Toronto; 3. Dept of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto.

Articular cartilage (AC) has a depth dependent zonal organization with a deep zone (DZ) that merges into a zone of calcified cartilage (ZCC). Mechanisms regulating mineralization have not been fully elucidated, and may involve inorganic polyphosphate (PP), a linear polymer of orthophosphate residues, and alkaline phosphatase (ALP), both located in the DZ. We hypothesize that PP is involved in regulating the location of mineralization in AC. PP, ALP, and mineral distribution were assessed by incubation with DAPI, naphthol phosphate, or Von Kossa stain, respectively, in histological sections of bovine native and in vitro formed cartilage. Native bovine DZ cartilage contained two regions with distinct ALP and PP distributions: upper deep zone (distant from ZCC; DZU) and lower deep zone (adjacent to ZCC; DZL). Cells from each region were isolated and five-fold lower ALP activities were observed in cells isolated from DZU, as compared to DZL. Cells were grown in in vitro 3D culture for up to 10 days, and PP and mineral content of DZU- and DZL-derived tissues were compared. In DZU, (originally low) PP levels appeared induced by β-glycerophosphate treatment within 2 days, declining as mineralization progressed. Tissue formed by DZU mineralized fully at day 10. Higher PP levels in DZL did not appear increased by β-glycerophosphate, and tissue showed biphasic organization: layer of proteoglycan-rich tissue above a calcified region. PP returned to original levels in the uncalcified region of DZL at day 10. ALP activity remained constant in DZU, but steadily declined in DZL tissues. When mineralization was inhibited with levamisole, an ALP inhibitor, PP levels decreased. Taken together, it appears PP is differentially regulated at different time points during mineralization of the DZ regions of bovine AC, implicating PP in the modulation of this process. Furthermore, phosphatases other than ALP may contribute to PP regulation in AC.

P-59: Development of a Serum Free Medium Optimized For Human Epithelial Cell Culture.

S Cortez Ghio1, D Larouche1, A Garnier2 & L Germain1.

1. Centre de recherche du CHU de Québec - Université Laval, Centre de recherche en organogénèse expérimentale de l'Université Laval/LOEX, Dépt de chirurgie, Faculté de médecine, Univ Laval; 2. Dépt de génie chimique, Faculté des sciences de génie, Univ Laval.

The culture of epithelial cells (i.e. keratinocytes) has improved treatments for patients suffering from severe burns. To properly culture human keratinocytes in vitro, fetal bovine serum (FBS) must be added to the culture medium. However, FBS exact biological composition is undefined and is known for batch-to-batch variations. FBS is also associated with a risk of pathogen transmission. For these reasons, discarding FBS usage in clinical contexts would be desirable. Although serum-free media (also called defined media) are commercially available, they are not effective in culturing keratinocytes for more than a few passages. Therefore, our objective is to develop and test a defined medium optimized for keratinocyte growth in vitro. Four candidate molecular factors capable of substantially promoting keratinocyte growth were initially identified from an experimental screening. This led to the development of an effective defined medium for culturing keratinocytes (DMK). This medium was then optimized in terms of cost-effectiveness by testing multiple concentrations of these factors, which allowed the formulation of four DMK derivatives (α, β, γ and δ). DMK and its derivatives were then ranked on their ability to sustain keratinocyte culture quality by testing additional cell populations and using daily population doublings and cell size over as many passages as possible as proxies. We observed that keratinocytes cultured in DMKβ were smaller and could be grown over more passages than those cultured in DMK, other DMK derivatives (α, γ, and δ) or FBS-containing medium. In addition, keratinocyte population doubling rates were similar across all media. The morphology of keratinocytes cultured in DMKβ was also comparable to that of those cultured in FBS-containing medium. Although more analyses are needed to characterize the effects of DMKβ on cultured keratinocytes, results thus far show that this new medium is very promising.

P-60: Using Axolotl Extracellular Matrix to Heal Mouse Skin Wounds.

É Villiard1, C Soto Valenzuela[2], JT Egaña2 & S Roy[1]

1. Dept. of Stomatology, Faculty of Dentistry, Université de Montréal, Montréal, QC; 2. Facultad de Ciencias, Pontifical Catholic University of Chile, Santiago, Chile.

Reconstructive medicine uses biomaterials to treat large skin wounds, promoting a better and a faster closing and healing. Those dressings are generally organ's ECM (extracellular matrix) of various organisms, including humans, pigs, amphibians and insects. ECM contain structural proteins, mostly collagen, and many growth factors (FGF, TGF-b, VEGF, PDGF, HGF) forming a scaffold promoting cellular colonization and proliferation. The use of a scaffold coming from a non-regenerating organism stimulates healing; what if this scaffold is made from a regenerating one? What if axolotl (Ambystoma mexicanum) skin ECM is used? It's known that the axolotl, an amphibian urodele, is able to regenerate complex structures (including skin) as an adult, perfectly and without scaring. Furthermore, the ECM scaffold from axolotl skin is easy to prepare using a standard decellularization protocol. When grafted in SKH-1 mice, axolotl ECM skin scaffold seems well tolerated (no apparent inflammation). The next step in this project is to compare the healing quality of ECM scaffolds (Integra® and axolotl ECM) by assessing angiogenesis, cellular migration and colonization in SKH-1 grafted mice.

P-61: Voluntary Running Exercise Attenuates Behavioural Signs of Pain and Reduces Pathological Nerve Sprouting in Degenerating Intervertebral Discs in a Mouse Model of Low Back Pain

SH. Lee1,2, M. Millecamps1,2, L.S. Stone1,2,3

1.The Alan Edwards Centre for Research on Pain; 2. Faculty of Dentistry, McGill University, Montreal, QC; 3. Faculty of Medicine, Dept of Pharmacology and Therapeutics, McGill University, Montreal, QC

Aim: Persistent low back pain (LBP) causes more global disability than any other condition. Due to the limitations of medications, patients seek alternative treatments such as exercise, yoga and meditation. SPARC (Secreted Protein, Acidic, Rich in Cysteine), an extracellular matrix protein, plays important roles in intervertebral disc (IVD) integrity. SPARC-null mice display accelerated disc degeneration associated with behavioral signs of axial and radiating LBP (Millecamps et al., 2015) and local nerve sprouting (Miyagi et al., 2014). In this study, we investigated how increased physical activity relieves LBP symptoms and pathological innervation in the SPARC-null mouse model. Methods: 8-month old SPARC-null and age-matched wild-type control mice had free access to a running or secured (sedentary) wheel for 4 months. Behavioral assays were performed to assess axial (grip test) and radiating (von Frey & acetone tests) discomfort. Lumbar IVD height and shape were analyzed by X-ray images. Innervation in lumbar discs was measured by PGP9.5- and CGRP-immunohistochemistry (-ir). Results: Axial and radiating pain in SPARC-null mice were reduced by running. X-rays confirmed altered disc shape and reduced disc height in SPARC-null mice; running reversed the former. The increased nerve fiber density observed in degenerating SPARC-null discs returned towards normal values following running. Conclusion: This study addresses the beneficial effects of running for LBP and its underlying mechanisms. In a pre-clinical model of LBP, both increased disc innervation and behavioral signs of pain were reversed by voluntary running exercise.

P-62: Nanostructure of Avian Eggshell (Gallus Gallus) and Correlation With Functional Properties

D Athanasiadou1, W Jiang1, D Goldbaum2, K Basu3, MS Pacella4, RR Chromik2, MT Hincke5, AB Rodrĺguez-Navarro6, H Vali3, JJ Gray4, KH Bui3 & MD Mckee1,3

1.Faculty of Dentistry, McGill University, Montreal, QC; 2.Dept of Mining & Materials Engineering, McGill Univ, Montreal, QC; 3.Dept of Anatomy & Cell Biology, McGill Univ, Montreal, QC; 4.Dept of Chemical & Biomolecular Eng, Johns Hopkins Univ, Baltimore, MD; 5.Dept of Cellular & Molecular Medicine, Univ of Ottawa, ON; 6.Dept de Mineralogía y Petrología, Universidad de Granada, Spain.

The functional properties of biomineralized structures found in Nature are based not only on the interactions between their hybrid components – both organic (mostly proteins) and inorganic (mineral) phases – but also on their hierarchical organization across different length scales. Here, we present a detailed nanostructural analysis of avian eggshell (chicken, Gallus gallus), and relate its variable nanostructure to differences in shell hardness. Furthermore, we describe changes in this nanostructure following eggshell dissolution, a shell-thinning and weakening process which occurs after fertilization and incubation of the egg (as required for chick skeletal growth, and hatching). The eggshell of the chicken has calcite as the predominant mineral phase, with abundant proteins (e.g. osteopontin) interspersed throughout its entire thickness. Electron backscatter diffraction and 2D X-ray diffraction showed that the eggshell consists of large columnar calcite units with significant (2.7°) internal misalignments. Here we show by atomic force microscopy (AFM) that these calcite crystal units have a fine nanostructure, ranging from approximately 30 nm to 75 nm depending on the region of the eggshell examined. The outermost region of the eggshell termed the vertical crystal layer had the highest nanoindentation hardness values associated with smaller nanostructure, whereas hardness values decreased towards the interior of the shell where nanostructure was larger. Transmission electron microscopy and electron tomography of focused-ion beam-cut sections of nonincubated eggs revealed fine mineral nanodomains (5-7 nm in diameter) in the outermost palisades region. Egg incubation resulted in decreases in nanodomain size in the innermost shell regions. In vitro analyses of synthetic calcite grown from solution in the presence of osteopontin revealed that occlusion of this one protein into calcite induced similar nanostructure, consistent with OPN's mineral-binding and inhibitory role. In conclusion, these observations provide details on the nanostructure of avian eggshell, and on protein-mineral relationships related to eggshell hardness.

P-63: Effects of ECM Concentration and Processing Methods on the Properties of Porous Decellularized Adipose Tissue Foams as Substrates for Adipose-derived Stromal Cell Culture and Delivery

CFC Brown1, B Turco2, M Cure1, L Flynn1,3

1. Dept of Anatomy & Cell Biology, Schulich School of Medicine and Dentistry; Western University; 2. Dept of Chemical Engineering; Queen's University; 3. Dept of Chemical and Biochemical Engineering, Western Univ.

Foams fabricated from decellularized adipose tissue (DAT) represent a promising pro-regenerative platform for adipose-derived stromal cell (ASC) delivery [1]. The current objective was to explore the effects of ECM concentration and processing methods on the scaffold physical properties and in vitro cellular response. METHODS: Human adipose tissue was decellularized and processed by (i) coarse mincing or (ii) cryomilling and enzymatic digestion to generate pure ECM-derived foams [1]. The scaffold physical properties were characterized over a range of ECM concentrations (10 – 50 mg/mL), and IHC was performed to assess ECM composition post-processing. Human ASCs were seeded under static or dynamic conditions on various formulations, and attachment, infiltration, proliferation and immunophenotype were assessed over 7-14 days in culture. RESULTS: Physical characterization revealed that all foams maintained their geometry following rehydration, and that porosity and equilibrium water content were inversely related to ECM concentration. Collagen I, IV, laminin, and fibronectin were detected in both minced and cryomilled foams. Cell infiltration was enhanced in dynamically-seeded cryomilled foams, but decreased with increasing ECM concentrations in all conditions. dsDNA quantification and Ki67 staining indicated that ASCs proliferated on all foams over 14 days, with enhanced proliferation on the 50 mg/mL DAT foams. These findings were supported by flow cytometry analysis of EdU incorporation in cells enzymatically-released from the foams at 7 days, with >96% of cells Edu+. At 25 mg/mL concentration, there were significantly more Edu+ cells on the minced foams. Immunophenotype analysis showed >93% expression of the MSC markers CD73, CD90, and CD105 over 7 days on the minced foams, with a significant reduction in CD90 and CD105 expression observed in the cryomilled foams. SIGNIFICANCE: Overall, DAT foams provide a supportive platform for ASCs, and ECM concentration and scaffold processing methods can be tuned to enhance infiltration or proliferation with maintenance of the MSC immunophenotype.

P-64: An Innervated and Vascularized Immunocompetent Tissue-Engineered Skin to Study Cutaneous Neuroinflammation

Q Muller1,2, MJ Beaudet2, E Schaeffer1, C Mueller1, F Berthod2# and V Flacher1#

1. CNRS-UPR3572/LabEx Medalis « Immunopathologie et Chimie Thérapeutique », Institut de Biologie Moléculaire et Cellulaire, France; 2. LOEX, centre de recherche du CHU de Québec-UL, and Dept of Surgery, Université Laval, Canada, # Equal contribution

Immune reactions in the skin are initiated by the cutaneous dendritic cells (DCs). The potential sensitizing effect of a compound can be predicted in vitro using human blood monocytes differentiated into DCs (Mono-DCs) or monocytic cell lines. However, these simplistic models remain inaccurate because the activation of cutaneous DCs by sensitizers may be triggered or modulated by microenvironmental interactions with multiple types of non-immune cells (1). Our goal is to develop an immunocompetent tissue-engineered skin (TES) that will combine DCs with all structural and functional element of the skin, i.e. an epidermal barrier laid upon a dermis containing a network of endothelial capillaries and nociceptive nerve fibers (2). Collagen-chitosan lattices were first seeded with fibroblasts and endothelial cells, then with precursors of nerve fibers derived from either human induced pluripotent stem cells (iPSC) or murine embryonic dorsal root ganglia (DRG). Finally, we introduced keratinocytes and Mono-DCs. We observed that in situ differentiated neurons grow axons towards the epidermis. Moreover, Mono-DCs settled as expected beneath the epidermis and remained sessile for several weeks. The model will be used to predict the irritant potential of chemical compounds, and the impact of nerves on DC activation.

1. Riol-Blanco L, Ordovas-Montanes J, Perro M, Naval E, Thiriot A, Alvarez D, et al. Nociceptive sensory neurons drive interleukin-23-mediated psoriasiform skin inflammation. Nature. 2014;510(7503):157-61.

2. Cadau S, Leoty-Okombi S, Pain S, Bechetoille N, andre-Frei V, Berthod F. In vitro glycation of an endothelialized and innervated tissue-engineered skin to screen anti-AGE molecules. Biomaterials. 2015; 51:216-25.

P-65: Loss of ENT1 Alters Cell Cycle Processes in The Annulus Fibrosus of The Intervertebral Disc.

MA Veras1, NA Tenn1, H Ii1, D Quinonez1, J Hammond2, SJ Dixon1, CA Séguin1
1.Dept of Physiology and Pharmacology, Schulich School of Medicine & Dentistry; Bone and Joint Institute, University of Western Ontario; 2. Dept of Pharmacology, University of Alberta

Equilibrative nucleoside transporter 1 (ENT1) facilitates the movement of nucleosides, such as adenosine, across the plasma membrane. Mice lacking ENT1 (ENT1-/-) exhibit a phenotype resembling diffuse idiopathic skeletal hyperostosis (DISH) in humans, with ectopic mineralization of paraspinal tissues and the annulus fibrosus (AF) of the intervertebral disc (IVD). The etiology of DISH remains unknown and no disease-modifying treatments exist. However, we have shown that AF cells from ENT1-/- mice exhibit decreased expression of mineralization inhibitors, increased cell proliferation and increased intracellular adenosine in vitro. To identify pathways associated with ectopic mineralization, we conducted microarray analysis of AF tissue from WT and ENT1-/- mice prior to AF calcification (2 months-of-age) and following AF calcification (6 months-of-age). GO and KEGG pathway analyses identified cell cycle and immune system deregulation in ENT1-/- AF tissues compared to WT control. ISMARA was used to predict transcriptional regulators and implicated the E2f family as potential effectors of cell cycle deregulation in ENT1-/- AF cells. qPCR analysis of E2f transcription factors in AF tissue confirmed upregulation of E2f1, E2f5, and Cdk2 – a cyclin-dependent kinase known to interact with E2f transcription factors - in ENT1-/- AF cells. Implicating adenosine receptor stimulation as an upstream effector of these changes, we also found a significant increase in the expression of Gnas in ENT1-/- AF tissue compared to WT. Furthermore, by PCNA immunostaining we found a striking increase in proliferating AF cells in ENT1-/- thoracic IVDs. The current study suggests that E2f transcription factors may mediate alterations in ENT1-/- AF cells. The association between cell cycle deregulation, E2f activity and ectopic mineralization will be further investigated in situ and in vitro. Together, these studies will identify cellular and molecular changes associated with the induction of ectopic mineralization in ENT1-/- tissues, with the goal of identifying therapeutic targets for DISH.

P-66: Phenotypic Characterization of a Bril-Cre Transgenic Mouse Model.

S Robinson, A Patoine, P Moffatt
Shriners Hospitals for Children and Department of Human Genetics, McGill University.

Conditional gene targeting in mice using CreloxP technology, allows for the spatial and temporal control of genetic modifications, and contributes to an outstanding extent to our comprehension of mechanism governing skeletal development, formation and integrity. The need for a well-characterized, diverse set of Cre driver lines is critical, as many previously described strains display characteristics that were not obvious on their initial generation. In the musculoskeletal field, only a few such Cre-expressing models are available to specifically ablate a gene of interest in bone forming cells, osteoblasts. Most of them had been generated with the expectation of being tissue- and even cell-type restricted, but recent studies underscored some unanticipated problems. Here we have generated such an osteoblast-specific Cre-driver based on the regulatory sequences of the Bril (Bone-restricted Ifitm-like) gene. Using a transgenic approach, founders were generated and analyzed for their cell-specific expression by crossing with a tomato red reporter mouse line. Also, we characterized the skeletal phenotype of the Bril-Cre transgenic to ensure that there was no unexpected bone defects introduced by the transgene. Preliminary results indicate that Bril-Cre represents a better and stricter bone- and osteoblast-specific Cre-driver.

23rd CANADIAN CONNECTIVE TISSUE CONFERENCE SPONSORS

Faculté de médecine dentaire
Université de Montréal

Hôpitaux Shriners pour enfants®
Shriners Hospitals for Children®
Canada

BIOMOMENTUM

McGill Faculty of Medicine Faculté de Médecine

Cell
ThéCell: Le réseau de thérapie cellulaire et tissulaire du FRQS