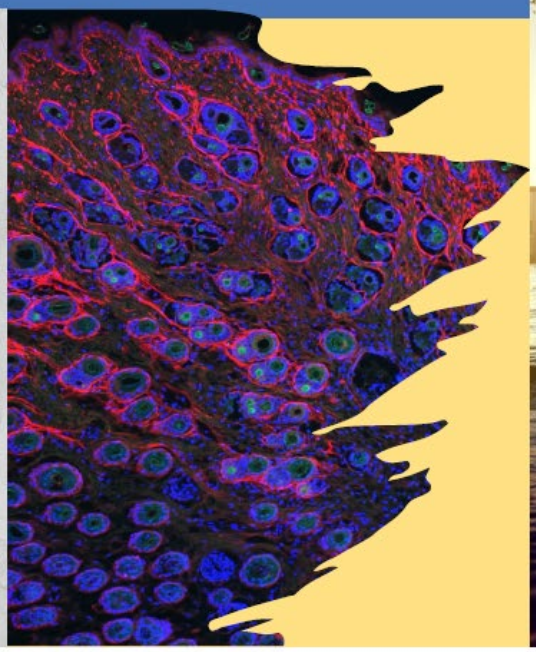
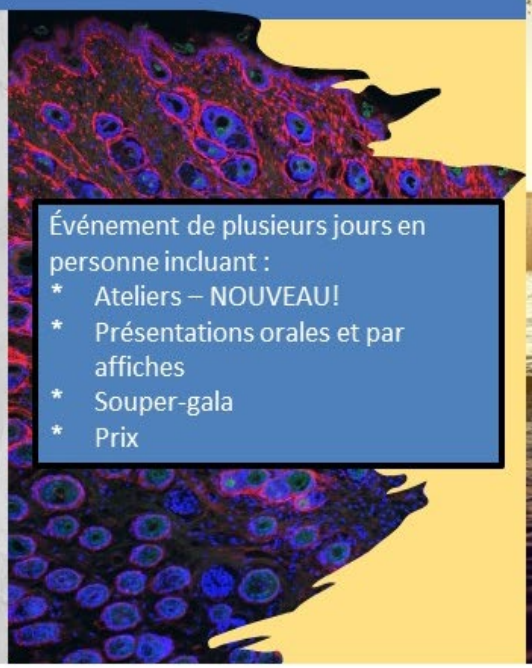
27th Annual Canadian Connective Tissue Conference



27iéme Conférence Annuelle Canadienne sur le Tissu Conjonctif

L'Inscription et la soumission des resumes sont maintenant ouvertes !!



Événement de plusieurs jours en personne incluant :

* Ateliers – NOUVEAU!
* Présentations orales et par affiches
* Souper-gala
* Prix

Message from Organizers

On behalf of the Canadian Connective Tissue Society, we're happy to invite you to the 27th Annual Canadian Connective Tissue Conference (CCTC), which will take place in Toronto from June 14th to 16th, 2023. It has been our pleasure to organize the first annual conference since 2019 and we look forward to welcoming you in Toronto in person!

The Scientific Program will foster knowledge exchange of research aimed at understanding the scientific and clinical aspects of diseases that affect bone, muscle, skin and joints. The CCTC is an especially important forum for trainees, who contribute the majority of podium presentations and all posters, and who will benefit from opportunities to network with colleagues, clinicians, and scientists in the field of connective tissue research.

We have designed new elements for this year's conference to complement a robust scientific program. We will begin the conference on Wednesday, June 14th, with a brand-new Workshop Day Focused on Careers outside Academia, Entrepreneurship & Patient Engagement. The Workshop will include informative sessions filled with a variety of speakers that we hope will inspire you to think in new ways. The Welcome Reception, which is scheduled at the end of the Workshop Day, and Gala Dinner, taking place on the evening of June 15th, will both be held at Hart House, a heritage building that is located on the University of Toronto campus. We've included these social events as part of the conference registration because we feel that connecting with colleagues in this way is invaluable for developing collaborations. The conference will conclude with awards for Best Poster and Podium Presentations, as well as two new awards, one for research that incorporates Equity, Diversity, and Inclusion principles and one for research that incorporates Patient Engagement.

Please join us in Toronto this June for an exciting return to the CCTC!

Adele Changoor and Marc Lavertu

Prof. Adele Changoor
University of Toronto
Co-chair of CCTC 2023

Prof. Marc Lavertu
Polytechnique Montreal
Co-chair of CCTC 2023

Message des co-organisateurs

Au nom de la Société canadienne du tissu conjonctif, nous sommes heureux de vous inviter à la 27e Conférence canadienne annuelle sur le tissu conjonctif (CCTC), qui aura lieu à Toronto du 14 au 16 juin 2023. Nous avons le plaisir d'organiser la première conférence annuelle depuis 2019 et nous sommes impatients de vous accueillir en personne à Toronto !

Le programme scientifique favorisera l'échange de connaissances sur la recherche visant à comprendre les aspects scientifiques et cliniques des maladies qui affectent les os, les muscles, la peau et les articulations. Le CCTC est un forum particulièrement important pour les étudiants et les stagiaires postdoctoraux, qui contribuent à la majorité des présentations orales et à toutes les affiches, et qui bénéficieront d'occasions de réseautage avec des collègues, des cliniciens et des scientifiques dans le domaine de la recherche sur le tissu conjonctif.

Nous avons développé de nouveaux éléments pour la conférence de cette année afin de compléter et bonifier notre programme scientifique. La conférence débutera le mercredi 14 juin avec une toute nouvelle journée d'ateliers (workshop) axée sur Carrières en dehors du milieu universitaire, l'entrepreneuriat et l'engagement des patients. Cette journée comprendra des sessions informatives animées par une variété d'intervenants qui, nous l'espérons, vous inspireront de nouvelles façons de penser. La réception de bienvenue, prévue à la fin de la journée d'atelier, et le dîner de gala, qui aura lieu dans la soirée du 15 juin, se tiendront tous deux à Hart House, un bâtiment historique situé sur le campus de l'Université de Toronto. Nous avons inclus ces événements sociaux dans l'inscription à la conférence, car nous pensons que le fait de se connecter ainsi avec des collègues est inestimable pour développer des collaborations. La conférence s'achèvera par la remise de prix pour les meilleures présentations orales et par affiches, ainsi que par la remise de deux nouveaux prix, l'un pour les recherches qui intègrent les principes d'équité, de diversité et d'inclusion et l'autre pour les recherches qui intègrent l'engagement des patients.

Rejoignez-nous à Toronto en juin prochain pour le retour de la CCTC en personne!

Adele Changoor et Marc Lavertu

Prof. Adele Changoor
University of Toronto
Co-chair of CCTC 2023

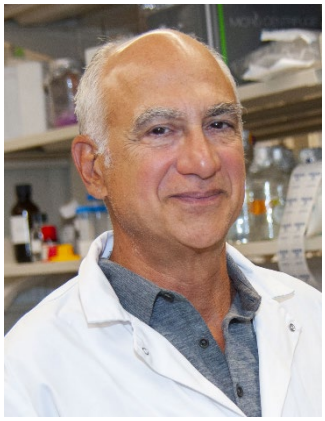
Prof. Marc Lavertu
Polytechnique Montreal
Co-chair of CCTC 2023

Message from President

Dear Friends, Colleagues, and all new trainees, it is with the greatest of pleasure that I welcome everyone back in person to our 27th annual Canadian Connective Tissue Conference!

Our mandate is to foster the careers of young Canadian scientists by providing trainees an opportunity to present their work and chair scientific sessions. Equally important is to provide a forum that will facilitate the ability of trainees to form a network of colleagues that can be relied upon during their future career paths.

I have attended about 20 of the 26 past conferences. These annual events are instrumental in enabling me to form and maintain relationships with scientific friends and colleagues; the support of these long-standing relationships, renewed annually at the CCTC, have allowed me to thrive and prosper within the Canadian Scientific community.



It is my sincere wish that all new trainees come away from this conference with the sense that they are now part of a larger community of friends and colleagues that will always be there for them, supporting their career. I encourage you to sit beside people you don't know and chat with them. Talk to as many people as you can during the poster sessions. The future of Canadian connective tissue research and our society is dependant on your ability to form and maintain networks, so please make the most of this opportunity to form a community for yourself.

Thanks for attending and supporting our community!

All the best, Morrie

Morris F. Manolson

CCTS, President.

Organizing committee for the 27th Canadian Connective Tissue Conference

Prof. Dieter Reinhardt
McGill University

Neha Dinesh, PhD Candidate
McGill University

Maya Ezzo, PhD Candidate
University of Toronto

Jodie Simard
University of Toronto

Abstract Reviewers

We would like to thank our panel of abstract reviewers who took the time to evaluate all the submitted abstracts for CCTC 2023

Dr. Margarete Akens, University of Toronto

Dr. Madhu Baghel, Bone & Joint Center

Dr. Adele Changoor, University of Toronto

Maya Ezzo, University of Toronto

Dr. Julie Fradette, University of Laval

Dr. Casimiro Gerarduzzi, University of Montreal

Dr. Michael Grant, Lady Davis Institute

Dr. Marc Grynpas, University of Toronto

Dr. Lisbet Haglund, McGill University

Dr. Caroline Hoemann, George Mason University

Sophia Huang, University of Toronto

Dr. Neda Latifi, University of Toronto

Dr. Marc Lavertu, Polytechnique Montreal

Dr. Morrie Manolson , University of Toronto

Dr. Chris McCulloch, University of Toronto

Dr. Veronique Moulin, University of Laval

Dr. Zofia Ostrowska-Podhorodecka, University of Toronto

Dr. Dieter Reinhardt, McGill University

Dr. Jason Rockel, University of Toronto

Mohammed Said, University of Toronto

Dr. Dong Ok (Donna) Son, University of Toronto

Fereshteh Sadat Younesi, University of Toronto

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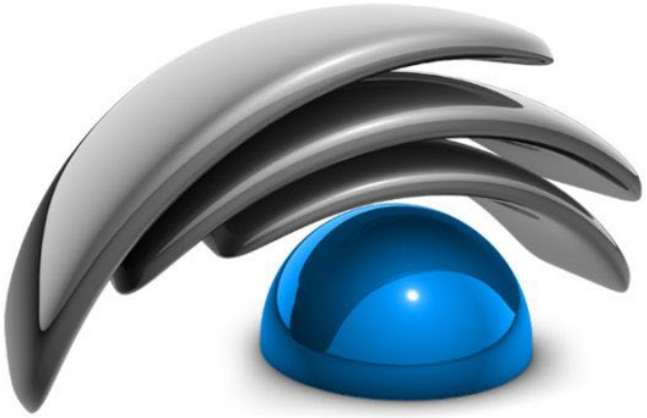

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Visit our booth!

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SKIN RESEARCH GROUP OF CANADA


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D'INGÉNIERIE
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TECHNOLOGIES
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Enabling Next Generation
RNA-Based Therapeutics

BCL BIOMATERIALS AND CARTILAGE LABORATORY

LBC LABORATOIRE DE BIOMATÉRIAUX ET DU CARTILAGE

The Changoor Lab

Sinai Health | Lunenfeld-Tanenbaum Research Institute

The 27th Annual Canadian Connective Tissue Conference
June 14-June 16, 2023

Overview

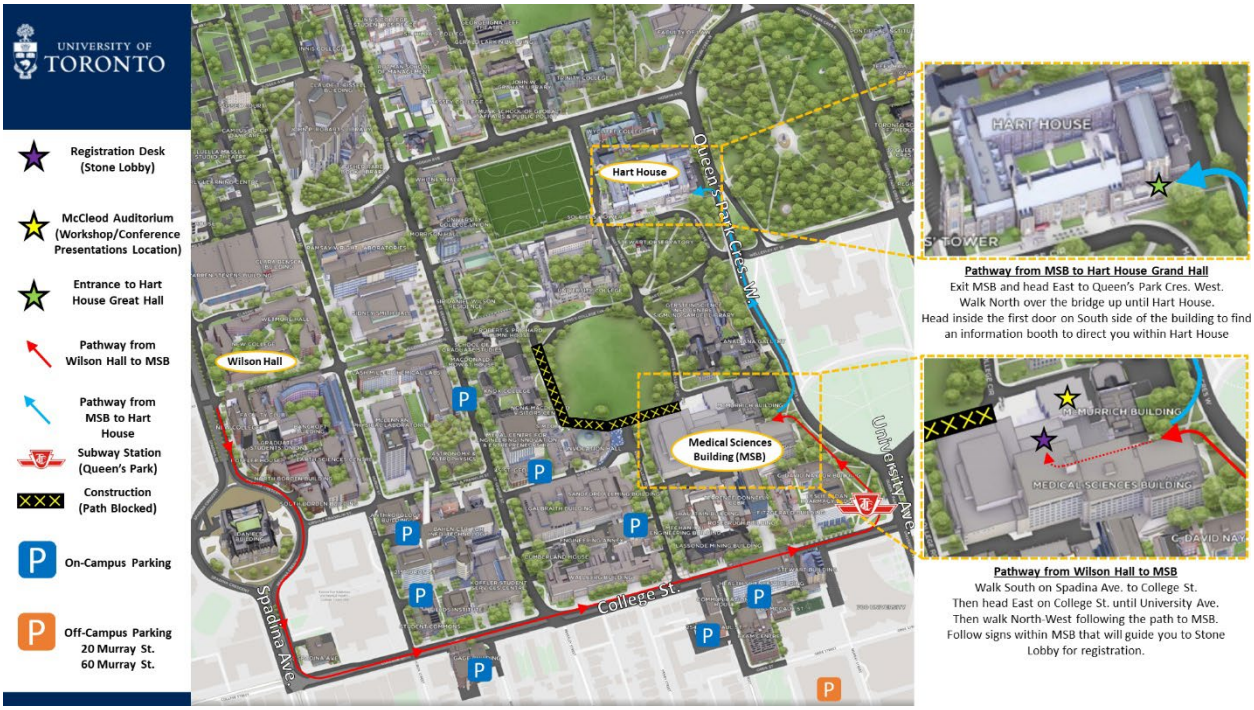
The first ever Canadian Connective Tissue Workshop with themes of Careers Outside of Academia, Entrepreneurship & Patient Engagement will be held on Wednesday, June 14th for all conference attendees. The objectives of the Workshop are to 1) Provide our members with new ideas concerning career paths outside of academia, and 2) Educate our members on patient perspectives in connective tissue research and set the stage for the inclusion of patients in future research. Interactive components will be used to engage attendees and guide them to think about how their research skill sets can be translated to industry careers and how promising research ideas may be commercialized. The Patient Engagement theme aims to educate attendees on how to incorporate patients into their research and how to effectively communicate scientific information to lay audiences.

"Mentorship Opportunity: Those selected for a pitch presentation will be mentored by Dr. Joseph Ferenbok, Associate Director of the Health Innovation Hub at the University of Toronto, who will help with presentation preparation."

Note: All oral presenters are requested to bring their presentation in a USB key, ready to be uploaded at registration desk on **Wednesday, June 14.** If unable to provide the presentation by USB key then the presenters should email it directly to **jodie.simard@utoronto.ca** by June 14.

Poster Judging: Poster judging will be during the second half of each poster session on Wednesday, June 14th and Thursday, June 15th. Posters can be taken down on Friday, June 16th (before 1:30 pm). Please pick up Velcro to hang your poster during check in on Wednesday, June 14th.

Conference Maps



Workshop and Conference Venue:
Medical Sciences Building
1 King's College Circle
Toronto, ON
M5S 1A8

Welcome Reception and Gala Dinner:
Hart house
7 Hart House Circle
Toronto, ON
M5S 3H3

Off-campus parking can be found at:

60 Murray St, Toronto, ON M5T 3L9

Or alternatively at:

20 Murray St, Toronto, ON M5T 1T7

Day 1	Wednesday, June 14 – Workshop Day Medical Sciences Building University of Toronto 1 King's College Circle Toronto, ON M5S 1A8
7:45- 8:30 h	Registration Opens
8:30- 8:40 h	**Welcome Remarks** **Dr. Adele Changoor & Dr. Marc Lavertu (CCTC 2023 Co-Chairs)**
8:40-10:15 h	**WORKSHOP SESSION I: Careers Outside of Academia** *Highlight: Experienced industry panelist with a variety of expertise* *Guest speakers & panel discussion* *Goal: To provide the audience with an opportunity to learn from those who have pursued careers outside of a traditional academic path*
08:40h	Moderator : Joseph Ferenbok, Unversity of Toronto & Health 2 Innovation Hub
08:50h	Martin Garon, Biomomentum Inc.
08:55h	Yasmin Mawani, Red Rock Regeneration
09:00h	Albert Yee, University of Toronto & Sunnybrook Health Sciences Centre
09:05h	Abdelahhad Barbour, Ostia Sciences
09:10h	Ryan Siu, MDPI

09:15h	Moderated Panel Discussion
10:15 – 10:30 h	Coffee break
10:30-12:15 h	**WORKSHOP SESSION II: Entrepreneurship** *Highlight: Pitch Presentations selected from online submissions with feedback provided by industry experts* Guest speakers & Pitch presentations *Goal: To educate the audience about issues related to the commercialization of research*
10:30h	Joseph Ferenbok, Unversity of Toronto & Health 2 Innovation Hub – Moderator & Presenter
10:45h	Claude Leduc, RNA Technologies & Therapeutics
11:00h	Lisa Wise-Milestone, Moderna Canada
11:15h	Moderated Panel Discussion
11:35h	Student Pitch Presentations: Abdellatif Elseoudi, Prohibitin as a Potential Biomarker to Predict Osteoarthritis Progression Mohadeseh Sartipi, Genetics and Gums: Personalized Medicine Approach to Oral Health Care Peter Suderman, Electroarthrography: An Accessible Method for Monitoring Joint Health
12:15-13:45 h	**Lunch and Poster Judging Session-1**
13:45-15:05 h	**WORKSHOP SESSION III: Patient Engagement in Connective Tissue Research** *Highlight: Perspectives from patients experiencing connective tissue diseases* *Patient speakers & panelist discussion* *Goal: To provide an opportunity for attendees to hear from patients living with connective tissue diseases and to provide information about how to include patients at various stages of reserach*

13:45h	Morris Manolson, Univeristy of Toronto & Patient Advocate – Moderator
13.55h	Dawn Richardson – CIHR Patient Engagement Representative
14:07h	Anna Samson, CIHR Patient Engagement Representative
14:19h	France Carbonneau, The Arthritis Society
14:31h	Morris Manolson, Canadian Association of Psoriasis Patients
14:43h	Panel Discussion – 15-20 minutes
15:05– 15:20 h	Coffee break
15:20 – 16:30 h	**WORKSHOP SESSION IV: Knowledge Translation-Three Minute Thesis Competition** *Goal: To highlight the importance of Knowledge Translation skills by having participants condense their research into a clear and concise presentation within three minutes and supported by only a single static slide.*
	Presentations by 19 trainees & winners selected by the audience
17:00-18:30 h	**Welcome Reception** **Keynote Speaker : Dr. Dennis Discher** **Connective Tissue Directed Cell Response – A Pan-Tissue View of Matrix Stiffness Effects** Great Hall, Hart House University of Toronto Hart House Circle Toronto ON M5S 3H3

Day 2 Thursday, June 15 – Scientific Program

Medical Sciences Building
University of Toronto
1 King's College Circle
Toronto, ON M5S 1A8

7:45-8:30 h	Breakfast
8:30-10:10 h	**SESSION I: Cartilage and Intervertebral Disc – I**
8:30 – 8:55 h	**Keynote speaker: Dr. Caroline Hoemann**
8:56 – 9:07h	Multiple-omics integrative analyses identified three endotypes of knee osteoarthritis by Jingyi Huang
9:08 – 9:19 h	Non-invasive electroarthrography prototype with re-usable carbon fiber fabric electrodes by Peter Suderman
9:20h – 9:31 h	Mechanical and histological signs of early cartilage degeneration following non-invasive ACL rupture in a rat model by Colleen Mathieu
9:32h – 9:43 h	CD109 differentially regulates ALK5 versus ALK1 signaling to increase protease expression and inhibit extracellular matrix production in articular cartilage by Shikha Chawla
9:44 – 9:56 h	Elucidating the effects and mechanisms of action of delta-9-tetrahydrocannabinol on knee osteoarthritis by Anca Maglaviceanu

9:57 – 10:08 h	Determination of urinary and synovial fluid C2C levels in total knee arthroplasty patients by Amit Sandhu
10:10-10:25 h	Coffee break
10:25 –11:25 h	**SESSION II: Cartilage and Intervertebral Disc- II**
10:25 – 10:50 h	**Keynote speaker: Dr. Lisbet Haglund**
10:51 – 11:02h	Regenerative approaches to treat disc degeneration and back pain by Saber Ghazizadeh Darband
11:03 – 11:14h	Development of an ex vivo disc model of degenerative disc disease for cell therapy by Sajjad Ashraf
11:15 – 11:26 h	Investigating the role of TRPV4 as a mechanoreceptor in the intervertebral disc by Taylor Shelton
11:30 – 12:30 h	**SESSION III: Cell Biology of Connective Tissues**
11:30 – 11:55 h	**Keynote Speaker** **Lifetime Achievement Award Winner: Christopher McCulloch**
11:56 – 12:07h	Direct contact with mechanically activated myofibroblasts drives macrophages into distinct transcriptional and functional states by Li Diao
12:08 – 12:19h	Characteristics of the extracellular vesicles from human intervertebral disc cells by Li Li

12:19 – 12:30h	Evaluating the profibrotic role of IL4Rα in monocytes and macrophages in pulmonary fibrosis by Megan Vierhout
12:30 – 14:00 h	**Lunch and Poster Judging Session-2**
12:30 – 14:00 h	CCTS Board meeting (only for board members)
14:00 – 15:50 h	**SESSION IV: Bone & Developmental Biology**
14:00 – 14:25h	**Keynote Speaker: Dr. Derek Rosenzweig**
14:26 – 14:37h	Molecular pathomechanisms of fibronectin mutations leading to spondylometaphyseal dysplasia by Neha Dinesh
14:38 – 14:49h	Over-expression of PITX1 induces periodontitis-like phenotype in transgenic mice by Mohadeseh Sartipi
14:50 – 15:01h	Heterotopic ossification following spinal cord injury: A novel mouse model by Rachad Aita
15:02 – 15:13h	Bone resorption markers in patients and mouse models of Osteogenesis Imperfecta: Systematic review and meta-analysis by Sirion Aksornthong
15:14 – 15:25h	Assessing the membrane injury and repair dynamics in osteoclasts by Chrisanne Dsouza
15:26 – 15:37h	A 3D, compartmental tumoroid model of patient-derived bone metastasis mimics tumor-stromal microenvironment by Derek Rosenzweig

15:45 – 16:00 h	Coffee Break
16: 00 – 17:05h	**SESSION V: Adipose Tissues & Stem Cells and Therapy**
16:00 – 16:25h	**Keynote: John Walker**
16:26 – 16:37h	Impact of obesity on cellular constituents and transcriptomic signature of infrapatellar fat pad in patients with knee osteoarthritis by Hayley Peters
16:38 – 16:49h	Role of fibrillin-1 in adipose tissue development and homeostasis by Iram Fatima Siddiqui
16:50 – 17:01h	Mechanically modified chromatins imprint memory of myofibroblastic transcription profiles in mesenchymal stromal cells by Fereshteh Younesi
18:30 – 21:30 h	**Gala dinner** **Keynote Speaker: Boris Hinz** Great Hall, Hart House University of Toronto Hart House Circle Toronto ON M5S 3H3

Day 3

Friday, June 16 – Scientific Program

Medical Sciences Building
University of Toronto
1 King's College Circle
Toronto, ON M5S 1A8

7:45 – 8:30 h	Breakfast
8:30 – 10:05 h	**SESSION VI: Connective Tissue Repair, Regeneration, Bioengineering -I** **Keynote Speaker**
8:30 – 8:55h	**Robin Poole Award Winner: Mohit Kapoor**
8:56 – 9:07h	Myofibroblast activation is induced through intracellular stress in a calcium-dependent process upon acute contact with profibrotic macrophages by Maya Ezzo
9:08 – 9:19h	Mesenchymal stromal cells grown in soft environment produce extracellular vesicles that suppress pro-fibrotic activation of macrophages and fibroblasts by Yan Hei Kelly Choi
9:20 – 9:31h	Mechanically active culture of patient-derived cruciate ligament cells for ligament tissue engineering by Tarek Klaylat
9:32 – 9:43h	Neutrophils mediate kidney fibrosis via extracellular matrix protein 1 by Jonatan Barrera-Chimal
9:44 – 9:56h	Tissue collagen degradation by endogenous MMP's is triggered by contractility inhibitors by TBA
9:57 – 10:08h	Identification of EphB4 as a critical mediator of tissue fibrosis by Brian Wu

10:10 – 10:25 h	Coffee Break
10:25h – 11:30 h	**SESSION VII: Connective Tissue Repair, Regeneration, Bioengineering- II**
10:25h – 10:50h	**Keynote Speaker: May Griffith**
10:51 – 11:02h	Effect of glycation on collagen properties for tissue engineering applications by Mina Vaez
11:03 – 11:14h	Doxorubicin-loaded nanoparticle bone cement for the treatment of metastatic spine disease by Ateeque Siddique
11:15 – 11:26h	Bone regeneration: A new application for DNA hydrogels by Nadeen Meshry
11:30 – 12:00 h	**CCTS Business Meeting/General Assembly (all CCTC participants)**
12:00– 13:30 h	**Lunch and Poster Session 3 – Judging**
13:30 – 14:50 h	**SESSION VIII: Skin & Matrix Biology**
13:30 – 13:55h	**Keynote Speaker:** Dr. Anie Philip
13:56 – 14:07	Repeated applications of mesenchymal stem cell-based biological dressings accelerate wound healing in a murine model of diabetes by Meryem Safoine

14:07 – 14:18h	Histological and mechanical characterization of skin in wound healing study with streptozotocin induced diabetic rats and application of chitosan and platelet-rich plasma biomaterial by Laura Ahunon
14:19 – 14:30h	A new antimicrobial drug, Ag373K augments keratinocyte migration by inducing vascular endothelial growth factor (VEGF)-A) release by Vida Maksimoska
14:31 – 14:42h	Role of N-linked glycans in short fibulins and LTBP-4 mediated matrix assembly and function by Valentin Nelea
14:45 – 15:00 h	Coffee Break
15:00 – 16:05 h	**SESSION IX: Machine Learning, AI and Computational Biology**
15:00 – 15:25h	**Keynote Speaker: Dr. Armstrong Murira**
15:26 – 15:37h	Generation of synthetic µCT images of rat lumbar vertebral fracture via deep convolutional generative adversarial networks by Allison Tolgyesi
15:38 – 15:49h	Unravelling PRP mechanisms of action: Integrating proteomics, metabolomics, and lipidomics using causal inference and deep graph neural network
15:50 – 16:20 h	**Awards Presentation and Concluding Remarks**

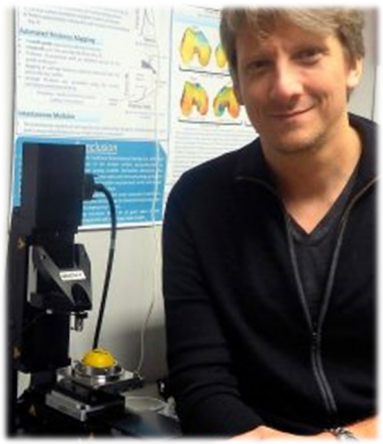
Guest Speakers: Careers outside of Academia

Moderator: **Dr. Joseph Ferenbok**

Dr. Ferenbok has an eclectic background that includes aspects of entrepreneurship, intrapreneurship, design, english literature, philosophy, engineering, computer science, humanities, biology, art history, surgery, and psychiatry. He is the founding Director of the Translational Research Program (TRP), Department of Laboratory Medicine and Pathobiology in the Temerty Faculty of Medicine at the University of Toronto. The TRP is a graduate program focused on training interdisciplinary trainees to systematically apply scientific knowledge to improve medicine, health & care. Dr. Ferenbok is an unconventional academic who loves to generate ideas to solve complex unwieldy problems. He is a pioneer in the translational community as co-founder and Associate Director of the Health Innovation Hub, the Temerty Faculty of Medicine's venture accelerator on health matters; and the Director of Education for Medical Innovations Toronto

Dr. Martin Garon

Martin Garon is an accomplished scientist and entrepreneur with extensive experience in the biomedical engineering industry. He is currently the President and Co-Founder of Biomomentum Inc. a Canadian-based company. Martin earned his Bachelor's degree in Physical Engineering from Polytechnique Montreal and went on to complete a PhD in Biomedical Engineering from the same institution. Throughout his graduate studies under the guidance of Prof. Michael Buschmann, Martin has received numerous scholarships and awards for his academic excellence. He also worked closely with industry, co-authored three patent applications, and was a finalist for the 2004 Young Innovator Award from the Canadian Arthritis Network. Following his PhD, he gained two years of industry experience before establishing Biomomentum in 2009 with his former labmate, Eric Quenneville. Since its founding, the company has received several prestigious awards, including in 2010 ranking first in the province of Quebec in the innovation and technology category of the Business Creation section of the OSEntreprendre Challenge. Currently, Biomomentum manufactures and commercializes the Mach-1™ mechanical tester for tissues and biomaterials to customers worldwide. This all-in-one, upgradable, multiaxial mechanical tester not only facilitates basic mechanical testing, such as compression, tension, bending, shear, friction, and torsion but also employs a unique proprietary technology developed by Martin that maps the 3D shape and mechanical properties of curved samples. Martin is also leading the way for Biomomentum's novel laboratory services division, which provide state-of-the-art mechanical testing, organ culture models, and biochemical and histological assessments to companies in the biotech sector.



Dr. Yasmin Mawani

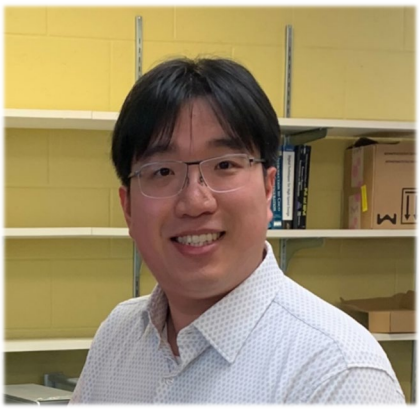
Yasmin Mawani has 9 years of experience working in the biologics and medical device fields. She is currently the Director of Product Development at Red Rock Regeneration Inc., a growing startup that focuses on the development and commercialization of bioimplants. Prior to working at Red Rock, she was a Biopharmaceutical Project Manager at SGS Life Sciences, the largest contract testing organization in the world, where she managed the analytical testing and stability studies for several large clients. Yasmin previously worked as a Scientist at Induce Biologics, a medical device startup that was focused on the development of a combination medical device. She completed her doctorate in medicinal inorganic chemistry at the University of British Columbia where her research focused on developing a more bioavailable treatment for osteoporosis using different ligands to complex with lanthanides.

Dr. Albert Yee

Dr. Albert Yee is the Holland Bone and Joint Program Chief and Division Chief, Marvin Tile Chair of Orthopaedic Surgery at Sunnybrook Health Sciences Centre. Dr. Yee is a Full Professor at the University of Toronto, Department of Surgery and a Full Member of the Institute of Medical Sciences with a cross-appointment in the Institute of Biomedical Engineering. He is the Vice-Chair of Research for the University Division of Orthopaedic Surgery and a Co-Director of the Department of Surgery Spine Program. Dr. Yee is a past President of the Canadian Orthopaedic Research Society (2016-17), the Canadian Spine Society (2019-21) and a Co-Chair of Bone & Joint Canada (2016-22). Dr. Yee has over 100 peer reviewed publications and has received academic honours including the Canadian Orthopaedic Foundation J. Edouard Samson Award (2011), Charles H. Tator Surgeon-Scientist Mentoring Award (2012) and the American British Canadian (ABC) International Travelling Fellowship, American Orthopaedic Association/Canadian Orthopaedic Association (2013). In 2019, he was also awarded the distinction of Fellow of International Orthopaedic Research (FIOR) by the International Combined Orthopaedic Research Society. His research focuses on translational orthopaedic and spine studies utilizing pre-clinical surgical models to evaluate minimally invasive therapeutics (e.g. photodynamic therapy, radiofrequency ablation in vertebral metastases). This work has led to first in human clinical trials and FDA approval and commercialization of new spine technology.

Dr. Abdelahhad Barbour

Dr. Barbour is a molecular microbiologist, co-founder, and CEO of Ostia Sciences Inc, a University of Toronto startup company. Inspired by Sir Alexander Fleming's discovery of penicillin, Dr. Barbour's research focuses on discovering beneficial bacteria that secrete antimicrobial compounds to combat infectious diseases and antibiotic resistance by developing innovative microbiome solutions. Dr. Barbour is an expert in lantibiotics, a class of antibiotics produced by beneficial microbes. During his post-doctoral research at the University of Toronto, Dr. Barbour discovered salivaricin 10, the world first phosphorylated lantibiotic, with dual antimicrobial and pro-immune functions. Dr. Barbour, together with Dr. Michael Glogauer, created Ostia Sciences to commercialize salivaricin 10 and the beneficial bacteria that produce it "*Streptococcus salivarius*" by developing new biotherapeutics to fight against the emerging antibiotic-resistant superbugs which are associated with respiratory infections and oral diseases.



Dr. Ryan Siu

Dr. Siu is the Journal Relations Manager of MDPI Open Access Publishing Canada Ltd. He completed his doctorate in Neurobiology at York University in 2020. His research focuses on synaptic plasticity in cellular and animal models. He joined MDPI in 2021 as an assistant editor and later publishing manager in 2022. Dr. Ryan Siu is here to share his experience transitioning from academia to publishing.

Guest Speakers: Entrepreneurship

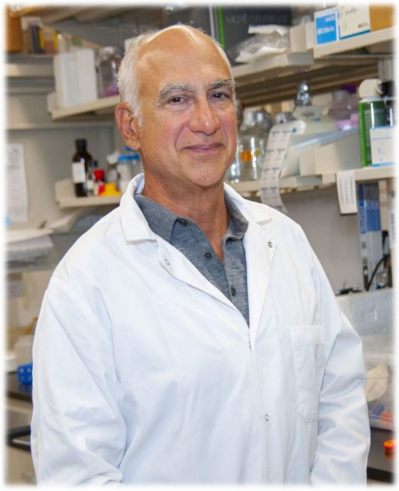
Dr. Claude Leduc

Claude is a Co-Founder and President – CEO of RNA Technologies & Therapeutics. He is a Bio-Pharma professional CEO / COO with 34 years of international experience and achievement in private and Fortune 500 Companies. During his career, Claude served various positions in sales, marketing, and international business development a Syntex Labs, Serono Labs and Biomatrix-Genzyme Biosurgery. He is a serial entrepreneur and investor and lead as CEO, teams succeeding in various development stages, from R&D, Preclinical and clinical programs, to market launch at BioSyntech, Skeltex Technologies, Axcellon Biopolymers, MRM Proteomics and Ortho Regenerative Technologies. Claude raised more than $70M+ in equity and debenture notes in private and public settings during his startups CEO positions.

Dr. Lisa Wise-Milestone

Dr. Milestone is the Strategic Lead for Pandemic Preparedness Initiative at Moderna Canada. She leads the execution of Moderna's Canadian strategy for biomanufacturing and pandemic preparedness. Lisa previously worked at FACIT, an entrepreneurial commercialization-venture group focused on advancing Ontario's life sciences innovations. She served numerous roles at FACIT, including corporate affairs, communications, government relations and project management. Prior to joining FACIT, Lisa was a Manager at KPMG LLP, where she led engagements for companies applying for Canada's Scientific Research and Experimental Development (SR&ED) tax credit program. Lisa has more than 10 years' experience in academic research settings, having completed a PhD in bioengineering from the University of Toronto and a postdoctoral fellowship at Sunnybrook Research Institute.

Guest Speakers: Patient Engagement in Connective Tissue Research



Moderator: **Dr. Morris F Manolson**

Dr. Manolson is a Professor and Interim Vice Dean, Research at the Faculty of Dentistry at the University of Toronto and President of the Canadian Connective Tissue Society. His research, focusing on preventing excessive bone loss associated with osteoporosis, inflammatory arthritis and periodontal disease, is funded by CIHR and NSERC, has produced three patent applications and over 60 peer reviewed papers which have accumulated over 5000 citations. He received the "Quality of Life" award from the Institute for Musculoskeletal Health and Arthritis, and the Canadian Institute of Health Research-Institute for Gender Health/Ontario Women's Health Council Senior Investigator Award, both in recognition of his work towards preserving bone health in arthritis and osteoporosis. With respect to patient engagement, Morrie was a board member the Canadian Skin Patient Alliance (2014-2017) and Chair of the board of directors of the Canadian Association of Psoriasis Patients from 2017 to 2022. He is currently a Patient Advisory Committee Member for the Psoriatic Arthritis Research Program at Toronto Western Hospital and a member of the Steering Committee for the Skin Investigation Network of Canada (SkIN Canada).

Dr. Dawn Richards

Dr. Richards, PhD, is the founder of Five02 Labs Inc. Her firm provides traditional services including project management and preparation of grants, manuscripts, corporate and lay language materials; along with those to incorporate the patient perspective and through the development of relationships with patients and patient organizations. Clients are from all sectors. With a PhD (Analytical Chemistry) from the University of Alberta, Dawn has worked in a variety of roles during the past 20 years, however her diagnosis with rheumatoid arthritis almost 15 years ago instigated a journey to intertwine her passion for science with making the most of her diagnosis. As a patient advocate and volunteer, Dawn is Vice President of the Canadian Arthritis Patient Alliance and a member of The BMJ's Patient Panel. She served as an IMHA Research Ambassador from 2014 – 2019 and was the first Patient Advisor of the Canadian Medical Association's Wait Time Alliance. Dawn advocates for arthritis awareness, access to treatment, the importance of research and the inclusion of patients both in decision-making and as research collaborators.

Anna Samson

Anna Samson (they/she) is a 23-year-old desi, queer, disabled person living near Toronto, Canada. They are a writer and an advocate for disability and mental health. They have ankylosing spondylitis, fibromyalgia, depression, anxiety, PMDD, and more. They have experienced symptoms since adolescence and now permanently use a cane and frequently use a walker to ambulate. They are passionate about using their intersectional lived experiences to raise awareness for chronic illnesses, disabilities, and mental health.

Anna is also an ambassador with Take a Pain Check where they raise awareness for chronic illnesses and disabilities in young people. They are also a patient partner with CIHR-IMHA's Patient Engagement Research Ambassador program where they collaborate with other patient partners to advocate for patient concerns in research. They are also a contributing writer for Health Union where they write articles sharing their lived experience with axial spondyloarthritis, chronic dry eye, and insomnia.

France Carbonneau

France Carbonneau,MBA-Research is an Innovation Ambassador at Arthritis Society Canada and provides input and feedback on innovation initiatives in the arthritis field. She currently volunteers as an information officer for the Information Line of the Society where she provides advice, support and reliable information to patients living with all types of arthritis conditions. France also facilitates virtual support groups for the Society for patients across Canada. France is an osteoarthritis and fibromyalgia patient who is passionate about advancing arthritis research and innovation.

Abstracts for Three Minute Thesis

Welcome Reception

Great Hall, Hart House
University of Toronto
Hart House Circle
Toronto ON M5S 3H3

Keynote Speaker: Dr. Derek Discher

Dr. Dennis Discher

Dr. Discher began at Penn in 1996 after a PhD at UC Berkeley & UC San Francisco and following an NSF Fellowship in computational biophysics at U British Columbia and Simon Fraser Univ. His lab focused first on biophysics of membranes and polymer mimetics, and then discovered matrix elasticity effects on stem cell differentiation and nucleus mechanosensing. Recent efforts focus on mechanobiology of DNA damage and genome variation, as well as 'self' pathways in macrophage attack of solid tumors. He is an elected member of the US National Academy of Medicine, the US National Academy of Engineering, the American Association for the Advancement of Science, and is on the Editorial Board of *Science*.

Talk title: Connective tissue directed Cell response – A Pan-tissue view of Matrix Stiffness effects

Solid tissues differ in their mechanics, with some being soft like the brain, some being rigid like cartilage and bone, and most falling somewhere in between, such as muscle. A good guess of course is that connective tissue amount is a key determinant of such mechanical differences, but ongoing questions include which if any components are most relevant, and can any mechanical effects of matrix on cells be established separate from biochemical effects? Our group and many others have been addressing these and additional questions over the past two decades in the context of mature tissue, disease, and to some extent development. Materials beyond polystyrene culture dishes have been key. Cytoskeletal forces among other pathways seem to have roles in modulating mechanisms and might present opportunities for some control. Tissue water content, viscosity, and viscoelasticity are further properties to consider, and modern single cell omics methods are adding insight to what seems to be a useful pan-tissue perspective.

SESSION I: Cartilage and Intervertebral Disc- I

Keynote Speaker: Dr. Caroline Hoemann

Dr. Hoemann (Ph.D.,MIT 1992), was Director of Cartilage Repair in a Montreal-based Biotech company for 5 years, where she co-invented and helped translate BST-CarGel®, a biomaterial device for articular cartilage repair, to the clinic. Supported by fellowships from the Canadian Arthritis Network and Fonds de Recherche Québec Santé, she transitioned back to academics to develop a research program centered on understanding the role of surgical wounding and biomaterial-induced inflammation in bone and cartilage repair at Polytechnique Montréal (2002-2017) after which she took a position in the Department of Bioengineering at George Mason University. Her laboratory is investigating how biomaterials interface with the innate immune system to drive tissue remodeling and regeneration. Her team is currently studying the role of thromboinflammation arthritis and acute respiratory distress syndrome, and is developing a novel cultured blood clot precision medicine tool that can be used to monitor personalized drug-induced immune responses. Another area of research is using scanning acoustic microscopy, an ultrasound imaging technique, to study how post-traumatic knee injury initiates remodeling of the cartilage-bone interface. Dr. Hoemann has over 80 peer-reviewed manuscripts, 7 book chapters, 8 patent applications (5 granted), serves on editorial boards of Cartilage and OA&C Open, is a Fellow of the ICRS and ICORS, and co-founder of Chitogenx, a company specializing in regenerative medicine in Orthopedics.

SESSION I: Abstracts for Oral Presentations

ABSTRACT #17:
Multiple-omics integrative analyses identified three endotypes of knee osteoarthritis

Jingyi HUANG[1], Ming LIU[1], Hongwei ZHANG[1], Feng PAN[2], Guang SUN[1], Andrew FUREY[1], Proto n RAHMAN[1], Graeme JONES[2], Guangju ZHAI[1]

[1]Memorial University of Newfoundland, St. John's; [2]University of Tasmania, Hobart, Australia

Background: Osteoarthritis (OA) is a heterogenous disorder, but there is still no consensus on how to classify OA patients into subgroups.

Objectives: This study aimed to identify endotypes of knee OA(KOA) and explore potential mechanisms through an integrative analysis of metabolomics, genomics, and transcriptomics.

Methods: Fasting blood and cartilage samples were obtained from OA and healthy controls recruited from the Newfoundland and Labrador population. Metabolomics, genomics, and transcriptomics data were generated by LC-MS/MS, microarray, and RNA-Seq.

Results: 409 KOA patients and 120 controls were included in the metabolomic analysis. Based on concentrations of 622 metabolites, the Uniform Manifold Approximation and Projection (UMAP) analysis grouped participants into four separate clusters (G0:110, G1:157, G2:141, G3:121). Among them, G0 was considered as the control cluster because it included the majority of the healthy controls (96/120), G1/G2/G3 were considered as three OA subgroups. This clustering had a sensitivity, specificity, and accuracy of 96.5%, 80%, and 92.8%, respectively, to distinguish KOA patients from controls. The combination of lactic acid, dehydroepiandrosterone sulfate, and the average value of two diglycerides had an area under the curve (AUC) of 0.996 in the ROC analysis for distinguishing KOA from controls, which was validated in an independent cohort with an AUC of 0.88. The combination of succinic acid and the average of five lysophosphatidylcholines could distinguish G2 vs. G1/G3 with an AUC of 0.929. The combination of butenylcarnitine (C4:1) and hexoses (H1) could distinguish G1 vs. G3 with an AUC of 0.865. GWAS analyses of these seven metabolic markers found 126 SNPs with minor allele frequency >1% were associated with all these markers at GWAS significance level ($p<5\times10^{-8}$) except for C4:1 and H1. These SNPs were directly located in or nearby 35 genes, and 24 of them had RNA-Seq data in >80% of the study participants. 10 out of these 24 genes had significantly higher expression levels in OA-affected cartilage than controls. Together, our data suggested that some of the metabolic pathways that contribute to the endotypes of KOA could be attributed to the genetic variation, which warranted further study.

Conclusions: Our data demonstrated that seven metabolic markers could discriminate KOA patients from healthy controls and separate KOA patients into three endotypes, which might partially be attributed to their genetic differences. While validation is needed, our results provided new insights into the mechanisms of the OA heterogeneity and had a great potential in clinical utility.

ABSTRACT #27:

Non-invasive electroarthrography prototype with re-usable carbon fiber fabric electrodes

Peter SUDERMAN[1,2], Hani NAGUIB[1], Marc GRYNPAS[1,2], Adele CHANGOOR[1,2]

[1]University of Toronto, Toronto; [2]Lunenfeld-Tanenbaum Research Institute, Toronto

Background: Osteoarthritis is a disease of articular joints characterized by progressive cartilage degradation. Detection of early cartilage degradation may identify opportunities for preventative treatment. There is a need for a sensitive and accessible method for assessing cartilage quality. Electroarthrography is the non-invasive measurement of electrical signals produced by cartilage through electrodes placed on skin around a joint. Cartilage electrical signals arise from the response of its hydrated extracellular matrix to compressive loading and reflects cartilage composition, structure, and function. Previous electroarthrography research required the application of individual, single-use electrodes, limiting clinical applicability. This study aims to create a re-usable prototype device that can capture electroarthrography signals at multiple sites around a joint in a single step process.

Methodology: A prototype device that could be installed around a knee in a single step was created using electrodes custom-made with conductive carbon fiber fabric (PX35, Zoltek). Data was captured using a BioRadio (Great Lakes Neurotechnologies) and simultaneous recording of force was captured using a Wii Balance Board (Nintendo). Three volunteers with no history of knee injury or pain (21 – 42 years old, BMI: 17.9 – 25.8) had electroarthrography measurements taken from both tibiofemoral joints at four locations on medial and lateral aspects of each joint. Measurements were captured as the volunteer was instructed to shift weight side to side to produce repeated compressive loading. Ten weight shifts for each limb were utilized for analysis. Signals were normalized to axial load filtered and drift removed. Intraclass correlation coefficients (ICC (2,1)) were calculated within weight shifts for each knee and between knees of each volunteer to assess repeatability. Results are reported as average ICC +/- standard deviation.

Results: Electroarthrography signals, normalized to force, were observed to be between 2.32 – 26.74 µV/kg. The average individual limb ICC was 0.739 +/- 0.186, reflecting moderate agreement between weight shifts. The average volunteer ICC was 0.812 +/- 0.040, indicating strong agreement between measurements obtained from both joints. Qualitative assessments of electroarthrography signals indicate that in addition to average signal amplitude, the change in signal amplitude with respect to the number of weight shifts may be an important factor for cartilage assessment.

Conclusions: These data demonstrated that the prototype could be deployed in a single step and be used to successfully capture electroarthrography signals produced by knee cartilage. Future work includes refining the prototype to optimize measurement sites and reduce noise, followed by validation in both normal and osteoarthritic joints.

ABSTRACT #29:

Mechanical and histological signs of early cartilage degeneration following non-invasive ACL rupture in a rat model

Jasmine DOAN[1], Sotcheadt SIM[1], Colleen MATHIEU[1], Alex PIETERS[1], Eric QUENNEVILLE[1], Mart in GARON[1]

[1]Biomomentum Inc.

Introduction: Anterior cruciate ligament (ACL) tears are common injuries that increase the incidence of post-traumatic osteoarthritis (PTOA). This study aims to explore the early pathology of PTOA at four weeks after non-invasive ACL-rupture in a *in vivo* rat model. Through mechanical and histological characterization of cartilage following injury, this model can mimic an accurate pathology portrait of PTOA which could be used to screen potential therapeutics.

Methods: Five 14-week-old Lewis rats were tested at the INRS-LNBE animal facility using protocols in accordance with the Canadian Council on Animal Care. Animals were randomly assigned in two groups: Control (N=1) and ACL-rupture (N=4). Using a tibial-compression protocol, a custom-fixture, and a mechanical tester (Biomomentum), animals in the ACL-rupture group received a non-invasive ACL injury under anesthesia. Their right hindlimb was fitted in the fixtures and underwent initial preconditioning followed by a 2mm vertical displacement at 8mm/s. Four weeks after injury, the animals were euthanized and both their hindlimbs were divided into three subgroups (Control n=2, Rupture n=4, and Contralateral n=4). Automated indentation and thickness mappings were carried out on freshly dissected femoral condyles and tibial plateaus using a 400µm grid to retrieve the cartilage' instantaneous modulus and thickness over 25-40 points on each surface. Next, all specimens were fixed (10%NBF), decalcified (0.5N HCl/1%glutaraldehyde) and paraffin embedded. Slides collected at 350-400µm intervals were stained with SafO/FG and H&E, and scored with a modified Mankin scoring system (Normal cartilage: 0 to fully degenerated: 17). Statistical analyses were performed using a linear model with R with significance set at $p<0.05$.

Results: Observations from the animal's tiptoeing and the dissection of right hindlimb joints at necropsy confirmed ACL-rupture. At four weeks, instantaneous modulus was significantly reduced in the medial-anterior region of both condyles and tibial plateaus in injured joints ($p=0.006$ and $p=0.005$ respectively). In femoral condyles, the cartilage was thicker on the entire surface in the Rupture group ($p<0.001$) suggesting cartilage swelling. Cartilage histology revealed mild surface irregularities and a slight to moderate decrease in SafO/FG staining with modified Mankin scores ranging from 0 to 6. Overall, results indicate that early cartilage degeneration occurred equivalent to mild signs of PTOA.

Conclusion: Non-invasive ACL rupture was successfully induced in a rat model, resulting in some visible differences between the Control and Rupture groups related to cartilage swelling, stiffness, and integrity. Next step is to validate the utility of this model in evaluating the efficacy of potential treatments.

ABSTRACT #34:

CD109 differentially regulates ALK5 versus ALK1 signaling to increase protease expression and inhibit extracellular matrix production in articular cartilage

Shikha CHAWLA[1], Sayeh KHANJANI[1], Kenneth W FINNSON[1], Meryem BLATI[1], Anie PHILIP[1]

[1]McGill University, Montreal

Introduction: Transforming growth factor-beta (TGF-β) is a pleiotropic cytokine that plays a crucial role in cartilage repair and homeostasis. Abnormally active TGF- β signaling has been observed in articular chondrocytes during the pathogenesis of osteoarthritis (OA). Our group has previously described the role of CD109, a novel TGF-β co-receptor in negatively modulating TGF-β signaling pathway in the skin. The current study aimed to delineate the role of differential CD109 expression levels, in modulating the balance between TGF-β signaling via ALK1 versus ALK5 signaling pathways and controls extracellular matrix expression (ECM) *in vivo* in murine articular chondrocytes.

Methods: Articular cartilage tissue was collected, and primary chondrocytes were isolated from CD109 Knockout (CD109 KO) and wild-type mice. Isolated chondrocytes and cartilage tissue were used to analyze TGF-β signaling pathway components by calculating the levels of Activin Receptor-like Kinase (ALK5) versus Activin Receptor-like Kinase (ALK1) and Smad2/3 versus Smad1/5 using Western blot and immunohistochemistry (IHC). Furthermore, chondrocyte function was evaluated by calculating the expression of collagen (type II versus type I and X), aggrecan, collagenase (MMP-13), and aggrecanase (ADAMTS-5), at the protein and mRNA levels by Western blot, real-time qRT-PCR, or immunocytochemistry (ICC), and IHC.

Results: We observed that articular chondrocytes isolated from CD109 KO mice demonstrate significantly augmented ALK5 levels, as well as enhanced gene expression levels of type II collagen and aggrecan. Instead, loss of CD109 expression in knockout mice demonstrated significantly reduced ALK1 levels, TGF-β1-induced Smad1/5 phosphorylation, and decreased protein expression levels of cartilage ECM degrading enzymes MMP13 and ADAMTS5. Interestingly we observed similar levels of proteoglycan and type I collagen expression in CD109 KO and wild-type mice.

Conclusion: Our findings suggest that CD109 differentially regulates TGF-β signaling pathway by modulating ALK5 versus ALK1 levels and inhibits ECM protein production and increases protease expression in articular chondrocytes *in vivo*. In conclusion, we propose the probable role of CD109 in regulating TGF- β mediated cartilage function and homeostasis.

ABSTRACT #81:

Elucidating the effects and mechanisms of action of delta-9-tetrahydrocannabinol on knee osteoarthritis

Anca MAGLAVICEANU[1,2,3], Jason ROCKEL[1,2], Helena FILIPPINI[4], Ewa WASILEWSKI[2,5], Melissa LEWIS-BAKKER[2,5], Sarah GABRIAL[1,2], Evgeny ROSSOMACHA[1,2], Johana GARCIA[1,2], Nizar MAHOMED[1,2,5], Timothy LEROUX[1,2,5], Hance CLARKE[2,6], Robert BONIN[4], Lakshmi KOTRA[2,4], Mohit KAPOOR[1,2]

[1]Division of Orthopaedics, Osteoarthritis Research Program, Schroeder Arthritis Institute, University Health Network, Toronto, ON; [2]Krembil Research Institute, Toronto; [3]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON; [4]Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, ON; [5]Toronto Western Hospital, University Health Network, Toronto, ON; [6]Department of Anesthesia and Pain Management, Toronto General Hospital, University Health Network, Toronto, ON

Objectives: Osteoarthritis (OA) is a whole-joint disorder that affects the articular cartilage, synovial membrane, and their cell constituents. Some patients use cannabis to alleviate pain symptoms, despite little being known about its effects. Joint cells express receptors that can bind Δ^9-tetrahydrocannabinol (THC), a prominent phytocannabinoid. In this study, we investigated the effects and signaling mechanisms of THC on pain and joint degeneration using pre-clinical OA mouse models and human OA cells.

Methods: Destabilization of medial meniscus (DMM) mice were administered THC (1, 5, or 10 mg/kg) orally 5 days/week or intra-articularly (IA) 1 day/week for 9 weeks. Monosodium iodoacetate (MIA) mice were administered THC (5 or 10 mg/kg) orally 5 days/week for 3 weeks. Von Frey tests were performed longitudinally on DMM and MIA mice to assess pain. DMM joints were evaluated for cartilage degeneration/synovitis (OARSI scoring) and αSMA/nuclear Ki67 expression (immunohistochemistry [IHC]). *In vitro*, flow cytometry was used to detect Annexin V^+ (apoptotic) primary human FLS and chondrocytes after treatment with 0-10 µM THC for 48 hrs. RNA sequencing was performed on THC-treated human OA chondrocytes and fibroblast-like synoviocytes (FLS) to determine differentially expressed genes (DEGs), and their associated pathways and transcription factors (TFs).

Results: IA injection of THC worsened cartilage degradation, increased synovitis, and had no effect on pain in DMM mice (n=5/group). Oral administration of THC at all doses attenuated cartilage degeneration, with 10 mg/kg THC decreasing synovitis (n=10-15/group) and synovial αSMA expression (n=6/group) in DMM joints, and reduced pain in DMM (n=15/group) and MIA (n=10/group) mice. *In vitro*, 2.5 µM THC significantly increased the percent Annexin V^+ OA FLS (n=5) and Annexin V^+ chondrocytes non-significantly (n=5). RNA sequencing revealed 73 differentially-expressed genes (≥1.5-fold) in OA FLS (n=4) and 21 genes in chondrocytes (n=4) with 1µM THC. Analyses showed that extracellular matrix (ECM)- and lipid/steroid/cholesterol-related pathways were enriched in the upregulated and downregulated genes of both cell types, respectively. Sterol regulatory element binding transcription factor 2 (SREBF2) was the most highly enriched TF associated with differentially-expressed genes reduced by THC in both FLS and chondrocytes.

Conclusions: Oral administration of 10 mg/kg THC reduced pain, cartilage degeneration, synovitis, and synovial αSMA expression in DMM/MIA mouse knee joints. Non-cytotoxic THC treatment of OA FLS and chondrocytes modified gene expression associated with ECM and cholesterol-related pathways, with SREBF2 being a potential signaling target. Upcoming studies will identify signaling mechanisms of THC in OA joint cells and impacts on cell functions.

ABSTRACT #24:

Determination of urinary and synovial fluid C2C levels in total knee arthroplasty patients

Amit SANDHU[1,2], Osvaldo ESPIN-GARCIA[1,2,3,4], Jason S. ROCKEL[1,2], Starlee LIVELY[1,2], Kimberly PERRY[1,2], Nizar N.MOHAMED[1,2,5], Y.Raja RAMPERSAUD[1,2,5], Anthony PERRUCCIO[1,2,5,6], A. Robin POOLE[7], Rajiv GANDHI[1,2,5], Mohit KAPOOR[1,2,5]

[1]Division of Orthopaedics, Osteoarthritis Research Program, Schroeder Arthritis Institute, University Health Network, Toronto, Ontario, and Canada; [2]Krembil Research Institute, University Health Network, Toronto, Ontario, Canada; [3]Department of Epidemiology and Biostatistics, Western University, London, Ontario, Canada; [4]Dalla Lana School of Public Health and Department of Statistical Sciences, University of Toronto, Toronto, Ontario Canada; [5]Department of Surgery and Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada; [6]Institute of Health Policy, Management and Evaluation, Dalla Lana School of Public Health, University of Toronto, Toronto, Ontario, Canada; [7]Department of Surgery, Faculty of Medicine and Health Sciences, McGill University, Montréal, Quebec, Canada

Introduction: Total knee arthroplasty (TKA) is a common treatment for end-stage osteoarthritis (OA); however, ~30% of patients show little improvement in pain or function 12 months post-TKA. Previous studies suggest that urinary C2C levels exhibit a positive association with cartilage degradation in the knee joint: increased levels were also positively associated with risk of emergent KOA, its onset and progression. Relationships of C2C in urine and synovial fluid with post-surgical patient outcomes remain to be determined. This study investigated associations of urinary (u) and synovial fluid (sf) C2C levels at time of TKA with 1-year post-surgical pain and function.

Methods: Both urine and synovial fluid were obtained from 493 patients with a primary diagnosis of knee osteoarthritis and undergoing TKA, as part of the Longitudinal Evaluation in the Arthritis Program (LEAP) Biomarker Exploration cohort study (Division of Orthopedics, Schroeder Arthritis Institute, Toronto). Demographic, anthropometric, and clinical information was also collected. C2C levels were measured by IB-C2C-HUSA™ with urine levels normalized to creatinine. Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain and function subscale scores were recorded at baseline and 1-year post-surgery. Pain and function outcomes considered were: 1) change in scores between the time points, and 2) categorical percentage change (>66.7%, >33.3 to ≤66.7%, and ≤33.3%) denoting “strong”, “moderate” and “mild/worse” responses, respectively. Regression models adjusted by age, sex, body mass index (BMI), metabolic comorbidity status, and one or more joints affected at baseline were fitted to assess associations between urinary/synovial C2C levels and pain/function.

Results: A total of 493 subjects were considered for analysis. Higher baseline uC2C-HUSA levels and a lower ratio of baseline sfC2C-HUSA to uC2C-HUSA were independently significantly associated with improvements in WOMAC pain and/or function by linear multivariable modeling. However, only lower ratios of sfC2C-HUSA to uC2C-HUSA were significantly associated with improvements in WOMAC function. In both univariable and multivariable ordinal modeling, lower ratios of sfC2C-HUSA to uC2C-HUSA were associated with an increased likelihood of a subject being categorized in the strong or moderate groups, where TKA was beneficial.

Conclusion: From this large sample set, uC2C-HUSA levels at the time of surgery were significantly correlated with absolute change in WOMAC pain at 1 year. The ratio of sfC2C to uC2C levels was consistently associated with changes in WOMAC pain and function at 1 year, regardless of modeling evaluated. Overall, intra-patient baseline ratios of sfC2C-HUSA to uC2C-HUSA and uC2C-HUSA may help inform of patient outcomes following surgery.

SESSION II: Cartilage and Intervertebral Disc- II

Keynote Speaker: Dr. Lisbet Haglund

Dr. Haglund is a basic scientist and a tenured Professor in the Department of Surgery, Division of Orthopaedic Surgery at McGill University, Montreal, Quebec, Canada. Lisbet has a strong interest in musculoskeletal research spanning from tissue injury, degeneration, and inflammation to regenerative medicine, and she has been actively involved in bone, cartilage, and intervertebral disc research throughout her career. She has developed a research program aiming to enhance our understanding of the molecular mechanisms leading to pain in spine pathology. More precisely, to develop molecular markers to follow disease state, progression, or effect of treatment, and most importantly to develop novel therapeutic interventions for painful spine conditions. Lisbet is a member of the McGill Scoliosis and Spine Group, and she is working closely with the clinical team. This has allowed her to generate an extensive cell and tissue bank of symptomatic (surgical) and non-symptomatic (organ donor) cells and tissues. Her research program is currently funded through the Canadian Institute for Health Research (CIHR), the Shriners Hospital for Children and the Arthritis Society

SESSION II abstracts for oral presentations

ABSTRACT #32:

Regenerative approaches to treat disc degeneration and back pain

Saber GHAZIZADEH DARBAND[1], Matthew MANNARINO[1], Hosni CHERIF[1], Lisbet HAGLUND[1,2]

[1]Department of Surgery, McGill University, Montreal,; [2]Shriners Hospital for Children-Canada, Montreal

Background: Low back pain is experienced by ~ 80% of individuals at some time in their life and is globally the number one cause of years lived with disability. This age-related health problem is associated with intervertebral disc (IVD) degeneration in many individuals. There is growing recognition that senescent cells accumulate with ageing and during tissue degeneration, where they contribute directly to disorders including heart disease, cancer, and osteoarthritis. They adopt a so-called senescent associate phenotype (SASP) and produce high levels of inflammatory and pain-mediating factors. The goal of this study was to evaluate Senolytic components as a potential treatment for low back pain.

Methods: Treatment of SPARC-/- mice began at 3–4 months of age when the female and male animals start showing signs of IVD degeneration and low back pain. Animals were treated by oral gavage bi-weekly for 5 months with RG7112 and O-vanillin alone or as a combination treatment. Grip strength (axial discomfort), acetone-evoked behavior (cold allodynia) and mechanical sensitivity to von Frey filaments (radicular pain) were assessed. High-resolution micro-CT scans of the spine were obtained to evaluate IVD volume and bone quality.

Results: As expected SPARC -/- animals developed progressive age-dependent IVD degeneration and back pain. Senolytic drug treatment prevented a loss in disc volume with the combination treatment showing the strongest effect. Pain behavior analysis showed that cold allodynia, radicular pain, and axial discomfort were significantly reduced by the Senolytic drugs with the combination treatment showing the largest effect. Furthermore, treatment significantly reduced the release of inflammatory factors from the IVDs, with combination treatment showing the most profound effect.

Discussion and significance: o-Vanillin and RG-7112 reduced pain and improved disc homeostasis in SPARC -/- mice. A combination of the two drugs further improved the outcomes and resulted in an additive effect. Senolytic combination therapies could potentially revolutionize the treatment of back pain for millions of patients worldwide and be one step closer to offering a preventative treatment for individuals at risk of low back pain or avoiding/prolonging the time before the need for invasive surgery.

ABSTRACT #35:

Development of an ex vivo disc model of degenerative disc disease for cell therapy

Sajjad ASHRAF[1,3], Evan KOTLER[1,2,3], Peter ZASTAWNY[4], Lea ZILA[4], Stephen D. WALDMAN[4], J. Paul SANTERRE[2], Rita A. KANDEL[1,2,3]

[1]Lunenfeld-Tanenbaum Research Institute, Toronto; [2]University of Toronto, Toronto; [3]Mount Sinai Hospital, Toronto; [4]Ryerson University, Toronto

Degenerative disc disease (DDD) is a prevalent and debilitating condition that is associated with the progressive deterioration of the intervertebral disc's (IVDs) structure and function, resulting in chronic back pain and reduced mobility. Stem cell injections for DDD is an emerging therapeutic approach. A simple easy to use *ex vivo* disc model for screening potential cell therapy *in vitro* would be helpful. The current study describes the development of a bovine *ex vivo* model of damaged IVD and the use of this model to assess injected cell viability as the first step towards their use for cell therapy for regeneration of the nucleus pulposus (NP) tissue.

Caudal IVDs with adjacent vertebral body endplates were isolated from the bovine caudal spine and placed in either static culture, spinner flasks bioreactor, or the latter combined with mechanical loading (2%-4%) for two hours every day for 5 days to observe cell survival for both NP and annulus fibrosis (AF) tissues in *ex vivo* culture. Different methods, including chemonucleolysis, by injecting of collagenase A (0.01-0.05%), or injury using 18-gauge needle, were utilized to induce NP damage. Labeled NP cells were implanted into the degenerated discs and cultured for 5-7 days. The NP tissue was evaluated by light microscopy, and by fluorescent microscopy to identify the presence of injected cells, as well as by TUNEL assay for cell survival.

We observed NP and iAF cell death in normal discs after 5 days under each culturing method (static culture, spinner flasks bioreactor or under mechanical loading). Collagenase A causes NP tissue degradation and cell death in a dose dependent manner that could be observed by 5 days post injection. Needle injury also caused disc degeneration resulting in loss of matrix after 5 days of *ex vivo* disc culture, but the damage appeared less than that induced by collagenase injection. Also, there was less cell death in the remaining NP tissue. While the implanted labelled NP cells were unable to regenerate the NP tissue in seven days, the implanted cells were viable in that period, in the *ex vivo* disc model.

Our findings demonstrate an *ex vivo* bovine disc model provides a feasible platform for assessing the regenerative potential of NP cells in the context of DDD. Future studies will focus on optimizing culture conditions, cell delivery methods, and evaluating long-term functional recovery in the *ex vivo* disc model.

Acknowledgement: CIHR grant #479529

ABSTRACT #22:

Investigating the role of TRPV4 as a mechanoreceptor in the intervertebral disc

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Introduction: According to the *Global Burden of Disease* study, low back pain is the leading cause of disability worldwide, associated clinically with intervertebral disc (IVD) degeneration in 40% of cases. Mechanical loading is known to be a key regulator of IVD homeostasis. TRPV4 is a cell membrane Ca^{2+} channel that regulates intracellular calcium levels in response to mechanical stimuli. Recent studies demonstrated that TRPV4 is required for the induction of ECM gene expression in murine IVD cells in response to mechanical load. The current study investigated the role of TRPV4 in age-associated IVD degeneration using a novel knockout mouse model. We hypothesized that a loss of TRPV4 within the IVD would increase age-associated disc degeneration.

Methods: A longitudinal study design was used to assess age-associated disc degeneration in male and female mice. Lumbar spines from mice with conditional TRPV4 deletion in most cells of the IVD (*Col2Cre*;*Trpv4*$^{fl/fl}$) and wild-type controls were assessed for histopathological signs of IVD degeneration at 12, 18, and 24 months-of-age (male and female, n= 8/group). qRT-PCR was performed to measure extracellular matrix and inflammatory marker gene expression in thoracic IVDs.

Results: For both male and female, mice histopathological scoring demonstrated no differences in IVD structure between WT and *TRPV4-/-* mice at 12 or 18 months-of-age. At 24-months-of-age, male *TRPV4-/-* mice showed changes in IVD structure compared to WT, including loss of nucleus pulposus (NP) cellularity, loss of concentric annulus fibrosus (AF) lamellar structure, and loss of the AF-NP boundary. These changes were associated with a significant increase in degenerative scores in IVDs from *TRPV4-/-* mice compared to WT at the L4/L5 and L5/L6 spinal levels, driven primarily by histopathological changes in the AF. Gene expression analysis of thoracic IVDs from 24-month-old male mice demonstrated a trend of increased expression of the matrix degrading enzyme *Mmp-13* and inflammatory markers *IL-1beta* and *IL-6* in *TRPV4-/-* samples compared to WT. In contrast to male mice, at 24 months of age female *TRPV4-/-* mice showed a significant decrease in lumbar IVD degeneration compared to WT mice. While the mechanism underlying these sex-specific effects remains to be fully elucidated, preliminary analysis demonstrates that TRPV4 expression is significantly increased in IVDs from female compared to male WT mice at this age.

Conclusions: Our findings suggest TRPV4 as an important mechanoreceptor in the IVD that contributes to the adaptive response to mechanical load, which may contribute to the pathobiology of disc degeneration.

SESSION III: Cell Biology of Connective Tissues

Keynote Speaker and Lifetime Achievement Award Winner: Dr. Chris McCulloch

Dr. McCulloch is professor of dentistry at the University of Toronto and since 1984 has contributed 335 papers, 150 presentations and 4 patents that relate to matrix biology and associated diseases of matrix disorders that affect human health. He uses animal models and cultured cells to examine and define fundamental regulatory circuits that define the function and mechanics of connective tissue matrices, particularly in the context of periodontal diseases. As a principal investigator on >30 MRC/CIHR research grants, he has developed and employed high resolution tools to define the contribution of fibroblasts in matrix remodeling in soft connective tissues. He has established a network of collaborators and trainees along with industrial partners to provide optimal support, know-how and reagents for the conduct of research focusing on how cell-matrix interactions help to define how inflammatory diseases contribute to matrix pathology.

SESSION III abstracts for oral presentations

ABSTRACT #37:

Direct contact with mechanically activated myofibroblasts drives macrophages into distinct transcriptional and functional states

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Background: Fibroblasts and macrophages (Mϕ) are key in the extracellular matrix (ECM) formation and remodeling following organ injury, but aberrant crosstalk can contribute to the development of fibrosis. Mϕ provide cytokines like TGF-β1 that stimulate fibroblast activation into contractile myofibroblasts (MF). We have published that MF activation by Mϕ-derived TGF-β1 requires spatial proximity and a 'scar-stiff' tissue environment. However, little is known how, in turn, MFs control Mϕ phenotypes in the context of tissue repair and fibrosis.

Hypothesis: Mechanically activated MFs control distinct Mϕ states in contact dependent/independent signaling processes.

Methods: Mϕ were obtained by treating mouse bone marrow-derived monocytes with M-CSF in vitro for 5 d. Subcutaneous fibroblasts were isolated from Col1a-GFP reporter mice. To mechanically establish fibroblast and MF populations, fibroblasts were cultured on skin-soft or scar-stiff gelatin-coated silicone substrates for 2 passages, respectively. Mϕ were then co-cultured for 3 d with fibroblasts and MFs on the respective substrates in setups that allowed either direct contact or media sharing. Cells were analyzed using immunofluorescence confocal microscopy and flow cytometry. Fibroblastic cells and Mϕ were flow-sorted for subsequent RNA sequencing, further analyzed for principal components, differentially expressed genes and enrichment of signaling pathways and transcription factors binding motifs.

Results: Fibroblasts but not Mϕ cultured alone on stiff substrates exhibit profibrotic protein and RNA profiles absent from soft-cultured cells. Conversely, co-culture with fibroblastic cells results in significant changes in Mϕ transcriptomes, with unique features depending both on the activation state of the co-cultured fibroblasts and the ability to form direct contact. Specifically, (1) Mϕ in direct but not medium-shared-only co-culture with fibroblastic cells acquire an activated MAPK signaling profile. (2) Direct contact with fibroblasts results in suppression of stress response to stimuli and inter- and intra-cellular signal transduction. (3) In direct contact with MFs, Mϕ exhibit upregulated pro-fibrotic signaling pathways, mediated by IL-17, TNF, NF-κB, and C-type lectin. Immunofluorescence and flow cytometry analysis validate RNA sequencing data. For instance, Mϕ exhibit up to 1.6-fold significantly increased expression of CD206 in Mϕ co-cultured with MFs versus fibroblasts. Increased expression of Mϕ CD206 is 6~8-fold more pronounced in co-cultures with direct contact.

Conclusion: Direct contact with MFs generate a unique Mϕ polarization state that features a combination of proinflammatory and profibrotic signaling. The recognition of new profibrotic Mϕ polarization states in direct contact with MFs offers novel therapeutic targets for the prevention and treatment of fibrosis.

ABSTRACT #39:

Characteristics of the extracellular vesicles from human intervertebral disc cells

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Background: Extracellular vesicles (EV) are membrane-bound structures produced by all cells. They carry a variety of cargo, including nucleic acids, proteins, and lipids. EVs play important roles in cell communication and regulation. Human mesenchymal stem cell (MSC) secretome has been shown to be affected differentially by the presence of tissue with varying disease states, and we have also shown that conditioned media from human intervertebral disc (IVD) cells of different degeneration grades alter gene expression in MSCs. However, limited knowledge exists regarding IVD cell-derived EVs. This study aims to characterize EVs of non-degenerating, degenerating organ donor tissue, and symptomatic degenerating tissue from patients undergoing surgery for low back pain and to determine the cell source generating the most regenerative EVs.

Methods: IVD tissue was collected from organ donors and surgical patients with low back pain. IVD cells were isolated from nucleus pulposus (NP), inner annulus fibrosis (iAF), and outer AF tissue of organ donors with varying degrees of degeneration. NP and iAF cells were cultured together for EV collection. EVs were enriched using Amicon® Ultra-15 *Centrifugal Filter Units and then* purified by size-exclusion chromatography. Nanoparticle tracking analysis was conducted to measure EV size and concentration. Transmission electron microscopy (TEM) and Western blot were performed to examine EV structure and markers. Mass spectrometry was conducted to determine protein cargo.

Results: EV size and concentration increased slightly with an increasing degree of degeneration with cells of symptomatic-degenerating IVDs producing fewer EVs. TEM showed that the EVs had round and single-lobed flat structures. Western blot data confirmed the presence of EV markers Annexin-5, Flotillin-1, CD63, and CD81, and the absence of the mitochondrial protein Tom-20 and Golgi protein 58K Golgi. Preliminary mass spectrometry data detected clusters of EV-specific markers, glycoproteins, extracellular matrix proteins and proteoglycans.

Discussion and significance: Our initial data provided characteristic information about IVD cell-derived EVs. Unveiling the composition of the cargo and determining the condition resulting in the most regenerative EVs may offer new insights into the potential clinical applications of IVD cell-derived EVs as a treatment for IVD-related low back pain.

ABSTRACT #42:

Evaluating the profibrotic role of IL4Rα in monocytes and macrophages in pulmonary fibrosis

Megan VIERHOUT[1], Pareesa ALI[1], Vaishnavi KUMARAN[1], Anmar AYOUB[1], Safaa NAIEL[1], Takuma ISSHIKI[1], Anna DVORKIN-GHEVA[1], Joshua KOENIG[2], Manel JORDANA[2], Martin KOLB[1], Jean-Claude CUTZ[3], Asghar NAQVI[3], Nathan HAMBLY[1], Kjetil ASK[1]

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BACKGROUND: Idiopathic pulmonary fibrosis (IPF) is a fatal lung disease that leads to excessive scarring of the lung interstitium. Circulating monocytes have been found to be increased in patients with fibrotic disease and their quantity has been correlated with poor outcomes. There is evidence to support that these monocytes leave the blood and enter the lung tissue, where they differentiate into profibrotic (M2-like) macrophages. However, little information about the characteristics and nature of these monocytes is known. Here, we investigated the transcriptional profile of circulating monocytes derived from patients diagnosed with IPF and compared them to control subjects. We then used this information to further investigate the fibrogenic mechanism of action of macrophages in the lung.

METHODS: CD14 positive monocytes were isolated from the blood of IPF patients (n=50) and control subjects (n=12) and submitted for RNA sequencing. Tissue microarrays (TMAs) were created from formalin-fixed paraffin embedded lung tissues from IPF patients (n=70), on which immunohistochemistry (IHC) and fluorescent in-situ hybridization assessments were performed. To conduct mechanistic studies on macrophage polarization in the lung, we developed a polarization protocol in murine precision-cut lung slices (PCLS). A time course study was conducted using PCLS generated from wildtype and IL4Rα knockout mice. PCLS were cultured in the presence of an M2 hyperpolarization cocktail (IL-4, IL-6 and IL-13). The profibrotic activation status of macrophages was evaluated via arginase activity assays on PCLS homogenates. IHC and multiplex histological readouts were used to visualize macrophage phenotype in PCLS.

RESULTS: Transcriptomic results revealed that IL4Rα is upregulated in monocytes from IPF patients. Histological readouts on the human TMAs showed the presence of profibrotic macrophages (CD206 and CD163 positive) in the lungs of IPF patients, that are also monocyte derived (MAFB positive). PCLS from wildtype mice that were stimulated with the hyperpolarization cocktail showed a significant increase in arginase activity at all timepoints (24, 48, and 72 hours) ($p<0.05$), while this was not observed in IL4Rα knockout mice, demonstrating that this receptor is critical for polarization. These findings were confirmed with Arginase-1 and CD206 immunohistochemical staining.

CONCLUSIONS: Our results demonstrate that monocyte-derived profibrotic macrophages are present in the lungs of IPF patients. Mechanistic studies reveal that IL4Rα plays a critical role in the polarization of monocytes and macrophages in a profibrotic microenvironment, and constitutes a potential therapeutic target. Overall, these results provide insight into an alternative activation status in monocytes and macrophages in pulmonary fibrosis.

Session IV: Bone & Developmental Biology

Keynote Speaker: Dr. Derek Rosenzweig

Dr. Rosenzweig is an associate professor at McGill University, Department of Surgery, and staff scientist/principal investigator at the RI MUHC Injury, Repair and Recovery Program. Dr. Rosenzweig is also an FRQS Junior 2 Research Scholar. He has a strong background in molecular pharmacology, cartilage and spine biology, biomaterials, microfluidics and additive manufacturing. His lab focuses on leveraging biofabrication, bioengineering and biophysics technology to study musculoskeletal repair and regeneration as well as building human tissue models for therapeutic screening and development in the context of bone and spine metastatic disease. He has worked on federally, provincially and industrially sponsored research programs funded through Quebec Ministère de l'Économie, de l'Innovation et de l'Énergie, iTrans Med Tech, Cancer Research Society/CHIR, AO Spine North America, New Frontiers in Research Fund, NSERC, RSBO and TheCell.

Session IV abstracts for oral presentations

ABSTRACT #6:

Molecular pathomechanisms of fibronectin mutations leading to spondylometaphyseal dysplasia

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Aim: Fibronectin (FN) is a ubiquitous matrix glycoprotein essential for physiological development. The role of FN in cartilage development and function is not known. We and others have previously identified heterozygous dominant mutations in the fibronectin gene *(FN1)* as a cause for corner fracture type spondylometaphyseal dysplasia (SMD) with short stature and growth plate defects. The current study addresses the functional importance of FN in skeletal development and pathomechanisms underpinning SMD.

Methods and results: To determine the consequence of *FN1* mutations in SMD, induced pluripotent stem cells (iPSCs) were generated using fibroblasts from SMD patients (FN mutations p.Cys123Arg and p.Cys231Trp) and an unaffected control. These iPSCs were differentiated into mesenchymal stem cells (MSC) and chondrocytes. Differentiation of the iPSCs were validated by iPSC and MSC marker expression using flow cytometry. FN protein expression in these cells were analyzed by immunofluorescence microscopy demonstrating for the mutant cells impaired secretion and intracellular retention of FN. Analysis of MSCs by transmission electron microscopy revealed accumulation of large vesicular structures in FN mutant cells, that were covered with ribosomes in close association with the endoplasmic reticulum (ER). Immunostaining with markers of ER chaperones, Golgi, endocytic vesicles, autophagy, and lysosome, revealed that retained FN along with ER chaperones were present within large lysosomal vesicles in the mutant cells. The accumulation of mutant FN induced cellular stress with elevated unfolded protein response markers in the patient-derived cells. FN mutant cells exhibited mitochondrial dysfunction with alterations in structure, membrane potential and ATP/ADP ratio. RNA sequencing of MSCs and differentiated chondrocytes showed differential regulation of several 1000 genes for the mutant cells relative to the control. Importantly, there was a significant reduction in over 21 chondrogenic markers in the mutant cells. To assess the cause for impaired chondrogenesis, we evaluated mesenchymal condensation of mutant MSCs, a pre-requisite for differentiation of MSCs into chondrocytes. The FN mutants displayed delayed condensation with remarkable reduction in proteoglycan levels. This defect in mesenchymal condensation was partially rescued by exogenous treatment with plasma FN and TGF-β1, a well-known regulator of chondrogenesis which was also found to be significantly reduced in FN mutants.

Conclusion: Overall, the data demonstrate that mutations in FN lead to critical cellular and matrix associated defects which result in impaired MSC condensation and chondrogenic differentiation. Impaired MSC condensation was partially rescued by exogenous treatment of plasma FN and TGF-β1.

ABSTRACT #9:

Over-expression of PITX1 induces periodontitis-like phenotype in transgenic mice

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Purpose: Periodontitis is a severe chronic inflammation of the gums that can destroy the tooth-supporting apparatus triggering gingival recession, bone loss, tooth mobility, and eventually tooth loss. PITX1 is a gene that codes for the PITX1 protein that acts as a transcription factor aiding in the control of gene activity. It has been shown that the complete inactivation of PITX1 will cause improper hind-limb development, micrognathia of the mandible, and fusion of the 1st and 2nd mandibular molar while partial inactivation results in osteoarthritic-phenotypic mice and over-expression induces senile osteoporosis. In this study, we examined how the over-expression of PITX1 can cause a large number of periodontal issues.

Methods: Transgenic mice were engineered to over-express Pitx1 gene in bone cell lineage using the 2.3 kb fragment of the mColla1 promoter. At 3 months, 24 mice were analyzed, 12 wild types (6 males, 6 females) and 12 transgenic mice (6 males, 6 females). A Faxitron MX20 was used to take lateral and dorso-ventral radiographs of mice's skulls, a dual-energy X-ray absorptiometric was used to measure Bone Mineral Density, and a Mach 1™ micro-mechanical testing system was used to conduct three-point-bending tests. Histological analysis was done by embedding the tissues in paraffin and later staining them with a hematoxylin/eosin solution.

Results: After 3 months, we could observe many manifestations of periodontitis, such as severe teeth defects that included the fusion of the first and second mandibular molar and malocclusion of the teeth. The transgenic mice also showed teeth loss and decreased jawbone density and content. Histological staining also showed the decalcification of dentin and odontoblast degeneration.

Conclusion: In general, the over-expression of PITX1 causes a large number of periodontal issues like malocclusions, loss of bone density, and periodontal defects.

ABSTRACT #11:

Heterotopic ossification following spinal cord injury: A novel mouse model

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Background: Affecting about 900 000 individuals every year, spinal cord injury (SCI) is an incapacitating injury leading to profound neurological deficits. Moreover, SCI is associated with debilitating sequelae severely impacting quality of life. One increasingly recognized complication of SCI is heterotopic ossification (HO): the abnormal and painful formation of bone in soft tissues. SCI-associated HO is reported in 50% of patients with SCI and predominantly co-occurs with peripheral mechanical stress. Due to the knowledge gap in HO pathophysiology, current prevention strategies are suboptimal in suppressing HO growth or minimizing recurrence after surgical resection. To study SCI-associated HO, few animal models have been developed. A previously established model administers snake venom-derived cardiotoxin to induce peripheral injury in the context of SCI. However, the use of cardiotoxin introduces an exogenous substance which may confound findings. To remediate this, we developed the first preclinical model for SCI-associated HO using only mechanical injuries.

Methods: Ten-week-old C57BL/6J mice were subjected to a spinal cord transection at T9-T10 to induce an SCI, followed by a musculotendinous injury (MTI) by compressing and transecting the left quadriceps muscle and tendon (n=17). All right hindlimbs were uninjured and served as internal procedural controls. Mice were euthanized after 1 (n=7) and 4 weeks (n=10), and high-resolution micro-CT scans of hindlimbs were then used to quantify ectopic mineral volumes and native femur bone quality. Samples were then embedded in methyl methacrylate, and consecutive 5µm sections were stained with Von Kossa for mineralized soft tissues, alkaline phosphatase (ALP) for osteoblast activity, and tartrate-resistant acid phosphatase (TRAP) for osteoclast activity. Mann-Whitney U test was used for the statistical analysis.

Results: Quantitative micro-CT analysis at 1 and 4 weeks showed that SCI+MTI led to significantly higher ectopic mineral volumes than SCI alone at 1 week (**p=0.0111**), and at 4 weeks (**p<0.0001**). Mice with SCI displayed significant thinning of femoral cortical bone and reduced trabecular bone volumes in comparison to non-SCI mice. At loci of ectopic mineral formation in SCI+MTI mice, only TRAP activity colocalized with mineral deposits at 1 week. At 4 weeks, ALP and TRAP staining corroborated the colocalization of osteoblast and osteoclast activity at the site of ectopic bone mineral and bone formation.

Conclusion: This study represents a novel preclinical model of SCI-associated sequelae to study pathogenic mechanisms and assess novel therapies.

ABSTRACT #15:

Bone resorption markers in patients and mouse models of osteogenesis imperfecta: Systematic review and meta-analysis

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Osteogenesis imperfecta (OI) is a fragile bone disease caused by mutation in collagen-encoding genes. Interestingly, there was an inconsistency of alteration in osteoclast function among different types of OI and mouse models with different bone phenotype severity. This study aimed to systematically identify all the reports of osteoclast function in clinical and animal studies of OI and to use meta-analysis to quantitatively summarize the data. The search strategy included the relevant articles from Medline, Embase and Web of Science with the MeSH term of OI and parameters related to osteoclasts. The title and abstract screening were performed by using Rayyan Systematic Review Screening Software. Selected studies reported bone resorption markers in serum and urine of OI subjects. The standardized mean difference was used as the effect size for each study and random effects meta-analysis was performed using JASP software. The secondary analysis assessed the association between the level of collagen degradation markers with phenotype severity, age and sex of OI mice. We identified 14 clinical studies describing type I, III, IV, and XIII of OI. 12 animal studies were presenting 9 models of OI mice with Coll1a1 mutation ($Col1a^{1Jrt1/+}$, $Brtl^{-/-}$), Coll1a2 mutation ($Oim^{-/-}$, $Oim^{+/-}$), Wnt1 mutation ($Wnt1^{+/G177C}$, $Wnt1^{G177C/G177C}$) and other gene mutation ($Bril^{-/-}$, $Crtap^{-/-}$). For clinical and animal studies, the bone resorption markers were reported as levels of C-terminal cross-linked telopeptide of type 1 collagen (CTX-1), N-terminal cross-linked telopeptide of collagen (NTX) or deoxypyridinoline (DPD). Random effects meta-analysis of 49 independent groups of OI patients demonstrated higher bone resorption markers compared to age-match healthy subjects, with the overall effect size of 1.20 (CI: 0.40, 2.00). The effect sizes were significantly higher in young patients than the adult group. For animal studies, the overall effect size was 1.59 (CI: 1.07, 2.11), indicating significantly higher bone resorption markers in OI compared to wild-type mice. Linear regression demonstrated a negative correlation between the effect size and the age of mice. The effect size also increased with bone severity in mouse models of collagen mutation. There was no difference in bone resorption markers between male and female subjects from clinical and animal studies of OI. In conclusion, OI patients and OI mice have a higher level of collagen degradation markers, suggesting higher osteoclast activity, especially in younger subjects with a severe OI. This study demonstrated the translational relevance of OI mouse models and emphasizes the potential role of osteoclasts in the pathogenesis of OI.

ABSTRACT #14:

Assessing the membrane injury and repair dynamics in osteoclasts

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Introduction: Exercise is essential for the maintenance of musculoskeletal health. Osteoblasts and osteocytes are considered the key mechanosensitive and mechanoresponsive cells in the skeleton, however, the response of osteoclasts to the mechanical environment is less studied. Nevertheless, osteoclasts experience similar forces as other bone cells and are known to alter their behaviour in mechanically loaded or unloaded bones. This study aimed to characterize the effect of mechanical deformation and micro-injury in a single stimulated osteoclast.

Methods: Primary osteoclast cultures were obtained from 10–12-week-old mice. For mechanical deformation experiments, a single cell was stimulated with a micropipette. Osteoclasts were labeled with quinacrine to study vesicle release dynamics. or loaded with Fura-2 AM to study changes in cytosolic free calcium $[Ca^{2+}]_i$. Membrane injury was assessed by the degree of fura-2 dye loss. For shear stress studies, osteoclasts were bathed with trypan blue dye before (shear stress-dependent) or after (membrane repair) and mechanically stimulated by displacing 50% of media 'n' number of times.

Results: Mechanical stimulation results in release of quinacrine-positive ATP-containing vesicles. Both primary and secondary osteoclasts demonstrated vesicular release upon mechanical stimulation. However, primary responders had significantly faster, higher, and more sustained vesicular release than secondary responders. Mechanical stimulation resulted in an increase in $[Ca^{2+}]_i$ in the primary (mechanically stimulated) and secondary (neighboring) cells. Mechanical stimulation results in reversible plasma membrane tears in osteocytes and osteoblasts. Similarly, 79% of osteoclasts exhibited reversible membrane injuries, which were classified using k-means as severe or mild/moderate. In primary responders, severely injured osteoclasts had calcium transients with higher amplitude, higher full-width half-maximum, and higher area under the curve compared to mildly injured osteoclasts. In secondary responders, severe injuries of primary osteoclasts resulted in a significantly higher number of secondary oscillators than in the mild category. To assess the effect of shear-related forces, trypan blue-loaded osteoclasts were subjected to increasing shear stress. Increase in shear stress resulted in increase in the fraction of dye-positive cells. To assess membrane repair, osteoclasts were subjected to 20x shear stress and dye was added at different time intervals. The fraction of dye-positive cells decreased with increase in time, indicating repair.

Conclusion: Osteoclasts respond to mechanical stimulation proportionally to the degree of mechanical injury and can propagate mechanical signals to secondary responders. Like osteocytes and osteoblasts, they can sense shear stresses and repair themselves, thus highlighting their mechanosensitive and mechanoresponsive capacities.

ABSTRACT #4:

A 3D, compartmental tumoroid model of patient-derived bone metastasis mimics tumor-stromal microenvironment

Mansoureh MOHSENI GARAKANI[1,2], Michael WEBER[1], Michael R. WERTHEIMER[2], Abdellah AJJI[2], Derek H. ROSENZWEIG[1]

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Introduction: Bone is a frequent site of tumor metastasis. Complexity in bone-tumor interface with a heterogeneous microenvironment along with complex interactions between the metastatic tumor cells, bone cells, and their extracellular matrix affect cellular sensitivity/resistance to chemotherapeutics. Current systemic chemotherapy treatment may not be efficient for every patient and the development of new chemotherapeutics is ineffective with a slow emerging rate mainly due to the absence of appropriate physiological in vitro/in vivo cancer models. Therefore, the generation of more complex and physiologically relevant in vitro 3D models to develop new therapies is essential.

Method: We developed a 3D bone-tumor interface model for personalized therapeutic screening comprised of i) an electrospun nanofibrous poly lactic acid, PLA, mat treated with electric discharge plasma active species and seeded with human osteoblasts to mimic a bone tissue ii) a layer of an alginate-gelatin hydrogel embedded with either MDA-MB 231 aggressive breast cancer cell line or patient-derived spine-metastasis tumor cells, isolated from bony spinal metastases, which is overlaid, like an organoid, representing the tumor compartment to create a physiologic 3D interface model. We applied this hybrid 3D co-culture model as a migration assay tool to assess and validate the migratory behavior of different patient-derived bone metastasized cells as well as their equivalent immortalized cell lines and then evaluate the impact of two different chemotherapeutics, Doxorubicin and Cisplatin, on migration and metabolic activity of both patient and immortalized cells.

Results: We observed a considerable amount of migration and invasion of tumor cells toward the target bone tissue over the course of 5 days. By screening both patient cells and an immortalized cell line with chemotherapy drugs in our model, we found a reduction in cellular metabolic activities which may be blocked with both Doxorubicin and Cisplatin; however, higher efficiency or lower IC50 was obtained with Doxorubicin. Gene expression analysis of the MDA-MB 231 breast cancer cell line that migrated through our 3D hybrid model proved epithelial-mesenchymal transition (EMT) as a critical biological mechanism occurring in cancer metastasis through increased expression of mesenchymal markers involved in the metastasis process.

Conclusion: Our innovative 3D model enables mimicking “natural” multi-cellular interactions in a synthetic 3D environment based on separate compartments that resemble native tissues in the human body. Our data indicate that this 3D hybrid model can mimic tumor migration in vivo and has great potential to be applied as a drug screening tool for personalized medicine.

Session V: Adipose Tissues & Stem Cells and Therapy

Keynote: Dr. John Walker

Dr. Walker completed his graduate training under the co-supervision of Dr. Douglas Hamilton and Dr. Lauren Flynn through the Department of Anatomy & Cell Biology at Western University. In his doctoral research, he investigated the intrinsic heterogeneity of dermal fibroblast populations and the effects of the extracellular matrix microenvironment in mediating cellular responses during wound healing using both in vitro and in vivo models. John has since continued his training as a post-doctoral fellow under the mentorship of Dr. Flynn. In his current projects, he is investigating the interaction of mesenchymal stromal cells with their environment, with a focus on their immunomodulatory and pro-angiogenic properties. He is particularly interested in understanding how these populations promote regeneration in vivo by applying interdisciplinary approaches combining biomaterials-based strategies with advanced biological characterization methods.

Keynote Talk: Mesenchymal Stromal Cells as a Therapeutic Population for Soft Tissue Regeneration

Adipose tissue shows a remarkable capacity to expand and regress throughout mammalian life. Despite this plasticity, it typically fails to regenerate following injury, and is instead replaced by functionally inferior scar tissue. With a need for improved therapeutics for soft tissue regeneration, mesenchymal stromal cells (MSCs) have garnered much interest due to their ability to stimulate regeneration through paracrine factor secretion. In particular, adipose tissue is a rich source of MSCs, termed adipose-derived MSCs (ASCs), which can be harvested from commonly discarded surgical waste. While ASCs represent a promising tissue-specific cell source for adipose regeneration, the positive results of pre-clinical studies have not yet been matched clinically, emphasizing the need for further research to better understand the mechanisms through which MSCs promote regeneration *in vivo* and to develop strategies to more effectively harness their pro-regenerative capacities.

In an effort to improve ASC therapy for adipose tissue regeneration, the Flynn lab has focused on enhancing ASC functionality by modulating culture conditions and improving delivery through the use of innovative biomaterials derived from decellularized adipose tissue (DAT). Recently, our team developed a novel cell-assembled biomaterial system, in which ASC-seeded DAT

microcarriers are stimulated to fuse *in vitro* to generate larger constructs containing a high density of well-dispersed ASCs. These engineered tissues have the potential to be applied as culture models or used to provide a supportive microenvironment for localized delivery. Notably, when implanted into nude mice, the cell-assembled scaffolds enhanced tissue integration, angiogenesis, and vascular stability compared to traditionally-seeded DAT scaffolds and unseeded controls.

Another main facet of our research has been to develop a better understanding of ASC-host cell interactions *in vivo* using implant models. In our recent work, we delivered syngeneic DsRED^{+} mouse ASCs on DAT scaffolds into the inguinal region of immunocompetent mice. Using this model of subcutaneous adipose regeneration, we observed rapid clearance of ASCs from the implant site, with no long-term effects of seeding on vascular perfusion or macrophage phenotype. However, flow cytometry analyses revealed that ASC seeding promoted a shift toward regenerative macrophage polarization up to two weeks post-implantation, when the ASCs were most abundant. Further analysis revealed that the proteome of ASCs remained stable following implantation, suggesting that their rapid clearance is due to immune targeting rather than an apoptotic response to adverse physical conditions.

In ongoing projects, we are combining the cell-assembly approach with testing in immune competent animal models as a strategy to enhance localized ASC persistence and help direct their pro-regenerative functionality for enhanced therapeutic efficacy.

Session V abstracts for oral presentations

ABSTRACT #1:

Impact of obesity on cellular constituents and transcriptomic signature of infrapatellar fat pad in patients with knee osteoarthritis

Hayley PETERS[1,2], Pratibha POTLA[2], Jason S. ROCKEL[2], Keemo DELOS SANTOS[2], Shabana VOHRA[2], Starlee LIVELY[2], Kim PERRY[2], Anthony PERRUCCIO[2], Nizar N. MAHOMED[2], Raja RAMPERSAUD[2], Rajiv GANDHI[2], Mohit KAPOOR[1,2]

[1]University of Toronto, Toronto; [2]Schroeder Arthritis Institute, University Health Network, Toronto

Purpose: Knee osteoarthritis (KOA) is the most common form of arthritis, characterized by cartilage degeneration, synovial inflammation, fibrosis, and subchondral bone remodelling. Obese individuals are at greater risk of developing KOA due to low-grade systemic inflammation and increased mechanical stress. The infrapatellar fat pad is the largest fat pad in the knee, however, its cellular composition and involvement in KOA pathogenesis is not well understood. This study utilizes single-nucleus RNA sequencing and advanced bioinformatics to identify key cell types, subsets and their transcriptomic differences within the fat pad of obese and non-obese patients with KOA.

Methods: Infrapatellar fat pad was obtained from late-stage KOA patients [KL grades III/IV; n=15; 53% females, 47% obese, BMI 30-40 and 53% non-obese, BMI 18.5-25] undergoing total knee arthroplasty. Tissue was homogenized and nuclei isolated by fluorescence-activated cell sorting (FACS). Nuclei underwent single-nucleus RNA sequencing using droplet-based Chromium Next GEM Single Cell 3′ Reagent Kits (v3.1). cDNA libraries were sequenced on an Illumina NextSeq 550 using the 150bp high output sequencing kit. Sequenced data was processed using Cell Ranger and reads aligned to the human transcriptome (GRCh38). Samples were merged for clustering and annotated based on canonical markers. Differentially expressed gene (DEG) testing determined gene signatures. Validation experiments will be performed, including immunohistochemistry and flow cytometry, to confirm the presence of identified cell populations in fat pad.

Results: Clustering analysis of sequenced data identified several cell types present in OA fat pad. Fibroblasts (43.3%), macrophages (20.8%), adipocytes (18.5%), and endothelial cells (11.6%) were identified as major cell populations, each with multiple subsets. Interestingly, individual fibroblast cluster analysis revealed nine subsets with unique transcriptomic signatures. When investigating fibroblast subsets based on BMI (obese versus non-obese) or sex, distinct differences in select gene expression patterns were observed. We are currently employing advanced bioinformatics and functional assays to elucidate the function and transcriptomic profiles of identified fibroblast subsets in KOA pathogenesis.

Conclusions: Using single-nucleus RNA sequencing, we have identified distinct cell subsets of fibroblasts, adipocytes, macrophages, and other cell types in fat pad of patients with knee OA. We have also uncovered differences in transcriptomic profiles within the fibroblast population based on BMI and sex. Further exploration of the contributions of these transcriptomic profiles to fibroblast subclusters and their function within the fat pad with OA will be investigated.

ABSTRACT #3:

Role of fibrillin-1 in adipose tissue development and homeostasis

Iram Fatima S. SIDDIQUI[1], Muthu L. MUTHU[1], Dieter P. REINHARDT[1]

[1]McGill University, Montreal

Fibrillin-1 is present in various tissues including adipose tissue, where it assembles into bead-on-the-string microfibrils. Mutations in fibrillin-1 lead to the connective tissue disorder Marfan syndrome, in which the adipose tissue is often severely affected. These patients can be either lipodystrophic or overweight and even obese. This wide-ranging phenotypic spectrum on fat tissue development motivated the investigation of the role of fibrillin-1 in adipose tissue development and maintenance. The Reinhardt lab has previously shown that fibrillin-1 hypomorph mice were overweight and developed insulin resistance. To gain more insights into fat tissue development and maintenance, we extend the study to understand the role of fibrillin-1 at the early and late stages of adipogenesis.

We have developed and analyzed a novel adipose tissue-specific fibrillin-1 knockout mouse model with deleted fibrillin-1 only in mature adipocytes using AdipoQ-Cre mice crossed with floxed Fbn1 mice (*Fbn1-AKO*). To fully understand the role of fibrillin-1, we are in the process of developing a second adipose tissue-specific fibrillin-1 knockout mouse model with fibrillin-1 deleted at the early mesenchymal stem stage using PDGFRα as the Cre driver crossed with the floxed Fbn1 mice (*Fbn1-PKO*). To elucidate the downstream pathways, we isolated primary mesenchymal stem cells from bone marrow (BM-MSCs) and from adipose tissue (ASCs) harvested from wild type mice.

Overall body weight and white adipose tissue weight of *Fbn1-AKO* mice were significantly reduced compared to their litter mate controls. DEXA analysis further showed a significant reduction in fat mass and fat percentage in the *Fbn1-AKO* mice compared to the controls. Isolated BM-MSCs were positive for CD 29, CD 44 and CD 106, whereas ASCs were positive for CD 29, CD 90.2 and CD 44, which confirms stem cell identity. Oil red staining performed for isolated BM-MSCs and ASCs after exogenous addition of a central fibrillin-1 subfragment containing an integrin-binding RGD motif showed a significantly reduced level of adipogenesis. Contrary, a subfragment with an inactive RGA motif did not affect adipogenesis. Consistent results were obtained from perilipin immunostaining of the BM-MSCs and ASCs treated with these fibrillin-1 subfragments. These data indicate that fibrillin-1 is an important mediator of adipogenesis and that integrins are involved in adipogenic inhibition.

Further knowledge of the role of fibrillin-1 in fat tissue development and maintenance will help identifying the underpinning mechanisms for profound metabolic disturbances including insulin resistance and diabetes caused due to abnormalities in adipose tissue in Marfan syndrome and related disorders.

ABSTRACT #80:

Mechanically modified chromatins imprint memory of myofibroblastic transcription profiles in mesenchymal stromal cells

FereshtehSadat YOUNESI[1,2], Andrew MILLER[3], Nicole BEREZYUK[1,2], Natalie ANDONIAN[2],

Thomas H. BARKER[3], Boris HINZ[1,2]

[1]St. Michael's Hospital, Toronto; [2]University of Toronto, Toronto; [3]Biomedical Engineering, University of Virginia, USA

Background: Mesenchymal Stromal Cells (MSC) are used in cell therapy to reduce scarring following burn injuries. Autologous MSC are scarce and must be multiplied from biopsies to achieve the billions of cells required for therapy. However, growth on conventionally stiff cell culture surfaces or bioreactor suspension beads mechanically activates MSC into pro-fibrotic myofibroblasts (MF) – while losing regenerative potential. We published that prolonged culture ('priming') on 'skin-soft' substrates prevents MF activation of MSC even after transfer to 'scar-stiff' environment. We have published that priming on skin-soft substrates imprints lasting 'mechanical memory' that prevents MF activation after delivery to hypertrophic rat wounds. We now explore the mechanisms of mechanical memory with a focus on chromatin accessibility and DNA methylation mediated by DNA methyltransferases (DNMTs).

Hypothesis: Mechanically induced chromatin accessibility and DNA methylation result in lasting expression pro-fibrotic genes and reduced expression of regenerative genes in MSC.

Objective: To enhance MSC regeneration potential by erasing mechanical MF memory.

Results*:* Transcription of MF and osteogenic genes was enhanced, and expression of regeneration-associated gene was reduced in stiff- versus soft-primed MSC. Concomitantly, global DNA methylation was higher and chromatin compactness was lower in stiff- than soft-primed MSCs. Compared to soft-priming, stiff-priming enhanced the accessibility of chromatin regions predicted to bind mechanosensitive and pro-fibrotic transcription factors . Conversely, accessibility for DNA binding of factors regulating MSC stemness was reduced. Expression of DNMTs increased upon stiff priming of MSC and DNMT knockdown attenuated osteogenic lineage commitment of mechanically primed MSC. Global DNA methylation, chromatin accessibility, and the transcription profiles established for 3 passages on one substrate stiffness condition were all preserved after switching to the respective other stiffness condition for 2 more passages.

Conclusions*:* Mechanical cues alter transcription profiles and epigenetic DNA modifications, which are both memorized. Currently, we are studying how gene regulatory networks driven by mechanically induced epigenetic changes correlate with transcription profiles and consolidate the regenerative potential of MSC

Gala Dinner

Great Hall, Hart House
University of Toronto
Hart House Circle
Toronto ON M5S 3H3

Keynote Speaker: Dr. Boris Hinz

Dr. Boris Hinz

Dr. Hinz is the Keenan Research Chair in Fibrosis Research at St. Michael's Hospital and University of Toronto Distinguished Professor in Tissue Repair and Regeneration. Dr. Hinz holds a PhD degree (1998) in Cell Biology and Theoretical Biology from the University of Bonn, Germany. From 1999 to 2002, he was postdoctoral fellow with Dr. Giulio Gabbiani, University of Geneva, Switzerland and then lead a research group at the Ecole Polytechnique Fédérale de Lausanne (EPFL). In 2009, he joined the Faculty of Dentistry, University of Toronto and is cross-appointed with the institutes of Medical Sciences and Biomedical Engineering. Dr. Hinz is secretary and inaugural board member of the Canadian Connective Tissue Society since 2012. He studies the role of contractile myofibroblasts in physiological tissue repair and in causing pathological tissue fibrosis. The findings of his lab are published in top-tier journals, including Cell, Nat Mater, Nat BME, Cell Stem Cells, Nat Med., with >22,286 citations and an h-index of 67 (Scopus). He published 145 peer reviewed articles, 15 book chapters, edited 2 books, and was invited to >300 seminar and conference talks plus >350 congress abstracts. His translational research led to the creation of two startup companies specialized in anti-fibrotic coatings for silicone implants and novel "soft" cell culture devices.

Talk title: Myofibroblasts: Taking Care of Business

To rapidly restore mechanical stability of tissues after injury, different cell types are activated into myofibroblasts that secrete and stabilize extracellular matrix into scar tissue. Rapid repair comes at the cost of reduced tissue function due to the inability of the myofibroblast to truly regenerate organs. When extracellular matrix accumulation and contraction become progressive and manifest as organ fibrosis, stiff scar tissue can lead to the loss of organ function. Pivotal for the formation and persistence of myofibroblasts are mechanical stimuli arising during tissue repair and chronic presence of inflammatory cells. I will provide an overview on how mechanical factors orchestrate the activation of myofibroblasts. I will focus on myofibroblast interactions with macrophages and extracellular matrix in persisting wound environments, using lung injury and implant fibrosis as paradigms. By understanding and manipulating myofibroblast mechanoperception and intercellular communication, we will be able to devise better therapies to reduce scarring and support normal wound healing in organ and implant fibrosis. I will provide some examples how our lab translated such strategies into commercialized intellectual property and spin-off companies.

Session VI: Connective Tissue Repair, Regeneration, Bioengineering -I

Robin Poole Award Winner and Keynote Speaker:

Dr. Mohit Kapoor

Dr. Kapoor is the Co-Director of the Schroeder Arthritis Institute (the largest multidisciplinary, Arthritis Institute in Canada) that integrates clinical care, education and research. He is the Tony and Shari Fell Platinum Chair in Arthritis Research. He is also the Canada Research Chair (Tier 1) and Professor of Orthopedic Surgery at the University of Toronto. Dr. Kapoor's translational research program is directed towards: (1) Understanding the complex cellular and molecular mechanisms associated with joint destruction during osteoarthritis; (2) Identifying reliable biomarkers for early identification of patients
with osteoarthritis to enable early intervention; (3) Identifying novel therapeutic targets to stop/delay osteoarthritis and restore joint function. His research program is funded by various organizations including the Canadian Institute of Health Research (CIHR), Canada Research Chairs Program, Canadian Foundation for Innovation (CFI), Natural Sciences and Engineering Research Council of Canada (NSERC), The Krembil Foundation, The Arthritis Society, Stem Cell Network, etc. He sits on review panels & boards of various research and funding organizations across the globe. He has over 100 research publications in high ranked scientific journals including Nature Medicine, Science Translational Medicine, Nature Reviews Rheumatology, Annals of The Rheumatic Diseases, etc.

Keynote talk: Omic Profiling in Osteoarthritis

Osteoarthritis (OA) is the most common form of musculoskeletal disease affecting millions of people worldwide. The endogenous mechanisms associated with OA pathogenesis are not well understood. Currently, there are no reliable diagnostics tests for early detection of OA and no approved disease modifying therapies for the treatment of OA. Omic technologies such as genomics, proteomics, transcriptomics, metabolomics etc have significantly evolved over the past few decades and are increasing being applied to better understand the complex pathophysiological mechanisms associated with OA. This talk will cover some latest advances in omic technologies and their utility in understanding the complex OA mechanisms, endotypes and phenotypes.

Session VI abstracts for oral presentations:

ABSTRACT #47:

Myofibroblast activation is induced through intracellular stress in a calcium-dependent process upon acute contact with profibrotic macrophages

Dahea LEE2, Jun WANG1, Gilbert PECORARO1, Joao FIRMINO1, Pardis PAKSHIR1, Boris HINZ1,2

1University of Toronto, Toronto; 2St. Michael's Hospital, Toronto

Background: Fibrosis (organ scarring) is responsible for >40% of disease-related deaths worldwide. Key effector and target cells in fibrosis are fibroblasts activated to myofibroblasts to produce and contract collagen into detrimental scar tissue. We published fibroblasts establish contact with mechanically attracted macrophages (Mϕ) and found that first contact with Mϕ results in fibroblast contractile events. However, the mechanisms of contact-induced acute fibroblast contraction are unknown. We hypothesize a touching Mϕ initiates fibroblast contraction and acute intracellular stress through an increase in intracellular calcium levels.

Methods: The change in acute fibroblast stress and myofibroblast activation was quantified before and after contact formation with Mϕ by measuring the nuclear translocation of mechanosensitive transcription factor YAP and Smad proteins. In addition, changes in real-time fibroblast contractions were computed from the decrease of micropatterned crosses for high throughput and the displacement of embedded fluorescent beads on a collagen matrix for high resolution. Fluorescent reporters were used to follow contact-induced cytosolic calcium changes as a fast indicator of contractile response. We inhibited Cx43 gap junctions formed between Mϕ and fibroblasts that – in principle – allow passage of calcium. Both, calcium dyes and gap-junction permeable tracers were assessed by immunofluorescence and flow cytometry. Mechanosensitive channels were inhibited, and contractile and calcium behaviours of fibroblasts upon Mϕ contact were computed.

Results: Fibroblasts in short contact with Mϕ exhibited 1.5-fold higher nuclear levels of YAP and 1.7-fold increased contraction speed compared to control fibroblasts. Increased fibroblast stress upon Mϕ contact correlated with a 1.6-fold enhanced intensity of cytosolic calcium transients. Fibroblasts and Mϕ both expressed Cx43 and exchanged gap junction permeable tracers. Consequently, inhibition of Cx43 or mechanosensitive channels in co-cultures of fibroblasts and Mϕ affected the regularity and frequency of fibroblast calcium increases. Myofibroblast activation was marked by increased Smad translocation to the nucleus acutely and increased alpha-smooth muscle actin expression and incorporation subsequently, through immunofluorescence and western blot.

Conclusion: Our results show Mϕ contact formation enhances fibroblast cytoskeletal stress and contractile activities in a calcium-dependent process and initiates the myofibroblast activation process. We propose the molecular components that mediate Mϕ-fibroblast interaction as potential targets for anti-fibrosis strategies.

ABSTRACT #48:

Mesenchymal stromal cells grown in soft environment produce extracellular vesicles that suppress pro-fibrotic activation of macrophages and fibroblasts

Yan Hei Kelly CHOI[1], Dong Ok (Donna) SON[1], Kyle LAM[1,2], Boris HINZ[1,2]

[1]St. Michael's Hospital, Toronto; [2]University of Toronto, Toronto

Rationale: Fibrosis contributes to approximately 45% of deaths in developed countries. Fibrotic scarring is initiated in all organs by tissue injury, followed by ongoing inflammation and a dysregulated wound healing process. Transplantation of mesenchymal stromal cells (MSC) with regenerative properties has been explored as anti-fibrotic treatment. Cell culture expansion following biopsy is essential to produce enough MSC for therapy. However, conventional expansion of MSC on stiff culture surfaces induces their activation into scar-forming myofibroblasts and reduces regenerative potential. Using a rat model of exacerbated skin wound healing, we found that transplantation of human MSC expanded on 'skin-soft' culture substrates affects the numbers and phenotypes of rat macrophages (Mϕ) in early (1-4 d) wound granulation tissue. Soft-grown human MSC also reduced fibrogenesis in later wound healing phases (5-10 d) despite being undetectable 4 d after transplantation. We propose that mechanical environment determines how MSC secretomes affect fibrotic activation of Mϕ and fibroblasts. We will focus on the extracellular vesicle (EV) fraction of secreted factors.

Hypothesis: Soft mechanical environment generates MSC secretory profiles that instruct Mϕ polarization and fibroblast phenotypes with scar-suppressing activities.

Objective: To elucidate how EV produced by MSC under different mechanical conditions differentially affect Mϕ and subcutaneous fibroblasts *in vitro*.

Methods: MSC were isolated from human umbilical cords and sub-cultured on skin-soft and scar-stiff culture substrates. To be consistent with our rat model of wound healing, fibroblasts were explanted from rat subcutaneous tissue and Mϕ were obtained by treating rat bone marrow-derived monocytes with rat M-CSF *in vitro*. EV (exosomes and microvesicles) were isolated from human MSC conditioned supernatants and concentrated to treat cultured rat Mϕ and fibroblasts. Mϕ were assessed using morphometry, flow cytometry and immunofluorescence staining of polarization markers (e.g., CD68, CD206) and functional tests. Myofibroblast activation of rat fibroblasts was assessed using Western blotting and immunofluorescence staining for activation markers (e.g., α-smooth muscle actin, ED-A fibronectin), and functional contraction assays.

Results: Mϕ cultured with EV from soft-grown MSC show enhanced expression of CD206 and elongated morphology compared to circular-shaped Mϕ receiving EV from stiff-grown MSC. Cytokine assays revealed enhanced secretion of anti-inflammatory cytokines by Mϕ treated with EV produced by soft-grown MSC. Transwell migration assays demonstrated attraction of Mϕ by medium containing EV from soft-primed MSC. Concomitantly, soft-grown MSC EV inhibit TGF-β-induced myofibroblast activation and contractile activity of rat fibroblasts.

Conclusion: Soft-grown MSC exert anti-scarring actions by secreting factors that control Mϕ polarization and myofibroblast activation.

ABSTRACT #50:

Mechanically active culture of patient-derived cruciate ligament cells for ligament tissue engineering

Tarek KLAYLAT[1,2], Mustafa FAKIH[1,3], Noémie JOANNETTE-LAFRANCE[3], Paul MARTINEAU[1,2], Derek ROSENZWEIG[2], Rahul GAWRI[1,2]

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INTRODUCTION: Anterior cruciate ligament (ACL) ruptures are common musculoskeletal injuries, especially among athletes. Owing to the biomechanics and poor blood supply, ligament tissues fail to properly heal and regenerate after injury, and surgical repair is required. The current standard of care is surgical reconstruction with auto/allografts, but the graft failure rate is high due to repeat injuries, thus creating a need for alternative therapies. Tissue engineering regenerative medicine (TERM) applications for ligament and tendon tissues is an emerging field, using scaffolds, bioactive compounds, and cell-based therapies. In addition, cell-seeded bioengineered ligament implants have been shown to be superior to ones without cell seeding. The optimal sources of cells for tissue engineering of ligament tissues remain a challenge, with stem cells from different sources currently under investigation with limited success. The use of primary ligament cells for TERM applications for ligament regeneration has dual limitations of limited cell numbers upon isolation and loss of phenotype on cell passaging. Overcoming these limitations would allow us to use bioengineered ligament implants, thus enhancing surgical outcomes and the quality of life of populations at risk of ACL rupture.

HYPOTHESIS AND AIMS: We hypothesize that culturing cells on continuously expanding surfaces increases cell number while maintaining phenotype. We investigate cell number expansion using a biaxial strain device that provides continuously expanding surfaces thereby expanding cells while avoiding passaging and the resulting dedifferentiation.

METHODS: We isolated primary human ligament cells from resected surgical samples and seeded them on the biaxial strain device with a 10-day continuous stretching protocol. Fixed surface area culture dishes with cells passaged to match the increasing cell growth surface served as control. At the end of the protocol, the cells on the biaxial strain device were still in passage P1, whereas the cells on the surface area matched culture dish were in P3. The relative gene expression of collagen type-I and III, tenascin C, and scleraxis and cell morphology changes were assessed.

RESULTS: Our data shows that the primary human ligament cells seeded on the biaxial strain device maintain their morphology and show an increase in relative gene expression of collagen type III, tenascin C, and scleraxis, compared to the cells grown on the static dishes, which dedifferentiate with an increase in passage number.

CONCLUSION: This strategy of expanding cells on continually expanding growing surfaces provides an effective way to increase cell number and maintain phenotype for TERM applications for ligament tissue.

ABSTRACT #62:

Neutrophils mediate kidney fibrosis via extracellular matrix protein 1

Jonatan BARRERA-CHIMAL[1], Nathalie HENLEY[1], Casimiro GERARDUZZI[1,2]

[1]Centre de Recherche de l'Hôpital Maisonneuve-Rosemont, Montreal; [2]University of Montreal, Montreal

Background: Acute kidney injury (AKI) is a common complication in hospitalized patients. AKI survivors are at increased risk of chronic kidney disease (CKD) due to maladaptive repair leading to fibrotic scarring. Increased infiltration of neutrophils is observed in the early days after ischemic AKI, however, their role in fibrotic repair and CKD transition remains unclear. **Objective**: To investigate the role of neutrophils in fibrotic remodeling following AKI. **Methods:** Thirty male C57BL/6 mice were divided in three groups: Sham (n=10), mice subjected to 25 min of bilateral renal ischemia (IR, n=10) and mice with bilateral renal IR and neutrophil depletion (IR neutro$^-$, n=10). Neutrophil depletion was induced by a single injection of anti-Ly6G antibody at day three after renal ischemia. Five mice from each group were followed-up for eight weeks to evaluate CKD progression and five mice were studied at day 5 after IR for kidney RNA-sequencing. For *in vitro* studies we used the NIH3T3 fibroblast cell line stimulated with recombinant Extracellular Matrix Protein 1 ECM1 (20 ng/mL) to assess for myofibroblast markers and HL-60 cells differentiated with retinoic acid and stimulated with PMA to evaluate for neutrophil extracellular trap (NET) release. **Results:** Eight weeks after renal ischemia, the mice from the IR group transitioned to CKD as was evidenced by a 50% increase in plasma creatinine and urea, interstitial fibrosis, and increased mRNA levels of various fibrotic molecules. Neutrophil depletion at day three post-ischemia protected the mice against AKI to CKD transition. RNA-sequencing identified extracellular matrix remodeling pathways to be downregulated following neutrophil depletion, particularly ECM1, a secreted glycoprotein with an important role in connective tissue organization, was one of the most down-regulated genes. Through immunofluorescence we show that ECM1 colocalizes with neutrophils following kidney injury. *In vitro*, recombinant ECM1 or transfection with ECM1 expressing vector (vECM1) stimulated the transformation of fibroblasts into myofibroblasts, as evidenced by increased levels of fibronectin, collagen I and alpha-SMA. The injection of ECM1 expressing vector (vECM1) into the kidney accelerated renal fibrosis. In addition, ECM1 overexpression in neutrophil-differentiated HL-60 cells accelerated NET release after PMA stimulation. **Conclusions:** Our data shows that acute neutrophil infiltration following kidney injury contributes to fibrotic repair, likely by releasing ECM1 and ultimately leading to myofibroblast activation and fibrotic remodeling.

ABSTRACT #63:

Tissue collagen degradation by endogenous MMP's is triggered by contractility inhibitors

Dennis DISCHER[1], Karan SAINI[1], Susanna BELT[1]

[1]University of Pennsylvania

Clinical use of contractility inhibitors is growing and includes inhibitors of myosin specific to cardiomyocytes that somehow – surprisingly – reduce fibrotic collagens made by fibroblasts. More generally, stiff tissues such as normal adult heart have far more fibrillar collagen than soft tissues such as brain or embryonic heart, but cell and molecular mechanisms remain unclear in collagen homeostasis. We hypothesized that contractile forces of cells in tissues as well as externally-imposed tensions suppress collagen degradation and thereby help maintain collagen levels and stiffness. Isolated tissues from mature mouse and embryonic chick hearts that spontaneously beat show collagenase-sensitive second harmonic generation (SHG) image intensity scales non-linearly versus tissue stiffness. Importantly, myosin-II inhibition triggers rapid decreases in SHG signal and tissue stiffness unless inhibitors of endogenous matrix metalloproteinases (MMPs) are also added. Chick hearts beating at ~5% strain likewise lose collagen when contractile beating is blocked, based on measurements by SHG and mass spectrometry proteomics. Isolated tendons are degraded by exogenous MMP and bacterial collagenase but degradation rate is again suppressed for physiological strains of ~5%. Tissue strain seems to sequester collagen cleavage sites because molecular permeation and mobility prove strain-independent whereas artificial collagen cross-links accelerate strain-dependent collagen degradation via collagen unfolding. Tension-suppressed degradation thus contributes to tissue stiffness scaling with collagen.

ABSTRACT #65:

Identification of EphB4 as a critical mediator of tissue fibrosis

Brian WU[1,2,3], Starlee LIVELY[1,2], Noah FINE[1,2], Chiara PASTRELLO[1,2], Sayaka NAKAMURA[1,2], Jason ROCKEL[1,2], Anca MAGLAVICEANU[1,2,3], Osvaldo ESPIN-GARCIA[1,2], Pratibha POTLA[1,2], Poulami DATTA[1,2], Laura TANG[1,2,3], Akihiro NAKAMURA[1,2,4], Rajiv GANDHI[1,2], Jianping WU[5], Boris HINZ[3,6], Igor JURISICA[1,2,7], Mohit KAPOOR[1,2,3]

[1]Schroeder Arthritis Institute, Toronto; [2]Krembil Research Institute, Toronto; [3]University of Toronto, Toronto; [4]Queen's University, Kingston; [5]University of Montreal Hospital Research Centre, Montreal; [6]St. Michael's Hospital, Toronto; [7]Slovak Academy of Sciences, Bratislava

Pulmonary fibrosis (PF) is a pathology comprising the overproduction of extracellular matrix (ECM) which disrupts lung architecture and function, and is a core pathology associated with interstitial lung diseases including idiopathic pulmonary fibrosis (IPF). Mechanisms associated with excessive ECM accumulation during PF remain unclear, however we have recently identified a crucial role for tyrosine kinase receptor EphB4 in the development of PF. To this aim, we generated mice with conditional deletion of *Ephb4* in fibroblasts and utilized a preclinical animal model of PF, RNA sequencing, NanoString, computational analysis, and functional assays of mouse and human (donor and IPF) lung fibroblasts. *Ephb4*-deletion and pharmacological inhibition protected mice against PF development. Reduced expression of genes involved in fibrosis/ECM production, ER cargo concentration, and protein trafficking were identified in fibroblasts isolated from *Ephb4*-deficient fibrotic mouse lung by RNA-sequencing. RNA sequencing of human lung fibroblasts identified elastin as a key ECM component involved in EphB4 signaling during fibrosis. Our study is the first to identify the fibrotic role of EphB4 in the development PF, and to link EphB4 expression to ECM production.

Session VII: Connective Tissue Repair, Regeneration, Bioengineering -II

Keynote Speaker: Dr. May Griffith

Dr. Griffith is a member of the Institute of Biomedical Engineering, Université de Montréal, and the Maisonneuve-Rosemont Hospital Research Centre. She holds the Canada Research Chair in Biomaterials and Stem Cells in Ophthalmology and Caroline Durand Foundation Research Chair in Cellular Therapy of the Eye. MG is known for pioneering work in translational regenerative medicine and in situ tissue regeneration. In a human clinical trial, she and her multinational, multidisciplinary team developed the world's first cell-free biosynthetic corneal implants that promoted human corneal tissue and nerve regeneration. Since then, she completed a second clinical trial on patients at high risk for graft failure, incorporating an inflammation-suppressing polymer network into her biomaterials. MG and her team are working on injectable hydrogels that are applied as a liquid but quickly adhere and gel to repair tissue defects. Then its similarity at a molecular level to a natural tissue framework promotes tissue regeneration.

Session VII abstracts for oral presentations

ABSTRACT #49:

Effect of glycation on collagen properties for tissue engineering applications

Meisam ASGHARI2, Emilie KHATTIGNAVONG1, Liisa HIRVONEN3

1University of Toronto, Toronto; 2McGill University, Montreal; 3University of Western Australia

Introduction: Collagen is the major extracellular matrix protein and its properties are modulated by the presence of crosslinks. Collagen scaffold is a great option for tissue engineering applications, however, a major limitation related to the engineering of collagen scaffolds is reduced biomechanical property and rapid degradation. This has been a long-standing problem in tissue engineering and various solutions have been brought forward to crosslink collagen. However, the cross-linkers that are used widely in the industry can be toxic, or too weak to hold the desired structure together.

During collagen in vivo biosynthesis, two main crosslinking pathways stabilize collagen fibrils: enzymatic-mediated crosslinking and glycation-mediated crosslinking. Advanced-Glycation-End-products (AGEs) formation results from long-time exposure of proteins such as collagen to reducing sugars. In this research, we explored the following hypothesis: The accumulation of AGEs in collagen decreases its proteolytic degradation rates while increasing its stiffness.

Methods: To test this hypothesis, we engineered Type I collagen scaffolds. AGE-mediated crosslinking was induced in collagen gels by exposing them to -the most reactive sugar in the body- Methyl Glyoxal (MGO). The formation of AGEs into the scaffolds was confirmed by performing autofluorescence measurement of scaffolds, competitive ELISA assay and Fluorescence Lifetime Imaging (FLIM) with an inverted confocal laser scanning microscope (CLSM). We used a Live/Dead® assay to measure cell viability after seeding NHDF cells over glycated collagen scaffolds. Furthermore, we investigated the impact of AGE-mediated crosslinks on collagen properties such as water sorption, structural ordering, and elasticity using a combination of Atomic Force Microscopy (AFM) and ATR-FTIR. The enzymatic degradability of collagen by collagenase was evaluated by time-lapse digital imaging.

Results: The accumulation of MGO-derived AGEs was found to increase exponentially in our collagen scaffolds as a function of MGO concentration. As MGO concentration increased, the fluorescence lifetime of glycated collagen decreased. The glycated scaffolds demonstrated pivotal cellular compatibility towards human dermal fibroblasts. Glycated scaffolds were found to absorb water at a much higher rate. In addition, the topology of collagen fibrils as observed by AFM became a lot more defined and promoted localized fibrillar alignment following glycation. Despite collagen scaffolds' increased elasticity, fibrils' elasticity decreased with glycation. Finally, we recorded a much slower degradation rate in the glycated scaffolds.

Conclusion: We demonstrated how we can harness invitro, the crosslinking potential of MGO, which is a natural phenomenon, rapid and highly efficient, to enhance the properties of engineered collagen scaffolds.

ABSTRACT #59:

Doxorubicin-loaded nanoparticle bone cement for the treatment of metastatic spine disease

Megan COOKE[1], Michael WEBER[1], Derek ROSENZWEIG[1]

[1]McGill University, Montreal

INTRODUCTION: Bone cement is used to fill defects created by the surgical resection of spinal metastases. Tumor cells remain hidden and drive cancer recurrence. Doxorubicin (DOX) is a common chemotherapeutic drug; however, it is cardiotoxic. Mesoporous nanoparticles are gaining attention for targeted drug delivery to bypass the negative side effects associated with systemic drug administration. Our objectives were to develop a nanoparticle cement for the local release of DOX and to test its ability to suppress cancer cells.

METHODS: DOX release from the cement was measured over a period of 4 weeks using a TECAN Infinite M200 PRO plate reader to quantify the concentration of DOX. The cement constructs were incubated with 2D and 3D cultures of cancer cell lines and cell viability was determined by alamarBlue and Live/Dead assays. Cell migration and spheroid growth was assessed in 3D spheroid culture.

RESULTS: A sustained DOX release profile was achieved with the addition of nanoparticles to the bone cement. The release profile of DOX from nanoparticle cement can be modified by changing the amount of the drug loaded onto the nanoparticles and the proportion of nanoparticles in the cement. Cancer cells treated with the cement constructs showed a dose- and time-dependent inhibition. Furthermore, cell migration and spheroid growth were impaired.

CONCLUSIONS: We determined that nanoparticles are essential for a sustained DOX release profile from bone cement. The release can be tweaked for an optimal drug delivery system. Our findings demonstrate that chemotherapeutic cements can inhibit cancer cells and impair their migration.

ABSTRACT #66:

Bone regeneration: A new application for DNA hydrogels

Nadeen MESHRY[1], Naara MONTEIRO[2], Ana C. ERVOLINO-SILVA[2], Roberta OKAMOTO[2], Christopher MCCULLOCH[1], Karina CARNEIRO[1]

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Regenerating large bone defects such as those secondary to inflammation or surgical tumor resection is an ongoing challenge. With over two million annual bone grafting procedures worldwide, there has been increasing demands for regenerative solutions(1). Although autologous grafts are still the gold standard, they are not free of important limitations including donor site morbidity and limited availability. These drawbacks are the driving forces behind a body of research dedicated to exploring osteoconductive biomaterials as replacements for autologous grafting. The field of DNA nanotechnology offers DNA-based nanoparticles as promising and potentially osteoconductive materials for bone grafting(2). Accordingly, the objective of this research was to investigate DNA hydrogels as biopolymeric scaffolds for bone repair. Complementary single DNA strands were used to form a hydrogel at room temperature, as characterized by rheology experiments. The rate of hydrogel degradation was assessed using polyacrylamide gel electrophoresis. Cell viability and attachment were investigated by incubating mouse calvarial osteoblasts on the DNA hydrogels; and osteogenic differentiation was assessed using RT-qPCR for osteogenic genes (ALP, Collagen-1, OCN, Runx2). The hydrogels were tested in 5-mm calvarial defects in rats. Data showed that the rate of degradation can be controlled by adjusting the DNA molar concentration of the hydrogels: the half-life of 2x concentrated hydrogels was 13.5d while that of 1x concentrated hydrogels was only 6.6d. Cell spreading was limited; however, the DNA hydrogels supported cell viability and osteogenic differentiation, with expression levels greater than negative controls at 14 days. Finally, in rat calvarial defects, histological and μ-CT images showed that hydrogels promoted bone repair, with increased bone-to-tissue volume compared with the buffer group at 28d. This research indicates that DNA hydrogels are promising biomaterials for bone regeneration. Future work will include optimizing DNA hydrogels for bone repair and defining the mechanisms by which DNA hydrogels are osteoconductive.

References:

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SESSION VIII: Skin & Matrix Biology

Keynote Speaker: Dr. Anie Philip

Dr. Anie Philip is a full professor at McGill University and a Senior Scientist at the Research Institute of the McGill University Health Center. She currently serves as a Co-Director of the

'Skin Investigators Network of Canada' (SkIN Canada) and as a Board Member and immediate Past President of the 'Skin Research Group of Canada'. Dr Philip's research focuses on understanding the cellular mechanisms underlying the dysregulation of cellular signaling pathways in fibrosis, osteoarthritis, and squamous cell carcinoma. Her most significant research contributions include the discovery of CD109 as a TGF-b co-receptor and inhibitor of tissue fibrosis and as a potent promoter of squamous cell carcinoma. Dr Philip has published over 100 scientific articles and her research is supported by CIHR Project and Network grants, NSERC Discovery and Alliance grants, and FQRNT grants. She serves on the review panels of various research funding organizations in Canada, the US, and Europe

Session VII abstracts for oral presentations

ABSTRACT #92:

Repeated applications of mesenchymal stem cell-based biological dressings accelerate wound healing in a murine model of diabetes

Meryem SAFOINE[1], Caroline PAQUETTE[1], Gabrielle-Maude GINGRAS[1], Julie FRADETTE[1,2]

[1]Centre de recherche en organogenèse expérimentale de l'Université Laval/LOEX, Québec; [2]Department of Surgery, Faculty of Medicine, Université Laval, Québec

Introduction:
Long term diabetes can lead to diabetic foot ulcers unresponsive to the actual standards of care. New strategies are actively being developed to enhance wound repair using cell-based approaches. Multiple cell types are being investigated, with adipose-derived stromal/stem cells (ASC) representing an optimal cell source considering their abundancy and therapeutic properties. ASC can be used to produce biological dressings which can act as releasing platforms to deliver steady secretion of growth factors onto the wound bed. However, cell culture requires the use of fetal bovine serum (FBS) which should be removed when considering clinical applications.

Methods:
Using the self-assembly approach of tissue engineering, we produced ASC-based dressings under a completely serum-free system from ASC extraction to biological dressings production. The biological dressings were repeatedly applied every four days on full thickness 8-mm splinted skin wounds created on the back of diabetic mice. Two diabetic murine models were used: polygenic diabetic NONcNZO10/LtJ mice and streptozotocin-induced diabetic K14-H2B-GFP mice displaying a fluorescent epidermis allowing tracking of reepithelization using an in vivo imaging system.

Results:
NONcNZO/LtJ mice global wound closure kinetics evaluated by macroscopic imaging showed that ASC-based dressings accelerated wound closure by a week, the treated group reaching 98.7 ± 2.3 % global closure compared to 76.4 ± 11.8 % for the untreated group at day 20. K14-H2B-GFP mice showed an acceleration of reepithelization kinetics for the treated group, especially at early time-points. On histological sections, treated wounds exhibited healed skin of better quality with a more organized, homogeneous and 1.6-fold thicker granulation tissue along with a 1.5-fold increase in its collagen content. Treated wounds displayed a well-differentiated epidermis, 2.2- to 4.6-fold thicker compared to the untreated wounds. Neovascularization, assessed by CD31+ labeling, was 2.5-fold higher in the treated wounds.

Conclusion:
We described an entirely serum-free production system of naturally derived scaffold-free ASC-based biological dressings optimal for clinical translation. These dressings act on multiple processes of wound healing including granulation tissue formation, reepithelization, and neovascularization, and represent promising candidates for difficult to heal diabetic wounds.

ABSTRACT #77:

Histological and mechanical characterization of skin in wound healing study with streptozotocin induced diabetic rats and application of chitosan and platelet-rich plasma biomaterial

Laura AHUNON[1], Anik CHEVRIER[2], Nicholas GILBERT[3], Julie Fradette[4, 5], Marc LAVERTU[1,2]

[1]Biomedical Engineering Institute, Polytechnique Montréal, Montreal, QC, Canada; [2]Chemical Engineering Department, Polytechnique Montréal, Montreal, QC, Canada; [3]Biomomentum Inc., Laval, QC, Canada. [4]Centre de recherche en organogenèse expérimentale de l'Université Laval/LOEX, Québec, [5]Department of Surgery, Faculty of Medicine, Université Laval, Québec.

The Biomaterial and Cartilage Laboratory (BCL) has been developing a biomaterial that was proven effective for orthopaedic applications in regenerative medicine. The biomaterial (CS-PRP) is composed of freeze-dried chitosan (CS) and platelet-rich plasma (PRP, a fraction of the blood), making an injectable material once mixed, that coagulates *in situ*. After establishing *in vitro* that CS-PRP promotes cell migration, the biomaterial was used *in vivo* to assess its wound healing properties in rats with healing impairment.

Methods: The pilot study included 5 male Sprague Dawley rats injected with streptozotocin, with 2 full-thickness excisional wounds on their back with a silicone splint around the wounds to prevent contraction and therefore favour healing by re-epithelialization. Inactive jelly was applied in 2 rats (as controls) and 3 rats had their wounds treated with CS-PRP.
Wound closure rate was followed with images taken every 4 days until euthanasia. All rats were sacrificed 19 days after initial wounding and the healed wounds were retrieved. For each rat, the skin rigidity and thickness were assessed by indentation (mechanical testing) in one wound and the other was dedicated to histology. Histochemical stains (e.g., Hematoxylin and Eosin, Masson's Trichrome, Picrosirius Red) and immunohistochemical labeling [e.g., CD31 for blood vessels, types I and III collagen, α-SMA (Smooth Muscle Actin) for myofibroblast, Arginase-1 for M2 macrophages] were performed to assess advancement of tissue healing.

Results: Only 2 showed sufficient hyperglycemia to be considered diabetic. One rat lost its splints on both wounds at day 8 post-surgery and the wounds closed rapidly through contraction. Rat skin was thinner in diabetic rats (2,2 +/- 0,4 mm) compared to healthy ones (2,8 +/- 0,2 mm). Healing of control wounds appeared less advanced than CS-PRP treated ones: wounds had more inflammatory infiltrate and more granulation tissue with thin mostly immature type III collagen fibers versus mature type I collagen fibers. There were more α-SMA positive cells in the upper regenerating dermis. Quantification of blood vessels and anti-inflammatory M2 macrophages is ongoing.

Discussion/Conclusion: Diabetic induction was not achieved in all rats and one rat lost its splints. Therefore, we ended up with one group with diabetic rats treated with CS-PRP (n=2) and a second group with healthy control rats (n=2). Given the small size of groups, the effects of diabetes and treatment are confounded. However, mechanical and histo/immunohistochemical methods were developed and lessons learned during this pilot study will be used to improve future larger studies.

ABSTRACT #78:

A new antimicrobial drug, Ag373K augments keratinocyte migration by inducing vascular endothelial growth factor (VEGF)-A release

Vida MAKSIMOSKA[1,2,3], Katrina VIZLEY[4], Carla J. SPINA[4], Katalin SZASZI[1,2,3]

[1]University of Toronto, Toronto; [2]St. Michael's Hospital, Toronto; [3]Keenan Research Institute; [4]Noa Therapeutics

Introduction: Failure of restoration of the skin barrier following injury leads to chronic wounds, a condition associated with reduced quality of life and high mortality. Since infection with antibiotic-resistant pathogens often complicates healing, there is an urgent need for new anti-microbial therapeutics that also promote re-epithelialization. Keratinocytes are essential for wound healing, as they migrate and proliferate to restore the skin barrier and secrete pro-healing molecules to augment functions of other crucial cells. Ag373K is a promising new antimicrobial drug that has high efficiency against bacteria common in chronic wounds. However, the effects of this drug on skin cells remains to be established. Our initial data suggested that at 5-10 μM, which is an efficient antimicrobial concentration, Ag373K is non-toxic and augments migration of a human keratinocyte cell line (HaCat). The **objective** of this study was to establish the mechanisms whereby Ag373K affects keratinocyte functions.

Methods and results: We performed a screen to identify changes in released cytokines and growth factors and to identify altered mRNA expression in HaCat cells treated with Ag373K. We found that the drug promotes secretion of several growth factors, including Vascular Endothelial Growth Factor (VEGF)-A. We also found increased levels of RhoA and Rac mRNA, two members of the Rho family of small GTPases that are key regulators of cell migration. Based on these findings, we **hypothesized** that autocrine effects of Ag373K-induced VEGF-A are key for altering Rho protein expression and activation, leading to augmented migration. Our data show that addition of AG373K or VEGF-165 to HaCat cells elevated expression of Rac and RhoA protein and enhanced their activity. Pharmacological or genetic inhibition of VEGF receptor 2 (KDR) efficiently prevented these effects. Interestingly, KDR inhibition also strongly reduced basal expression of Rac and RhoA, suggesting the VEGF-induced signalling is a key input for basal Rho protein signalling. A live imaging using a gap closing assay revealed that VEGF addition augments keratinocyte migration. Staining of F-actin showed earlier and larger lamellipodia in drug-treated cells. Further, inhibiting KDR prevented the effect of Ag373K on keratinocyte migration.

Conclusion: Taken together, these findings reveal that the potent anti-microbial agent Ag373K affects keratinocyte behaviour via VEGF-A release, which is central for its beneficial effects. Ongoing experiments are aimed at understanding the signalling pathways involved in VEGF-A-induced upregulation and activation of Rho proteins.

ABSTRACT #73:

Role of N-linked glycans in short fibulins and LTBP-4 mediated matrix assembly and function

Valentin NELEA[1], Heena KUMRA[1], Daniel WILLIAMSON[2], Robert HALTIWANGER[2], Dieter REINHARDT[1]

[1]McGill University, Montreal; [2]Complex Carbohydrate Research Center, University of Georgia, USA

Introduction: Elastic tissue require various extracellular proteins, including fibulin-4 and -5 and the latent TGFβ binding protein-4 (LTBP-4) long and short isoforms (LTBP-4L and LTBP-4S) to synthesize functional elastic fibers. Mutations in these proteins cause heritable diseases such as cutis laxa, and result in deficient elastic fibers and compromised skin elasticity. We recently demonstrated new mechanisms in elastogenesis involving a dual role of fibulin-4 in inducing a stable conformational and functional change of LTBP-4L, and promoting deposition of tropoelastin onto the elongated LTBP-4L. All these proteins are known to be N-linked glycosylated, but the role of the glycosylation during elastic fiber formation has not been determined.

Methods: Fibulin-4, fibulin-5, LTBP-4S and LTBP-4L proteins were recombinantly produced by HEK293 cells and purified chromatographically. Deglycosylation treatments were performed by protein enzymatic digestion with PNGase F enzyme. The binding affinity of the proteins was studied by surface plasmon resonance spectroscopy. Atomic force microscopy was used to visualize the proteins. Dynamic light scattering was employed to analyze protein conformations in solution. Glycoproteomic mass spectral analysis of *N*-glycan structures of LTBP4s and fibulins were also performed. Matrix assembly was assessed in cell culture experiments.

Results: Fibulin-5, but not fibulin-4 induces a similar but not identical extension and functional change of LTBP-4S, resulting in an increase in LTBP-4 assembly and in tropoelastin deposition. This provides a mechanism for the previous in vivo observations and suggest the existence of two separate development axes in elastogenesis (fibulin-4—LTBP-4L and fibulin-5—LTBP-4S). We next asked if N-linked glycans on fibulins and LTBP4s play a key role in fibulin-mediated LTBP-4 structure, function, assembly, and elastogenesis. We found that overall deglycosylation influences LTBP4 assembly as well as tropoelastin deposition. Loss of N-linked glycans from LTBP4L resulted in a complete abrogation of its binding to Fibulin-4 and its extension, whereas removal of N-linked glycans from Fibulin-4 did not affect its binding or its ability to extend LTBP-4L. In the other interaction axis, fibulin-5 N-linked glycans were found essential for promoting LTBP4S molecular extension, binding, and LTBP4 assembly, and tropoelastin deposition. N-glycosylation mutants of fibulin-4, when endogenously expressed, enhanced elastin assembly.

Conclusion: Our data elucidate new mechanisms which regulate the process of elastic fiber formation in including the fibulin-5—LTBP-4S axis and suggest that the presence of N-linked glycans in fibulins and in LTBP4s affect the LTBP4 assembly and tropoelastin deposition with glycans important factors for elastogenesis.

SESSION IX: Machine Learning, AI and Computational Biology

Keynote Speaker: Dr. Armstrong Murira

Armstrong is a molecular biologist with a PhD focused on bioinformatics and computational biology in HIV, a specialization that allowed him to contribute to significant research during his postdoctoral studies, particularly within Hepatitis C. This academic foundation gave him a profound appreciation for the intricacies of biological research and the potential for technological interventions.

Transitioning from academia to the pharmaceutical industry, Armstrong immersed himself in an array of roles. His diverse experiences spanned from business analytics and development to specialized work in machine learning (ML), specifically within clinical R&D. Each role provided him with valuable insights into the multifaceted nature of the industry. His interests range from basic, foundational research to clinical drug development. The fusion of these experiences led Armstrong to co-found Simmunome, where he currently serves as CEO. At Simmunome, he is leveraging his background in ML and bioinformatics, diving deep into biological complexities to find innovative solutions to challenges in biological and clinical R&D. Armstrong finds the work at Simmunome incredibly rewarding and is excited to see where the team's continued research and exploration will lead.

SESSION IX abstracts for oral presentations

ABSTRACT #69:

Generation of synthetic µCT images of rat lumbar vertebral fracture via deep convolutional generative adversarial networks

Allison TOLGYESI[1,2], Cari WHYNE[1,2], Michael HARDISTY[1,2]

[1]University of Toronto, Toronto; [2]Sunnybrook Research Institute, Toronto

Purpose: The complex ultra-structure, heterogeneous material properties, and ubiquity of flaws in bone matrix present challenges in characterizing bone fracture. Modeling bone failure is challenging as damage nucleation and propagation is stochastic. Bone damage has previously been successfully modeled with micro finite element models; however, these require extensive setup and are computationally expensive limiting their use for large scale bone quality simulations. Deep learning (DL) presents useful tools for modeling using a data-driven approach. Generative DL models can represent stochastic processes, allow for multi-scale modeling, and incorporate non-linear relationships to input variables. The present study aimed to develop a generative DL model to simulate failure through creation of synthetic 3D µCT images of fractured rat lumbar vertebrae.

Methods: A 3D conditional generative adversarial network with a modified Pix2Pix architecture learned the mapping between unloaded µCT images of rat lumbar vertebrae and their fracture patterns after load-to-failure testing. Thirty-nine cropped and resampled real 3D µCT image pairs (70µm voxel spacing) of unloaded and fractured rat vertebrae were used for training. Random image augmentations (mirroring, rotation) increased the training dataset to 429 image pairs. Eight real pairs were held out for validation. Training used a learning rate of 0.0002, batch size of one, Adam optimization, and ran for 1000 epochs. The generator was a 3D U-Net, and the discriminator was a four-layer 3D deep convolutional neural network for conditional-image classification. Training was run on a Nvidia Titan RTX GPU.

Results: After 1000 epochs (~1400s/epoch), generated training and validation images appeared realistic, with correctly oriented anatomical features (pedicles, spinous process), trabeculae and intervertebral discs. The location of fracture in real images was often correctly identified but presented as deformations in generated images without obvious fractures in the bone matrix. Other generated images featured a realistic deformation but did not correspond to the fracture pattern of the paired real image. Future work will increase the training dataset and incorporate sequential µCT images acquired during loading before fracture.

Conclusion: This generative DL model shows promise in creating realistic images of fractured rat vertebrae. Success of this approach may enable vertebral fracture predictions, which clinically could improve detection of unstable vertebrae in pathologic bone (metastasis, osteoporosis). Preclinically, understanding fracture mechanics of diseased bones and the effects of clinically used treatments (chemotherapy, radiotherapy, bisphosphonates) could be facilitated without the need of destructive testing. The DL approach allows for thorough (and feasible) computational study of vertebral fracture

ABSTRACT #93:

Unraveling PRP Mechanisms of Action: Integrating Proteomics, Metabolomics, and Lipidomics using Causal Inference and Deep Graph Neural Networks

Nardin NAKHLA[1], Saba DAFTARI[1], Armstrong MURIRA[1]

[1]Simmunome Inc.

Platelet-rich plasma (PRP) has emerged as a promising treatment for enhancing the healing of various occupational and sports-related injuries involving tendons, meniscus, and ligaments. Despite its clear outcomes, the underlying mechanisms of action remain elusive. To ensure safety and potency, it is essential to characterize PRP, focusing on aspects such as growth factor concentrations and platelet activation. Understanding the biological systems and mechanisms of action of treatments demands a comprehensive knowledge of real-time multiomics. Proteomics has taken center stage in the realm of biomarkers due to its direct involvement in causal interactions and proximity to phenotypic traits compared to genetics. Recent advances in high-throughput proteomics, resulting in vast amounts of data, have paved the way for machine learning models to unravel the complex biological systems, especially in terms of causal relationships in health and disease states.

In this study, we present a novel approach to characterizing the mechanism of action of PRP in tissue repair by modeling the causal interactions of tissue repair while integrating PRP proteomics with other modalities such as metabolomics and lipidomics. By simulating the cascades involved in inflammation, proliferation, and remodeling that ultimately lead to tissue repair, we examine these interactions on various levels, ranging from population down to single patient profiles.

Liquid chromatography-mass spectrometry (LC-MS)/MS is employed to measure proteomics, metabolomics, and lipidomics data. These datasets are then integrated into large networks that simulate the dynamic interactions underlying tissue repair. Machine learning algorithms, particularly deep graph neural networks, are utilized to infer causal relationships among the omic entities. The resulting graph representing tissue repair contains the 71 core proteins, 1000+ proteins and hundreds of metabolites and lipids identified from analyzing the PRP samples. With 85% inference accuracy, the resulting number of interactions relating the omic entities is approximately 76,000.

Our approach provides valuable insights into patient profiling, PRP formula optimization, and predicting clinical outcomes based on individual PRP profiles. By unraveling the mechanisms of action of PRP in tissue repair through the integration of multiomics data and machine learning algorithms, we can tailor PRP treatments to specific patient needs, potentially revolutionizing the field of regenerative medicine and paving the way for personalized healthcare.

Poster Presentations

Abstract #	Title	Presenting author	Poster Judging
2	The mechanism underlying the anti-mineralization property of matrix Gla protein: Role of its conserved serine residues	Kyoungmi Bak	Thursday
5	PCL scaffolds promote bone repair of mandibular defects in a rat model of bisphosphonate-related osteonecrosis of the jaw	Tarek Klaylat	Thursday
7	Systematic Review and Meta-Analysis of Collagen Post-Translational Modification in Osteogenesis Imperfecta	Priyesh Patel	Thursday
8	Functional role of fibronectin isoforms in chondrogenesis and skeletal development	Neha Dinesh	Wednesday
10	This isn't my vesicle. How did I get here? Localization of V-ATPases and Rabs	Ralph Zirngibl	Thursday
12	Transglutaminase 1 – a novel regulator of osteoclastogenesis	Sahar Ebrahimi Samani	Wednesday
13	Characterizing the Effect of Sub-clinical Priming Injuries on Enhanced Fracture Healing	Misghana Kassa	Thursday
16	Validation of Finite Element Analysis for Bone Remodeling Simulation With Ex Vivo Mechanical Testing Of Bovine Trabecular Bone	Mahsa Zojaji	Wednesday
18	CAN COSTAL COLLAGEN FIBER ORIENTATION EXPLAIN THE TENDENCY OF WARP IN STRUTS PREPARED FOR NASAL RECONSTRUCTION	SABA RAFIEIAN	Wednesday
19	Investigating the role of steroid hormones in the IVD	Jeffrey Hutchinson	Thursday
20	Crosstalk between cartilage and bone in adolescent idiopathic scoliosis	kai sheng	Thursday

Abstract #	Title	Presenting author	Poster Judging
21	Senolytic combination treatment for painful degenerating intervertebral discs.	Hosni Cherif	Thursday
23	Impact of SUMOylation on biomolecular modification of PHB1 in primary osteoarthritis	Abdellatif Abouelseoud	Thursday
25	Inflammation-inducible strategies for growth factor gene therapy to promote joint repair in a preclinical model of post-traumatic osteoarthritis	Anisha Thomas	Thursday
26	A Bioinformatics Approach to Understanding the Pathogenesis of Ectopic Spine Calcification	Fang Chi Wang	Thursday
28	Anti-inflammatory gene therapy strategies to promote joint repair in a preclinical model of post-traumatic osteoarthritis	Sepideh Taghizadeh	Wednesday
30	Link N Regulates Inflammasome Activity in the Intervertebral Disc through Interaction with CD14	Muskan Alad	Thursday
31	Synovial macrophage activation mediates pain experiences in experimental knee osteoarthritis	Garth Blackler	Thursday
33	Role of irisin in promoting resistance in chondrocytes to pro-inflammatory cytokine-mediated damage	Hayley Galsworthy	Thursday
36	Senescence and Inner Annulus Fibrosus Cells	Sajjad Ashraf	Thursday
38	Inflammation promotes release of vimentin from gingival fibroblasts in an Annexin A2-dependent manner	Zofia Ostrowska-Podhorodecka	Wednesday
40	Single nucleus RNA sequencing identifies distinct synovial fibroblast subsets with unique transcriptomic profiles in early and late-stage radiographic knee OA	Kabriya Thavaratnam	Wednesday
41	Analysis of Nuclear Scaling Properties between Multinucleated Osteoclasts and Mononuclear Pre-osteoclasts	Mohammed Said	Thursday
43	Role of P2 receptors in nucleotide-induced calcium signalling in murine appendicular and axial tenocyte cultures – implications for tendon mechanotransduction	Ryan Armstrong	Thursday
44	Fate and Function of Mesenchymal Progenitors in Musculoskeletal Regeneration	Wilder Scott	Wednesday

Abstract #	Title	Presenting author	Poster Judging
45	Matrix production and contraction: The myofibroblast Two-Face personality	Raquel Benitez	Thursday
46	Mechanical Stress Reduces Global Chromatin Condensation and Enhances DNA Methylation in Mesenchymal Stromal Cells	Nicole Berezyuk	Wednesday
51	Cold Plasma-Based Redox Therapy for Bone Tumor Growth Control and Bacteria Inactivation	Laura Bouret	Thursday
52	A Biocompatible and Biodegradable Hydrogel for an Infection-Free Wound and Recovery	Justin Matta	Wednesday
53	Targeting Bone Healing with a Novel Bioresorbable Borophene-Monetite Implant	Justin Matta	Thursday
55	Development of a novel inorganic polyphosphate-releasing thermoresponsive hydrogel aimed towards improving fracture outcomes.	Rayan Ben Letaifa	Thursday
56	Mechanical control of extracellular vesicle formation by mesenchymal stromal cells	Kyle Lam	Wednesday
57	Assessing the effect of design parameters on 3D-printed scaffolds for bone tissue repair	Alexandrine Dussault	Thursday
58	Collagen hybridizing peptide induce in vitro collagen fibril growth	Sophia Huang	Wednesday
60	Acute contact with macrophages induces lasting myofibroblast activation through mechanical stress-dependent YAP signalling	Dahea Lee	Wednesday
61	Establishment and evaluation of an ex vivo osteochondral-synovim model for assessing psoriatic arthritis therapies.	Atoosa Ziyaeyan	Thursday
64	Chitosan/platelet-rich plasma implants in a large arthroscopic model of meniscus repair	Margaux DELVAUX	Wednesday
67	Protein phosphatase 6 is a new regulator of GEF-H1/Rho signaling in kidney tubular cells	Negar Arghavanifard	Thursday

Abstract #	Title	Presenting author	Poster Judging
68	Biomechanical Properties of Bone and Cartilage during Prolonged Storage with the Missouri Osteochondral Allograft Preservation System	Isabel Li	Wednesday
70	Adjuvant Therapy Using Senolytic Drugs to Prevent Breast-To-Bone Metastasis	Eleane Hamburger	Thursday
71	A 3D culture model of intestinal cells and fibroblasts as a screening platform for therapeutics in IBD	Paraskevi Tselekouni	Thursday
72	To understand the dichotomy between synovial resident macrophages and infiltrating monocytes in osteoarthritis progression	Shahrzad Nouri	Wednesday
74	Differential interaction of LOXL1 variants linked to pseudoexfoliation syndrome with elastic tissue matrix proteins	Valentin Nelea	Thursday
75	MFAP4 forms calcium-dependent tetramers of homodimers which bind some, but not all, extracellular matrix proteins in a calcium-dependent manner	Valentin Nelea	Thursday
76	Integrin α7β1 represses differentiation of intestinal absorptive cells	Gabriel Cloutier	Wednesday
79	Mechanical priming on skin-soft elastomer substrates improves the wound healing potential of human mesenchymal stromal cells	Dong Ok (Donna) Son	Thursday
82	Cryopreserved Hair Follicles: An Abundant and Accessible Source for Autologous Mesenchymal Stromal Cells in Cell Replacement Therapies	Amatullah Fatehi	Thursday
83	Combined transcriptome and epigenome profiling reveal regulators of dermal fibroblast state switching	Thomas Kirk	Thursday
84	Defining transcriptomic differences between left and right ventricle-derived cardiac fibroblasts	Michael Dewar	Thursday
85	A novel and bioresorbable hematene-doped monetite scaffold for bone repair	Justin Matta	Thursday

Abstract #	Title	Presenting author	Poster Judging
86	Ex vivo mechanical over-loading of articular cartilage leads to extracellular matrix alterations in osteochondral cartilage plugs	Colleen Mathieu	Wednesday
87	Latent transforming growth factor binding protein – 2 deficiency improves cardiac function post-myocardial infarction	Fahad Ehsan	Thursday
88	Evaluating a novel storage protocol for preservation of fresh osteochondral allografts	Sarah Aloi	Wednesday
89	Inorganic polyphosphates: an immuno-modulatory therapeutic target in early fracture healing	Rayan Ben Letaifa	Thursday
90	Unlocking the power of macrophages through dectin-1: Novel yeast beta-glucan targets disease-driven phenotypes in lung fibrosis and cancer	Safaa Naiel	Wednesday
91	Evaluation of the immuno-tactic effects of inorganic polyphosphates as a therapeutic target for early fracture healing	Rayan Ben Letaifa	Thursday

Abstracts for Poster Presentations

Adipose Tissues and Blood Vessels

ABSTRACT #2:

The mechanism underlying the anti-mineralization property of matrix Gla protein: Role of its conserved serine residues

Kyoungmi BAK[1,2], Abhinav PARASHAR[3], Vincent RICHARD[4], Ophélie GOURGAS[1,2], Nawara OSMAN[1,2], Monzur MURSHED[1,2]

[1]McGill University, Montreal; [2]Shriners Hospital for Children-Canada, Montreal; [3]Adult Stem Cell Section, National Institute of Dental and Craniofacial Research, National Institute of Health, Bethesda, Maryland, USA; [4]Lady Davis Institute for Medical Research, Montreal

Vascular calcification is a frequent complication of aging and various chronic and genetic diseases. Despite the serious health risks associated with vascular calcification, currently there is no cure for it. Deficiency of matrix Gla protein (MGP), a strong inhibitor of soft tissue calcification, has been linked to vascular calcification. MGP carries two sets of conserved residues which undergo post-translational modifications – three N-terminal serine residues are phosphorylated (pSer) and four glutamic acid residues are γ-carboxylated (Gla). Recently, using genetic models, we demonstrated that the pSer residues play a key role for the anti-mineralization function of MGP. However, the detailed mechanism of action of these residues is not fully understood. Besides, it is not clear whether the two sets of conserved residues in MGP function in a cooperative manner. Also, the kinase that phosphorylates MGP remains unidentified.

To study the mode of action of MGP's pSer residues, I used an in vitro cell culture model mimicking vascular elastin calcification and analyzed a newly developed mouse model. To investigate the effects of charge and amino acid sequence of MGP's pSer peptide on the prevention of elastin calcification, mineralizing cultures were treated with various mutated derivatives of the peptide for 10 days and deposited minerals were quantified. To examine the inter-dependency of the post-translational modifications of MGP, we performed co-transfection experiments expressing the native or mutated forms of MGP lacking the conserved serine or the glutamic acid residues. The pulled-down MGPs from the culture media were analyzed by mass spectrometry to examine the status of the post-translational modifications. To identify the kinase that phosphorylates MGP, co-transfection experiments as above were performed with a vector expressing kinase selected by its target sequence. Lastly, we analyzed a new 'knock in' mouse model expressing a chimeric protein in which the N-terminal conserved serine residues of MGP have been added to the C terminal part of bone Gla protein (BGP), a protein related to MGP, which carries the Gla residues, but does not show any anti-mineralization function.

My analyses showed that the serine residues are the most critical functional residues in MGP and the anti-mineralization property of MGP's N-terminal peptide is not sequence- or charge-dependent but requires the presence of the phosphate moieties. Furthermore, I have identified Fam20c, a Golgi-localized kinase to be involved in the phosphorylation of MGP. Overall, my work provides a detailed molecular basis of how MGP prevents vascular calcification.

Bone and Developmental Biology

ABSTRACT #5:

PCL scaffolds promote bone repair of mandibular defects in a rat model of bisphosphonate-related osteonecrosis of the jaw

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INTRODUCTION: Intravenous administration of bone anti-resorption agent bisphosphonates (BP) is considered frontline therapy for skeletal complications of malignancy. However, patients taking third-generation BP zoledronic acid (ZA) treatment undergoing oral surgery are at high risk of developing BP-related osteonecrosis of the jaw (BRONJ). Polycaprolactone (PCL) is a biocompatible plastic that can be fabricated with interconnected pores resembling trabecular bone. The objective of this study was to characterize the repair of mandibular bone defects in BRONJ with PCL and chitosan-coated PCL (PCL/c) scaffolds.

METHODS: 6–8-month-old male rats were assigned to either control [weekly IP injection of PBS] or BRONJ [weekly IP injection of 0.13mg/kg ZA+3.8mg/kg body wt. dexamethasone (DEX)]. After 4 weeks, the first left and right mandibular molars were extracted, and weekly injections of PBS or ZA+DEX were continued for 4 weeks. PCL scaffolds with average pore diameter of 150µm were fabricated and coated with chitosan (PCL/c). Administration of PBS and ZA+DEX was stopped after 8 weeks, and rats were anesthetized to generate 5x2x3mm defects in the left and right mandible. PCL scaffolds were press fit into the LEFT and PCL/c scaffolds into the RIGHT defects. Animals were euthanized 6 weeks post-op, the jaw bones were harvested, and µCT analyses and histology for ALP and TRAP activities were performed.

RESULTS: Quantitative µCT showed significantly more bone in left (PCL) and right (PCLc) defects of control rats compared with BRONJ rats. There was significantly more bone in the left (PCL) mandible underlying PCL scaffolds than in the right (PCLc) mandibles underlying PCLc scaffolds, with no apparent difference between control and BRONJ rats. Quantitative analyses of ALP and TRAP showed reduced ALP and increased TRAP activity in BRONJ compared with control rats. Comparison of left (PCL) and right (PCLc) hemi-mandibles in BRONJ group showed little difference in ALP but significantly more TRAP in right mandibles adjacent to PCLc scaffolds.

CONCLUSION: The amount of regenerated bone (BV/TV) in control rats was only ~15% of that in the adjacent mandible and in BRONJ rats less than 2%. We doubt the yield would have increased by extending the healing time due to the presence of a prominent layer of connective tissue between the implant and mandibular bone. The presence of PCL scaffolds with interconnected pores of 150µm diameter was insufficient to induce robust repair of large mandibular defects in BRONJ rats. Future work will investigate the healing potential of PCL scaffolds with higher pore sizes.

ABSTRACT #7:

Systematic review and meta-analysis of collagen post-translational modification in osteogenesis imperfecta

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Background: Osteogenesis imperfecta (OI) is a rare connective tissue disorder characterized by bone brittleness and caused by a mutation in type 1 collagen encoding genes or collagen processing genes. The severity of OI can vary drastically. Type 1 collagen is composed of 2 Col1A1 chains and 1 Col1A2 chains that assembles into a triple helix. During the triple helix assembly, collagen undergoes multiple post-translational modification before being secreted. In OI, the delay in triple helix assembly results in a increase levels of post-translational modification. The structural difference in type 1 collagen between healthy and OI collagen differ beyond base change mutation but also due to increase levels of post-translational modifications. Studies relating OI genotype to phenotype severity were unable to find a strong correlation between them. We hypothesis that alterations in collagen post-translational modification is strongly correlated disease severity of OI.

Method: The objective of this systematic review was to systematically identify all studies measuring post-translational modification type I collagen in OI. In addition, patient information such OI type, age and sex will be used as covariates. Academic paper which quantitatively measured lysine hydroxylation, proline hydroxylation and hydroxylysine glycosylation levels are included. The academic database Medline and Embase were used and 362 unique papers were identified. After full text screening, papers were selected, from which the 40 papers screened to date.

Results: OI types II, III and IV accounts for 68% of the patient's population. The age of patients is not reported in 80% of the patient population and 88% patient's sex is not disclosed. The following collagen properties were reported: 36 described lysine hydroxylation, 28 described proline hydroxylation, 9 described hydroxyproline glycosylation. Thus, the data includes diverse patient population with regards to OI type. Gaps in knowledge in regard to patient age and sex reporting is identified.

ABSTRACT #8:

Functional role of fibronectin isoforms in chondrogenesis and skeletal development

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Aim: Fibronectin (FN) is a ubiquitous matrix glycoprotein essential for physiological development. We and others have previously identified heterozygous dominant mutations in the fibronectin gene *(FN1)* as a cause for corner fracture type spondylometaphyseal dysplasia (SMD) with short stature and growth plate defects. However, the functional importance of FN isoforms during physiological skeletal development is not clear and is hence the subject of the current study.

Methods and results: The physiological role of FN in skeletal development was analyzed by employing conditional knockout mouse models for **i)** cellular FN in cartilage (cFNKO), **ii)** plasma FN in hepatocytes (pFN KO), and **iii)** both FN isoforms (FNdKO). Bone samples harvested from these mice at several embryonic and postnatal developmental time points (E16.5 to 2 months) were analyzed through histological, immunohistochemical and computed tomography-based techniques. Deletion of FN isoforms in cartilage of the FNKOs was confirmed at embryonic day 16.5. Immunostaining revealed that pFN does not enter cartilage and that cFN is the major isoform present in the growth plate. Deletion of both FN isoforms in FNdKO mice resulted in significantly reduced body weight, reduced bone length (femur, tibia, humerus, radius and ulna) and whole-body length from early postnatal day 1 (P1) to the adult stage. Additionally, whole mount skeletal staining on P1 pups showed possible ossification defects in the vertebral bodies of the FNdKO. Assessment of the bone microarchitecture using micro-CT on 2-month-old mice revealed a significant reduction in trabecular and cortical bone volume, increase in overall bone porosity and reduction in bone mineral density of FNdKO. Both, cFNKO and pFNKO mice exhibited only some subtle changes in some of the analyzed bone parameters. Further, chondrogenic dysregulation was evaluated using two well established markers, collagen type II and type X, through indirect immunofluorescence and immunohistochemistry on tibia sections of mice at various time points. A gradual reduction in the collagen type II deposition and increase in collagen type X levels were observed in the growth plate of FNdKO. Additionally, the FNdKO also showed reduced trabecular bone mineralization at P1, consistent with the reduced total bone mass identified at adult stage through micro-CT.

Conclusion: The findings demonstrate that cFN is the major FN isoform in the epiphyseal growth plate during embryonic and postnatal skeletal development and that pFN does not enter cartilage even in the absence of cFN. However, the two isoforms are essential for trabecular bone formation, mineralization, and bone growth during skeletal development.

ABSTRACT #10:

This isn't my vesicle. How did I get here? Localization of V-ATPases and Rabs.

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V-ATPases are multi-protein pumps that generate a proton gradient across lipid bilayers which is used for optimizing biochemical reactions. V-ATPases can be grouped into isocomplexes based on the 'a' subunit that anchors the complex to the membrane providing the path for the protons. In mammals there are four 'a' paralogues (a1, a2, a3, a4), each thought to exhibit distinct subcellular localization. They are associated with vesicles inside the cell and can move. Small GTPases of the Rab family determine vesicle identity and regulate their movement, fusion, and fission. Recently it was shown that Rab7a interacts with the a3 subunit. This prompted us to re-examine subcellular localization of the V-ATPase in HeLa cells and to see how Rabs could possibly alter localization. To this end we tagged the different 'a' subunits and some candidate Rabs with fluorescent proteins to simultaneously visualize them inside cells. In the process of cloning the Rabs, we discovered expression of splice isoforms that have were predicted but not characterized. We found that the expression of the 'a'subunits is more overlapping than had been previously assumed and that Rabs do co-localize with the 'a' subunits. Some of the identified splice isoforms are predicted to act as dominant negative proteins and we are currently elucidating their functional significance.

ABSTRACT #12:

Transglutaminase 1 – a novel regulator of osteoclastogenesis

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Osteoporosis is a bone disease characterized by loss of bone mass (osteopenia) which results from imbalanced activity between bone-forming osteoblasts and bone-resorbing osteoclasts. Osteoclastogenesis involves two main stages, recruitment, and differentiation of monocyte-macrophage lineage cells into mononuclear pre-osteoclasts, followed by their fusion to multinucleated osteoclasts. We previously showed that pharmacological inhibition of transglutaminases – protein crosslinking enzymes – completely blocks osteoclast formation from bone marrow macrophages. We also demonstrated that during osteoclastogenesis three TG enzymes; TG1, TG2 and FXIII-A are active. *In vivo* and *in vitro* studies have demonstrated that the deletion of TG2 (*Tgm2*-/- mice) causes increased osteoclastogenesis. A double knockout of TG2 and FXII-A (*Tgm2*-/-;*F13a1*-/-) also has increased osteoclastogensis *in vivo* and *in vitro* which in cell cultures is reversed by a TG inhibitor. This suggests that TG1 may drive osteoclast differentiation. Here we confirm that the TG2 deficiency results in increased osteoclastogenesis *in vitro* and show that this increase can be reversed by a TG inhibitor suggesting that other TGs are responsible for driving the osteoclastogenesis in the absence of TG2. Assessment of total TG activity with 5-(biotinamido)-pentylamine as well as TG1 and FXIII-A activities in *Tgm2*-/- bone marrow flushes, bone marrow macrophages, and osteoclasts using TG-specific Hitomi Peptides (bK5 and bF11), showed an increase, specifically in TG1 activity. Aspartate proteases, such as cathepsins are involved in the degradation of organic bone matrix and can be produced by osteoclasts. Moreover, Cathepsin D was shown by previous work to be increased in TG2 null cells and is known to activate TG1 *in vitro*. We show that Pepstatin A, an aspartate protease inhibitor blocks osteoclastogenesis of wild-type and *Tgm2*-/- cells and decreases TG1 activity. Global TG1 deletion leads to postnatal lethality and thus the role of TG1 in bone is unknown. We investigated the effect of TG1 deletion on osteoclastogenesis via the generation of a conditional knockout of *Tgm1* in osteoclasts using $Tgm1^{flx/flx}$ and Cathepsin K-Cre mice. Our preliminary data from $Tgm1\text{-/-}^{CTSK}$ osteoclasts show significantly decreased osteoclast markers bringing robust novel evidence for the role of TG1 in osteoclast differentiation.

ABSTRACT #13:

Characterizing the effect of sub-clinical priming injuries on enhanced fracture healing

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BACKGROUND: Bone healing is essential for the success of various elective orthopedic surgical procedures. Failure of bone healing and its complications results in prolonged patient recovery, poor patient outcomes, and is a significant burden on the healthcare network. Bone is a regenerative tissue that relies on complex interdependent processes to restore and maintain its physiological and biomechanical properties. Bone healing is largely regulated by various complex interactions between the immune and skeletal systems. Modulating the innate immune system in favour of enhancing bone healing is an emerging field and can augment the current treatment modalities to reduce the burden of the disease. In this study, we aim to investigate, characterize, and optimize a priming strategy to accelerate bone healing in a murine model.

METHODS: Skeletally mature male C57BL/6 mice were randomly assigned to 6 experimental groups: control group receiving no priming stimulus (group 1), sham skin incision (group 2), sham skin + muscle incision (group 3), and groups 4, 5 and 6 each undergoing a 1mm diameter femoral window priming stimulus (RIGHT leg). A standardized 2mm x 1mm subcritical-sized bone defect was made on the contralateral (LEFT leg) 2 weeks later for groups 1-4, 6 weeks later for group 5, and 12 weeks later for group 6. Animals in all groups were euthanized 8 weeks after their left femoral bone defect, and both hindlimbs were harvested and scanned by μCT and analyzed for histology. Bone healing was quantified by analyzing the cortical thickness of the healing bone in the area of the defect, compared to the adjacent host bone (normalized thickness). A $P < 0.05$ was considered significant.

RESULTS: Complete healing of the bone defect only occurred consistently in groups 3 and 6. The other groups had varying degrees of bone healing with the mean normalized cortical thickness being significantly different between all the groups ($p<0.0005$). There was significantly greater bone formation in groups 3 (104%) and 6 (109%) compared to the control group (68%) ($P<0.0001$). There were no statistical differences in bone volume fraction or cortical thickness between groups 3 and 6.

CONCLUSION: In conclusion, our data suggest that a priming stimulus is effective in enhancing bone healing of a subsequent bone defect. Further studies are required to optimize and refine a priming technique that is minimally invasive.

ABSTRACT #16:

Validation of finite element analysis for bone remodeling simulation with ex vivo mechanical testing of bovine trabecular bone

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Introduction: The ability to accurately simulate bone remodelling would be an invaluable tool for comprehensive clinical analysis (pre- and post-treatment). The objective of this study was to develop validated finite element analysis (FEA) models required for bone remodelling simulation. Experimental data for validation was provided from *ex vivo* experiment testing of live trabecular bone cores under cyclic mechanical loading. [1]. Overall, this study demonstrates a promising approach to predicting bone remodelling based on *ex vivo* mechanical testing and structural topology optimization. This approach could have implications for designing orthopedic implants and treatments for bone diseases.

Methodology: Twenty-two trabecular bone cores were scanned and reconstructed with a voxel size of 20?m. Segmentation and 3D surface mesh refinement were completed before exporting to Abaqus/CAE (version 2017, Simulia, Johnston, RI, USA) for further mesh generation (quadratic tetrahedral with an edge length size of 0.2 mm). A loading of -3000 με was applied to the top nodes of the model at a loading rate of 3000 με/s when the bottom nodes were fixed in all directions. FEA Stiffness (K_{FEA}: N/mm) was calculated as a ratio of maximum reaction force to the maximum bone core deflection, and the FEA tissue elastic modulus was calculated based on the experimental stiffness (EXP) by linear scaling, as where K_{EXP} and K_{FEA} are the stiffnesses as determined from the experimental test and from the FEA, respectively; is the tissue modulus measured based on the tissue density ($?_{tissue}$: g/cm^3) and x-ray attenuation. The experimental and FEA stiffness and apparent elastic modulus values will be compared by calculating the percent difference between them.

Results: Day 0 apparent elastic modulus of each bone core was measured and applied to specimen specific FEA. Although the apparent elastic modulus of the load and no loadexperiment groups was not statistically different, there was a 7.3% increase in apparent elastic modulus in the load group at the end of the 21 day experiment.

Discussion: The combination of simulation and experimental testing can provide a powerful tool for understanding the mechanical properties of bone and developing effective treatments for bone diseases which this study highlights the importance of validating bone remodeling simulations with experimental data. So far, we measured and compared the initial and experimental elastic modulus values of bone cores. For the next steps, the FEA elastic modulus values will be predicted with a bone remodelling simulation and compared to the experimental measurements.

References: [1] B. A. Kunath, MSc thesis, 2022

Cartilage and Intervertebral Disc

ABSTRACT #18:
Can costal collagen fiber orientation explain the tendency of warp in struts prepared for nasal reconstruction?

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Introduction: In post-traumatic nasal reconstruction, costal cartilage is commonly used to re-create the central "L strut" support in the nose. However, the tendency of this tissue to warp when cut leads to a very limited supply of useful intraoperative graft tissue. It has been postulated that warping may be due to microstructural collagen fiber orientation. Orienting the direction of cartilage samples during graft preparation based on the arrangement of collagen fibers may help to balance cross-sectional forces and minimize warping. This research aims to quantify the relationship between collagen fiber orientation and warp by first characterizing the underlying structure of collagen fibers in human costal cartilage.

Methods: Diffusion Tensor Imaging (DTI) is an MRI technique that uses anisotropic diffusion to estimate the organizational structure of a tissue. DTI parameters were optimized to enable reconstruction of the three-dimensional network of collagen fibers in human costal cartilage (long rib and synchondrosis junction) within a 7 Tesla MRI scanner. To reveal the organization of the collagen fibers, specimens were treated with contrast agent (0.5% Prohance) and exposed to diffusion gradients 60 encoding directions ($b=1000$ s/mm^2) and 6 non-diffusion-weighted scans (b0) for 21 hours. Tractography was then used to visualize the collagen fiber network (DSI Studio Toolkit).

Result: A fiber orientation map of costal cartilage generated using tractography shows an arc shaped pattern of collagen fibers in the peripheral zones in the sagittal plane, a dominant fiber direction in the craniocaudal orientation in the midzone, and fibers running lengthwise along the rib cartilage. Release of the outer ring of collagen fibers when costal cartilage struts are cut may release tension in the tissue resulting in warp. Histochemical analysis can confirm collagen fiber orientation, and evaluate proteoglycan, and glycosaminoglycan distribution.

Conclusion: The elucidation of the relationship between anatomic morphology (fiber orientation) and biomechanical behaviour (warping) in cartilage may allow for optimized utilization of cartilage for nasal reconstruction. Guiding cuts based on structural knowledge could help to reduce operative time and the potential need for re-operation due to postoperative nasal deformity.

ABSTRACT #19:
Investigating the role of steroid hormones in the IVD

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Introduction: The most recent *Global Burden of Disease* study identified back pain as the most common cause of disability, with a socioeconomical impact estimated at $100 billion annually. Chronic back pain has a lifetime prevalence of over 80% in Canada. Though complex, back pain is associated with intervertebral disc (IVD) degeneration in 40% of cases. Despite its tremendous socioeconomic impact, the etiology of IVD degeneration is largely unknown and there are no disease-modifying treatments. The current research is based on clinical observations of increased IVD volume in competitive athletes and suspected anabolic steroid users. These findings are unexpected as increased IVD volume is not normally seen and may provide a therapeutic target for IVD degeneration. Moreover, anabolic steroid and growth hormone injections are being used clinically based on a reported decreased incidence and severity of patient self-reported back pain despite no biological investigations on their effects in the IVD. Given this, we hypothesize that exposure to steroid hormones will alter cell signaling within the IVD, resulting in increased cell proliferation and matrix synthesis, and decreased matrix degradation.

Methods: Nucleus pulposus (NP) and annulus fibrosus (AF) cells were isolated separately from bovine caudal IVDs and cultured in micromass under hypoxia (2% O_2). Cells were treated with increasing doses of 5α-dihydrotestosterone or 17β-estradiol (to 125 nM) for 72 h alone or following pre-treatment with TNFα (25 ng/mL) or IL-1β (10 ng/mL) (N=5). Cells were harvested for RT-PCR analysis of extracellular matrix genes and markers of degeneration.

Results: In our *in vitro* model system, acute exposure to neither 5α-dihydrotestosterone nor 17β-estradiol significantly altered the expression of extracellular matrix genes in NP or AF cells. To model the pro-inflammatory microenvironment of IVD degeneration, we pretreated cells with pro-inflammatory cytokines prior to steroid hormone treatment. IL-1β stimulation induced the expression of matrix degrading enzymes (MMP3, ADAMTS5) and pro-inflammatory cytokine (IL-6) in NP cells, while TNFα treatment induced a similar response in AF cells. In NP cells, exposure to either 5α-dihydrotestosterone or 17β-estradiol attenuated the pro-inflammatory response induced by IL-1β. In AF cells, exposure to 5α-dihydrotestosterone attenuated the pro-inflammatory response induced by TNFα, an effect that was not seen following 17β-estradiol treatment.

Discussion: Further exploration on the effects of steroid hormone exposure on IVD cell types in vivo, as well as the combined effects of ECM composition and mechanical loading may prove useful in the context of a novel therapeutic for IVD degeneration.

ABSTRACT #20:
Crosstalk between cartilage and bone in adolescent idiopathic scoliosis

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Facet joint osteoarthritis (OA) is prevalent in young patients with adolescent idiopathic scoliosis (AIS) and might contribute to the disease progression and perceived pain. Toll-like receptors (TLR) activation has been linked to cartilaginous tissue degeneration and the production of pro-inflammatory factors. We previously found a negative correlation between osteoarthritic severity and subchondral bone density in facet joints from AIS patients, similar to changes seen in adult OA patients. Studies have shown that subchondral bone undergoes active remodeling as it attempts to adapt to the loss of cartilage and maintain joint stability. However, the balance of bone turnover and bone synthesis is perturbed by inflammatory mediators resulting in bone resorption in the early phase of OA while leading to subchondral plate densification and formation of osteophytes in the late phase. Based on our previous findings, we hypothesized that Toll-like receptor (TLR) activation in facet joint chondrocytes is an important regulator of pro-inflammatory and osteoclastogenic factors leading to bone loss and cartilage degeneration. To investigate these pathological mechanisms, we analyzed the transcriptome profile of both isolated chondrocytes (CC) and osteoblasts (OB) from AIS and non-scoliotic facet joints. A total of 2855 DEGs of CC (1065 downregulated and 1790 upregulated DEGs) and 1358 DEGs of OB (798 downregulated and 560 upregulated DEGs) were identified between AIS and control samples (n=3). These upregulated CC DEGs were primarily enriched in Toll-like receptor signaling, cytokine-mediated signaling pathways, and calcium signaling pathways (GO and KEGG). Moreover, several GO terms related to bone remodeling were enriched significantly including osteoblast proliferation, osteoclast proliferation, ossification, trabecular formation, and rheumatoid arthritis (KEGG). The protein-protein interaction system of CC-upregulated DEGs also showed that TLRs were the key proteins in the first-ranked cluster, suggesting TLR activation could be the key biological event in the crosstalk between cartilage and bone. We further demonstrated that conditioned media from TLR-activated chondrocytes inhibit osteoblast mineralization while promoting osteoclast precursor cell proliferation *in vitro*. In summary, our data suggeste that scoliotic chondrocytes disrupt the homeostatic balance of bone resorption and synthesis through TLR activation. TLR inhibitors could have dual therapeutic outcomes including restoring cartilage health and preventing bone loss. A healthier facet joint can stabilize the spine, therefore potentially slowing down curve progression, and decreasing or avoiding the patient's need for surgery.

ABSTRACT #21:
Senolytic combination treatment for painful degenerating intervertebral discs.

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Introduction: Low back pain is a global health problem that is directly related to intervertebral disc (IVD) degeneration. Senolytic drugs (RG-7112 & o-Vanillin) target and remove senescent cells from IVDs in vitro improving tissue homeostasis. The objective of this study was to determine if combining the two senolytic drugs could more efficiently remove senescent cells, reduce inflammatory mediators, and relieve pain in cells from degenerating human IVDs, than either drug alone. Next-Generation Sequencing was also performed to reveal the pathways mediating the senolytic effect of single and combination treatments.

Methods: Pellet cultures of cells from painful degenerate IVDs were exposed to TLR-2/6 agonist Pam2CSK4 to further induce senescence. They were then treated with the senolytics o-Vanillin and RG7112 alone or combined. $p16^{INK4a}$, Ki-67, caspase-3, inflammatory mediators, and neuronal sprouting were assessed. RNA was extracted, and sequencing was performed using Next-Generation Sequencer NovaSeq 6000 PE100. Bioinformatics, including quality control, read alignment and transcript assembly, expression quantification, and differential expression analysis was used to analyze RNA-seq data.

Results & Conclusion: The senolytic combination significantly reduced senescent IVD cells, pro-inflammatory cytokines, neurotrophic factors, and neuronal sprouting in PC-12 cells when compared to the single treatments. Moreover, RNA-seq data analysis identified DEGs both in single and combination treatments and suggested potential pathways that mediate the effect of senolytics. Taken together, these results support the potential of senolytics as a promising treatment for IVD-related low back pain.

ABSTRACT #23:
Impact of SUMOylation on biomolecular modification of PHB1 in primary osteoarthritis

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Purpose: This study aimed to identify the impact of SUMOylation pathway mechanism, by underlying the nuclear trapping of mitochondrial protein; PHB1, in primary osteoarthritis (OA).

Methods: The presence of a SUMO interacting motif (SIM) in PHB1 proteins led us to examine the role of SUMOylation in primary OA. The subcellular localization of PHB1 and SUMO proteins in human knee joints was carried out by immunofluorescence staining and confocal microscopy with normal and OA articular chondrocytes. Several PHB1 constructs devoid of SIM, and/or a nuclear localization signal (NLS) were generated to examine the subcellular localization of PHB1 and its physical interaction with SUMO proteins. We evaluated the contribution of UBC9; the sole E2 SUMO ligase, by using immunohistochemical assay to identify the source of increased SUMOylation in primary OA. Moreover, X-ray imaging of the knee joints of transgenic *Ube2i* mice; overexpressing constitutively UBC9, were performed at different intervals (from 5 weeks up to 40 weeks).

Results: SUMO1 proteins were increased and strongly co-localized with PHB1 in the nuclei of OA chondrocytes, whereas in control cells, SUMO1 was predominantly in the cytosol. Of note, high UBC9 protein levels were detected in the knee joints of OA surgical cases. Transfection assays with different PHB1 constructs showed that overexpression of UBC9 alone is sufficient to induce the nuclear trapping of PHB1 when the SIM is present. Analysis of the x-ray examination of transgenic *Ube2i* mice showed formation of osteophytes and narrowing joint space with deformity in the joint bone surface.

Conclusion: These findings shed a new light into research fields such as cell senescence and aging, where SUMOylation pathway may aid in detecting OA at a nascent stage, which may help to identify novel therapeutic targets.

ABSTRACT #25:
Inflammation-inducible strategies for growth factor gene therapy to promote joint repair in a preclinical model of post-traumatic osteoarthritis

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Rationale: Osteoarthritis (OA) is a degenerative disease of synovial joints characterized by loss of articular cartilage, subchondral bone remodeling, and intra-articular inflammation with synovitis that leads to chronic pain. Despite its economic and health impacts, there are no disease-modifying therapies that alter OA progression. Many growth factors essential for joint development and homeostasis, including transforming growth factor beta (TGF-b) and insulin-like growth factor 1 (IGF-1), are dysregulated in OA; however, constitutive overexpression of any growth factor as a therapeutic can inadvertently cause joint destruction through effects on other joint tissues other than cartilage. Thus, an inducible method of growth factor expression is required to promote joint repair while avoiding deleterious outcomes in other joint tissues.

Hypothesis: The use of inflammation-inducible promoters to drive growth factor expression in OA joints is required to promote joint tissue repair.

Experimental Approach: We are cloning *Igf1* or *Tgfb1* open reading frames (ORFs) into helper-dependent adenovirus (HDV) plasmids downstream of either the inflammation-responsive ELAM-1 proximal promoter (HDV-ELAM1-*Igf1*, HDV-ELAM1-*Tgfb1*), which contains nuclear factor kappa B (NFkB) binding sites, or the constitutive elongation factor 1 (EF1) promoter (HDV-EF1-*Igf1*, HDV-EF1-*Tgfb1*). These vectors will be tested *in vitro* using cultures of primary chondrocytes treated with synovial fluid from human OA patients to monitor transgene expression. Next, the destabilization of the medial meniscus (DMM) surgical model of post-traumatic OA will be used to evaluate the effectiveness of these vectors. HDV vectors will be injected intra-articularly into joints of skeletally mature mice post-DMM. Sham-operated mice will serve as surgical controls (no injection). Mice will be evaluated post-DMM using: 1) behavioural assays; 2) histological staining and scoring; 3) phase-contrast mCT; and 4) RNA-seq followed by qRT-PCR and immunohistochemistry.

Significance: This inflammation-inducible strategy aims to mitigate adverse outcomes associated with continuous growth factor delivery in the OA knee joint and establish an effective gene therapy strategy capable of promoting tissue repair during active disease in OA joints.

ABSTRACT #26:
A bioinformatics approach to understanding the pathogenesis of ectopic spine calcification

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INTRODUCTION: In North America, 15%-25% of people over 50 suffer from diffuse idiopathic skeletal hyperostosis (DISH). DISH is a non-inflammatory spondyloarthropathy associated with mineralization of connective tissues along the anterolateral aspect of the spine. Diagnosis is based on the radiographic detection of ectopic spine calcification satisfying criteria developed in 1976. Unlike many forms of arthritis, DISH has a higher prevalence among males than females (~2:1 ratio). Despite the high prevalence of DISH, the etiology remains unknown and there are no disease-modifying treatments. Previous research in the Séguin lab showed that mice lacking expression of the equilibrative nucleoside transporter 1 ($ENT1^{-/-}$) display progressive mineralization of fibrocartilagenous tissues including the annulus fibrosus (AF) of the intervertebral disc (IVD), mimicking DISH in humans. This preclinical model provides a valuable tool to understand cellular changes and pathways associated with DISH pathogenesis.

HYPOTHESIS: Ectopic spine calcification in the $ENT1^{-/-}$ mouse is driven by dysregulation of pathways associated with fatty acid metabolism and oxidative stress, changes consistent with DISH in humans.

METHODS & RESULTS:

AIM 1- Unbiased proteomics and metabolomics analyses were previously performed on AF tissues isolated from wild-type (WT) and $ENT1^{-/-}$ mice at 2 months-of-age (onset of mineralization) and 6 months-of-age (established ectopic mineral). Metabolomics was also conducted on serum from mice at both ages. Integrated bioinformatic analysis is being used to identify differentially regulated proteins, metabolites, and cellular pathways associated with DISH pathogenesis. Our analysis to date identified a number of upregulated proteins associated with AF mineralization; immunohistochemistry is being used to localize targets of interest, including S100A8, S100A9, and caspases in regions of mineralization. Western blot will be used to verify signaling pathway activation, for example PI3K/AKT.

AIM 2- Metabolomic analysis of serum samples from DISH patients will investigate small molecule metabolites associated with DISH pathogenesis as potential biomarkers of disease. Working with clinical collaborators, serum samples from patients with DISH and age-matched controls (n=20/group) are being collected. Liquid chromatography-tandem mass spectrometry will be used to conduct unbiased metabolomic analysis, enabling both assessment of metabolites identified in our preclinical mouse model (i.e. LysoPCs) and the detection of novel metabolites associated with disease in humans. Based on data from the $ENT1^{-/-}$ mice, we anticipate this will highlight changes in fatty acid metabolism in DISH.

SIGNIFICANCE: This study aims to identify molecular changes correlated with spine mineralization in DISH and to translate these findings to develop clinical interventions to prevent or delay disease pathogenesis.

ABSTRACT #28:

Anti-inflammatory gene therapy strategies to promote joint repair in a preclinical model of post-traumatic osteoarthritis

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Background: Osteoarthritis (OA) is a multifactorial disease characterized by progressive cartilage and bone damage with intra-articular inflammation and synovitis. Cytokines such as interleukin-1b (IL-1b) and tumor necrosis factor-a (TNF-a) are particularly abundant in the synovial lining of the joint, and contribute to the pro-inflammatory environment within OA joints that contributes to joint destruction and chronic pain. Unfortunately, small molecule or recombinant protein therapies targeting these factors are limited by short half-lives in vivo. In this regard, gene therapy strategies utilizing viral vectors hold promise for OA treatment as sustained expression of target genes can be achieved following a single intra-articular injection.

Previous work from Dr. Grol and colleagues has shown that delivery of a helper-dependent adenovirus (HDV) expressing IL-1 receptor antagonist (IL-1Ra) can slow OA progression and improve pain outcomes in preclinical surgical models of OA; however, inflammation was not entirely abrogated, and efficacy was eventually lost. This suggests that a broader suppression of inflammation may be needed.

Hypothesis: Our hypothesis is that a combinatorial gene therapy approach targeting both IL-1b and TNF-a will more effectively slow OA progression, block inflammation, and prevent disease-induced pain compared to blocking either pathway alone.

Experimental Approach: IL-1Ra is an established endogenous inhibitor of the IL-1b pathway, whereas soluble TNF receptor 2 (sTNFR2) effectively blocks TNF-mediated signaling. We are producing a combinatorial anti-inflammatory vector by cloning *Il1ra* and *sTnfr2* cDNAs into an HDV plasmid downstream of the inflammation-responsive ELAM-1 proximal promoter (HDV-ELAM1-*Il1ra*-IRES-*sTnfr2*), which contains nuclear factor kappa B (NFkB) binding sites. In parallel, we are producing monotherapy vectors to serve as controls (HDV-ELAM1-*Il1ra*, HDV-ELAM1-*sTnfr2*). Expression of our transgenes in response to inflammation will be tested in vitro using transduced culture of human chondrocytes or synoviocytes treated with human OA synovial fluid. To test therapeutic efficacy, we will use the destabilization of the medial meniscus (DMM) surgical model of post-traumatic OA. Sham or DMM surgery will be performed, and, at 2- and 4-weeks post-DMM, the constructed HDV vectors and empty control vectors (HDV-Empty) will be injected intra-articularly. Mice will be evaluated at 8-, 14- and 20-weeks post-DMM using behavioral assays for pain and motor deficits, histological staining and scoring for joint tissue pathology and destruction, phase-contrast μCT imaging to quantify 3-D cartilage and bone structure, and RNA-seq followed by qRT-PCR and immunohistochemistry for molecular profiling of target pathways.

Significance: This work will provide insights into the role of inflammation in OA pathogenesis.

ABSTRACT #30:
Link N regulates inflammasome activity in the intervertebral disc through interaction with CD14

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Introduction: Intervertebral disc degeneration disease (IVDD) is a leading cause of chronic back pain. IL-1β, a key proinflammatory cytokine, is implicated in the progression of disc degeneration and pain. The NLRP3 inflammasome is an intracellular complex that activates caspase-1 to generate cytokines such as IL-1β. Toll-like receptors (TLRs) and their co-factors such as MD2 and CD14, important in ligand recognition, are principal activators of the inflammasome. Various ligands can activate TLRs and include DAMPs, PAMPs, Alarmins, LPS, etc. Recently studies have shown the involvement of the NLRP3 inflammasome in IVDD. Link-N (LN,16 aa) and its active portion(sLN,8 aa) have been shown both *in vitro* and *in vivo* to regulate markers of inflammation and pain markers in IVD models. However, whether LN/sLN can directly regulate inflammasome activity remains unknown.

METHODS: ***In vitro*– Western blot**: Isolated human NP cells (hNPs) were cultured as pellets or in 6-well plates and treated with Prime Growth® Disc Cell medium and incubated with LPS [1µg/ml] with and without LN. Lysates and Pellets were collected individually and processed for Western blotting to identify changes in Caspase-1, IL-1β, and P-NFκB. ***In vivo*** **– Histology:** IVD degeneration was induced in 12 skeletally mature New Zealand White rabbits, at the L2/3 and L4/5 into the NP. Two weeks post nucleotomy, the punctured discs were injected into the NP area with either saline or LN [25 µg] and were euthanatized at 12 weeks post-injection and their discs were excised for histological evaluation. IVD sections were incubated with antibodies targeting PGP9.5, and NLRP3. **Peptide Docking:** Peptide docking CD14 (crystal structure, 1wwl) was determined using the CABS-dock web server. Models were created using PyMOL (Schrodinger, LLC).

RESULTS: Western Blot demonstrated that LN/sLN inhibited LPS-induced NFκB activation in a dose- and time-dependent manner($n=4$, $p<0.01$). After 48 hrs. incubation, LPS-induced activation of active caspase-1 we observed significant dose-dependent decreases following co-incubation with LN/sLN($n=4$, $p<0.01$). Decreases in caspase-1 activation with LN/sLN were accompanied by reduced IL-1β maturation and secretion($n=4$, $p<0.01$). Rabbit IVDs demonstrated decreases in markers of pain and inflammasome activity when compared to saline-treated controls($n=3$). *In silico* modeling suggested that LN can interact with CD14 in the LPS-binding pocket.

CONCLUSIONS: These results may indicate that LN/sLN can regulate inflammasome activation in IVDD.

ABSTRACT #31:
Synovial macrophage activation mediates pain experiences in experimental knee osteoarthritis

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Synovial macrophages are thought to play a role in mediating joint pain in knee osteoarthritis (OA), but the underlying mechanisms are unknown. Our objectives were to test whether synovial macrophages mediate knee OA pain experiences and investigate the role of macrophage activation via STAT signaling. Male Sprague Dawley rats underwent knee destabilization surgery to induce experimental OA. RNA sequencing of sorted synovial macrophages was used to identify OA associated pathways. Liposomal clodronate or inhibitors of STAT1 or STAT6 were selectively delivered to synovial macrophages in a separate cohort of experimental OA animals. Evoked pain behaviors were assessed using pressure application measurement and electronic von Frey. Knee joint histopathology was performed to assess synovitis and cartilage damage. RNA sequencing identified a large role played by STAT signalling in synovial macrophages during experimental OA development. Macrophage depletion and STAT6 inhibition led to a marked improvement in pain behaviors compared to vehicle control at multiple timepoints. Synovial macrophage depletion reduced signs of synovial inflammation but led to increased fibrosis and vascularization, whereas STAT1 or STAT6 inhibition did not. Synovial macrophages play a key role in mediating pain experiences in joint destabilization-induced experimental knee OA. Selective drug targeting to synovial macrophages with STAT6 inhibitors may be a strategic approach to treatment of OA-related pain without accelerating joint damage.

ABSTRACT #33:
Role of irisin in promoting resistance in chondrocytes to pro-inflammatory cytokine-mediated damage

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Tissue engineering is an attractive approach for the regeneration and repair of damaged articular cartilage. For this to be a viable therapy, engineered cartilage must remain stable and be resistant to the pro-inflammatory cytokines present during early OA and which inhibit cartilage formation and promote degradation. While we have previously determined that myokines (muscle-derived biochemicals) can promote resistance to inflammation-induced degradation in engineered cartilage, it is unknown which myokines are involved in this mechanism. It has been recently reported that myokine irisin may protect cartilage from further damage during OA disease[1]. Here, we present a pilot study to assess the interaction of irisin with engineered cartilage in the presence of pro-inflammatory cytokine IL-1β; IL-1β is known to induce cartilage matrix damage during early OA.

Cartilage-like tissue pellets were engineered from primary bovine articular chondrocytes and cultured in chondrogenic media for 20 days, with treatment of IL-1β and/or irisin after Day 12. Pellets were digested with papain and sulfated glycosaminoglycan (GAG) content was quantified using the dimethylmethylene blue assay. DNA content was quantified via the QuantiFluor® assay kit (Promega) and used to normalize GAG content for further analysis.

While no differences were observed in GAG/DNA ratio between the samples cultured in the presence of irisin alone, there was a significant increase in DNA content as compared to the control. Furthermore, engineered tissues treated with both IL-1β and irisin yielded significantly higher levels of GAG and GAG/DNA ratio as compared to those treated with IL-1β only.

Our preliminary results suggest the possible role of irisin in promoting the stability of engineered cartilage in the presence of IL-1β through the increased production of GAG, a major cartilage matrix protein. These initial findings are aligned with a recent study that suggests recovery from OA-induced damage in human osteoarthritic chondrocytes in 3D culture[2]. Further studies are warranted to assess the role of irisin on relevant chondrogenic and matrix degradation markers through mRNA expression, protein quantification, and histology. Additionally, the observed significant increase in DNA content for pellets cultured in the presence of irisin as compared to controls suggests the possible role of irisin in promoting cell viability, increasing cell proliferation, or inhibiting cell apoptosis, and justifies further investigation.

1. Roggio *et al*, *Int. J. Mol. Sci.* **24**, 5126 (2023).
2. Vadalà *et al. Cells* **9**, 1478 (2020).

ABSTRACT #36:
Senescence and inner annulus fibrosus cells

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Intervertebral disc (IVD) degeneration is an irreversible process associated with accumulation of senescent nucleus pulposus (NP) cells. However, the role of senescence in annulus fibrosis (AF) degeneration is still not well understood. The inflammatory cytokine (TNFα) and reactive oxygen species (ROS) are crucial mediators of nucleus pulposus (NP) degeneration via activation of senescence pathways. TNFα is also known to induce the senescence associated secretory phenotype (SASP) in NP cells which results in secretion of cytokines, ROS and other factors. This current study describes the role of TNFα, H_2O_2, and NP SASP secretome in mediating inner AF (iAF) cell senescence.

NP and iAF cells were isolated from bovine caudal IVDs and seeded in monolayer culture. All experiments were initiated using passage 1 cells, as we have shown previously these cells still retain their phenotype. Senescence was evaluated by quantifying β-galactosidase staining (SA-β-Gal) and p16 and p21 expression by immunostaining. Gene expression was semi-quantified by qPCR. Protein expression of matrix macromolecules and proliferation (ki67) was evaluated by immunostaining.

iAF cells did not undergo senescence when treated with TNFα (40 ng/mL) at a concentration that induces senescence in bovine NP cell as indicated by lack of expression of p16 and p21. Furthermore, NP cells continued to proliferate as demonstrated by Ki67 staining. TNFα treated NP cells accumulated intracellular ROS and secreted more H_2O_2 as compared to iAF. Upon TNFα exposure, iAF cells showed higher gene expression of the superoxide scavengers *SOD1* and *SOD2*, and lower levels of *NOX4* gene expression, an enzyme that can generate H_2O_2, than NP cells. iAF cells treated with low dose H_2O_2 (50 µM) caused senescence, however unlike TNFα, H_2O_2 did not induce degenerative-like changes as there was no difference in *COL2*, *ACAN*, *MMP13*, or *IL6* gene expression or number of COL2 and aggrecan immunopositive cells compared to untreated controls. These results suggest that iAF cells have distinct degenerative and senescent phenotypes compared to NP cells. To evaluate the effect of SASP secretome on iAF cells, TNFα-treated NP cells were co-cultured with iAF. In contact co-culture the TNFα-treated NP cells did induce senescence in iAF, suggesting that senescent NP cells may be propagating senescence in healthy iAF cells via a paracrine effect. This may contribute to a positive feedback loop of disc degeneration. Further studies are required to explore the signaling mechanisms of H_2O_2 and TNFα in iAF cells in senescence.

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ABSTRACT #68:

Biomechanical properties of bone and cartilage during prolonged storage with the Missouri Osteochondral Allograft Preservation System

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Background: Osteochondral allograft transplantation is a surgical approach for treating cartilage lesions which involves replacing damaged cartilage with grafts composed of bone and cartilage obtained from a cadaveric donor. The procedure is limited by the availability of viable grafts. The Missouri Osteochondral Allograft Preservation System (MOPS) is a proprietary serum-free media reported to maintain viability for 56 days, however, there is limited data on the effects of MOPS on cartilage and bone biomechanical properties. This study aims to quantify biomechanical properties of cartilage and subchondral bone in ovine femoral condyles stored in MOPS.

Methods: Femoral condyles from 15 sheep (4-6 years old, 70 ± 15 kg) were preserved in either MOPS media or a Lactated Ringer's Solution-based media (SOC) and assigned to different storage times of 0, 14, 28 or 56 days for right lateral, right medial, left medial and left lateral condyles, respectively. All samples underwent Micro-CT scanning at 14.2 µm (Skyscan 1174) to quantify subchondral bone density and subchondral bone thickness. Afterwards, cartilage thickness was measured using a 26-gauge needle probe and Young's modulus determined by indenting cartilage with a 1 mm diameter spherical indenter to 10% at a rate of 0.2 mm/s (Mach-1 Micromechanical Tester). Statistical comparisons were made using a two-way ANOVA with treatment and storage duration as independent variables followed by LSD post-hoc tests (Statistica v.14). Results are reported as average ± standard deviation.

Results: No statistically significant differences were detected between MOPS and SOC stored samples at any given storage time. The subchondral bone density in samples stored for 28 days in MOPS was 0.77 ± 0.04 g/cm3 and SOC was 0.76 ± 0.02 g/cm3 which are both lower than 14 day MOPS-stored samples at 0.88 ± 0.06 g/cm3. Furthermore, SOC samples stored for 28 days had a significantly lower Young's modulus of 2.27 ± 0.51 MPa compared to 14 day MOPS samples at 4.29 ± 1.31 MPa. Comparisons were made only among medial or lateral condyles because these joint surfaces have intrinsically different biomechanical and physical properties. Values of $p < 0.05$ were considered as statistically significant differences.

Conclusion: MOPS samples exhibited moduli that were not statistically different compared to SOC at any given storage time and supports the positive outcomes of MOPS reported in previous literature. These data contribute to elucidating the effects of MOPS on cartilage and bone and supports efforts to increase the availability of grafts for clinical use.

ABSTRACT #86:
Ex vivo mechanical over-loading of articular cartilage leads to extracellular matrix alterations in osteochondral cartilage plugs

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[1]Biomomentum Inc.

Introduction: Articular cartilage lesions can occur after traumatic over-loading of joints and results in osteoarthritis (OA) long-term. This study aims to develop an ex vivo OA model allowing the screening of therapeutics. Characterization of this model will include mechanical, biochemical, and biological assessments of osteochondral cartilage plugs following a mechanical injury.

Methods: Equine femoral condyles from a slaughterhouse were used to harvest 20 osteochondral cartilage plugs (d=4.8mm) randomly assigned to either Control or Injured groups. Plugs were fixed in a custom-made holder to compartmentalise the bone and cartilage restricting crosstalk between them. Plugs were cultured up to 12 days in DMEM/F-12 Ham/0.1% BSA/13nM Dexamethasone (changed every 3 days) and incubated at 37°C/5%CO_2.

After 2 days, mechanical injury was inflicted to the Injured group via 50% compressive deformation at 100%/s using a mechanical tester (Biomomentum). Effect of injury on the mechanical properties of cartilage was assessed at day-6 (n=8) and day-12 (n=12) with unconfined compression (UC) followed by rotational friction with UC testing and coefficient of friction (COF) was defined at peak loading. Cell viability was evaluated at these timepoints as well in the surface and transition regions using Live/Dead imaging kit (Invitrogen). In addition, GAG release into the culture media was measured at every media change and at timepoints using a colorimetric DMMB assay. All collected data was submitted to an ANOVA Post-Hoc test for statistics.

Results: Fissures and 50% more dead cells (p=0.0008) were observed at the cartilage surface in samples submitted to mechanical injury. Injured plugs had an increased hydraulic permeability of 0.002 mm^2/(MPa.s) compared to 0.0007 mm^2/(MPa.s) for Control plugs at day-6 (p=0.010) and remained at 0.002 mm^2/(MPa.s) at day-12. In counterpart, the COF was similar to Control plugs at day-6 and increased by 2.4-fold at day-12 (p=0.0018). Maximum GAG release occurred during the first 3 days following the injury and was 2.3-fold higher than Control plugs (p=0.025). From day 9, it stabilized at 99.7 µg/sample to the same level as Control plugs.

Conclusion: Changes in mechanical, biochemical, and biological properties of cartilage at early timepoints suggest extracellular matrix (ECM) structural alterations due to the mechanical injury. Despite an increase in COF at day-12, a 6-day timepoint would be sufficient to mimic early signs of osteoarthritis. However, a complete validation of this model would require more data on other molecules of the ECM, such as collagen, before using it for therapeutics screening.

ABSTRACT #88:

Evaluating a novel storage protocol for preservation of fresh osteochondral allografts

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Background and Objectives: Osteochondral allograft transplantation is a surgical technique used to treat large focal cartilage lesions that involves resection and replacement of degraded cartilage with grafts harvested from a cadaveric donor. Successful transplantation is dependent upon maintaining a minimum 70% chondrocyte viability within the donor tissue compared to fresh control. This pilot study aims to 1) formulate a novel storage media for storing osteochondral allografts by identifying additives which mimic certain aspects of the native joint environment and extend chondrocyte viability beyond 14 days, and 2) characterize the effects of the proposed formulation on cartilage extracellular matrix components and bone quality. We hypothesize that chondrocyte viability will be extended by storing donor tissues in a supplemented DMEM-based media containing specific components that mimic the native environment of cartilage more effectively than the current standard used by Mount Sinai Allograft Technologies.

Methods: The effectiveness of cartilage preservation in DMEM cell culture medium containing glucose/L-glutamine/sodium pyruvate supplemented with hyaluronic acid and doxycycline was investigated using a rabbit model. Mature rabbit cartilage was stored at 4°C in both supplemented and standard Lactated Ringer's-based storage media. Analyses were done on two individual samples per timepoint (day 0, 7, 21 and 28), per storage condition. Confocal microscopy was used to analyze chondrocyte cells stained with Calcein AM and Ethidium Homodimer-1 for live/dead quantification. A custom-built program (MATLAB R2022a) was used to quantify live/dead cells stained with Calcein AM/Ethidium Homodimer-1, respectively. Effects on the cartilage extracellular matrix and bone quality will also be characterized using biochemical assays for collagen and glycosaminoglycan content, apoptotic cell content, biomechanical testing, and histological staining.

Results: Normalized to fresh control, cell viability was maintained well above 70% in cartilage stored up to 28 days in supplemented DMEM. When directly comparing viability numbers in both storage methods, absolute cell viability data displayed 6% higher viability after 21 days in supplemented DMEM storage and a 25% increase after 28 days versus LRS. Normalized to fresh control, cell viability was maintained above 95% in supplemented DMEM storage for the entirety of the 28-day period, whereas viability in LRS stored samples decreased to 67% by day 28.

Conclusion: The current standard used at Mount Sinai Allograft Technologies only allows tissue to be transplanted after a maximum of 14 days. Cartilage stored in DMEM media supplemented with hyaluronic acid and doxycycline exhibited superior viability after 28 days of storage when compared to the institutional standard.

Cell Biology of Connective Tissues

ABSTRACT #38:

Inflammation promotes release of vimentin from gingival fibroblasts in an Annexin A2-dependent manner

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Periodontitis is a high-prevalence, chronic inflammatory disease characterized by episodic tissue destruction, but the determinants of disease activity are not defined. In health, vimentin (Vim) is an exclusively intracellular intermediate filament protein that helps to maintain the structural integrity of periodontal connective tissue cells. But in periodontitis, Vim is released from gingival connective tissue cells into the extracellular space, amplifying the destructive effects of pro-inflammatory cytokines like IL-1β. The concentration of extracellular vimentin (ECV) is increased dramatically in gingival crevicular fluid in periodontitis, but it is not understood how inflammation promotes the release of ECV. We considered that inflammation-induced cell injury modeled by exposure of human gingival fibroblasts to serum or IL-1β perturbs the function of the plasma membrane-associated, phospholipid-binding protein Annexin A2 (AnxA2), which promotes ECV release via the non-classical protein secretory pathway.

Human gingival fibroblasts (hGF) were cultured with and without 10% fetal bovine serum or IL-1β (10 ng/ml). For estimating Vim release, the culture medium was collected and immunoblotted for Vim. The role of AnxA2 in Vim secretion was assessed by immunostaining and colocalization of AnxA2 in hGFs, siRNA depletion of AnxA2, and non-classical secretory pathway inhibition by neomycin (10 mM). Treatment of cultured hGF with the IL-1β or with 10% FBS compared with serum-free medium enhanced ECV release by 90% ($p=0.044$) and 84% ($p=0.033$), respectively. Treatment of cells with IL-1β or with FBS increased Vim and AnxA2 colocalization at the plasma membrane by 83% ($p<0.001$) as measured in non-permeabilized cells. Depletion of AnxA2 expression or neomycin-dependent inhibition of non-classical secretory pathway reduced ECV by ~85% ($p<0.001$). Vim export is stimulated by cell injury modeled by treatment with serum or a pro-inflammatory cytokine and involves an AnxA2-dependent secretory pathway. These data indicate that the release of Vim from fibroblasts in response to inflammation may amplify pro-inflammatory signaling.

ABSTRACT #40:

Single nucleus RNA sequencing identifies distinct synovial fibroblast subsets with unique transcriptomic profiles in early and late-stage radiographic knee OA

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Background/rationale: Osteoarthritis (OA) is a heterogeneous joint disease of unknown etiology. Synovium lines the joint capsule and is emerging as a contributor to joint destruction during OA. The synovium undergoes substantial changes during knee (K)OA pathogenesis including inflammation, hyperplasia and cellular proliferation; however, there remains a limited understanding of its contributions to OA during early and advanced stages of the disease. Thus, we sought to identify if distinct cell subtypes and their transcriptomic profiles exist in the synovium of early (KL1) versus late stages (KL3/4) of radiographic KOA.

Methods: Synovia from early- (KL=1; n=5) and late-stage (KL=3/4; n=4) radiographic KOA patients were subjected to single nucleus (sn)RNAseq and bioinformatics analyses. Canonical cell-specific markers were used to identify cell types from clustering analysis with prominent cell types re-clustered. Differentially expressed genes between subclusters within a cell type were determined based on top gene expression between early and late OA synovium, with some validated by immunohistochemistry. Pathway and gene ontology enrichment analyses were performed on fibroblast subclusters to identify prominent pathways, with putative upstream transcriptional regulators also identified. In vivo and in vitro methods are being used to assess transcription factor contributions to OA in fibroblast cultures and a mouse model.

Results: Fibroblasts constituted 50% of cells from early and late-stage KOA synovium. Clustering analysis identified 8 distinct fibroblast subclusters in the KOA synovium. Interestingly, we observed a phenotypic shift in fibroblast subsets from early to late stages of KOA (fibroblast clusters 1, 2, 4 and 6 were predominantly associated with early-stage while fibroblast clusters 0, 3 and 5 were predominantly associated with late-stage OA). Each of the fibroblast subsets exhibited unique transcriptomic profiles, some being cell surface markers confirmed to be differentially expressed in the synovium of early or late-stage KOA in vivo. Furthermore, pathway analyses suggest that the two major fibroblast subclusters, clusters 1 (early) and cluster 0 (late), may play crucial roles in ECM/fibrosis related pathways. Computational analysis has also identified putative upstream transcriptional regulators that may play key roles in ECM regulation and fibrosis. Current efforts are focused on targeting select transcription factor(s) using in vitro and in vivo gain and loss of function studies to identify their role in OA synovial pathology.

Conclusions: SnRNAseq analysis has led us to identify distinct synovial fibroblast subsets with unique transcriptomic profiles in early and late-stages of KOA that may play a key role in driving OA synovial pathology.

ABSTRACT #41:

Analysis of nuclear scaling properties between multinucleated osteoclasts and mononuclear pre-osteoclasts

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Allometry is the study of biological scaling: how different parts of an organism change during growth. In cells, researchers studied the scaling behavior of organelles in embryos and mitotic cells. However, the subcellular scaling properties of post-mitotic syncytia remains understudied. Osteoclasts (OCs) are the only syncytia that functionally degrade the calcified matrix of bone. Thus, my proposal analyzes the subcellular scaling properties between multinucleated OCs and mononucleated pre-osteoclasts (pOCs).

How does nuclear size scale in multinucleated osteoclasts as they grow in cellular volume, from mononucleated pre-osteoclasts?

To compare the nuclear dimensions in multinucleated OCs vs. mononucleated pOCs, I investigated the scaling properties of nuclear volume (V_{nuc}) with cell volume (V_{cell}) across OCs with nuclear number (*N*) of 1, 2, 3, 4, and 8. Nuclear scaling was measured and evaluated between OCs and pOCs by staining cells with DAPI (for nuclei) and FM1-43FX (plasma membrane) for spinning-disk confocal microscopy as well as PI (nuclei) and CellTracker-Green (cytosol) for flow cytometry.

Through spinning-disk confocal microscopy, nuclear volume appears to sublinearly scale ($\beta<1$) with cell volume as *N* increases from pOCs to multinucleated OCs. With flow cytometry, V_{nuc} appears to also sublinearly scale ($\beta<1$) with V_{cell} as *N* increases from pOCs to multinucleated OCs. Overall, my research suggests sublinear scaling of V_{nuc} with OC V_{cell} as pOCs fuse into multinucleated OCs and as *N* increases.

With nuclear volume sublinearly scaling with osteoclast size, this finding suggests that as cell size increases, syncytia constrict the increase in their perinuclear (or Voronoi) domains, in support of the cytoplasmic-to-nucleus domain theory. Furthermore, as DNA content is expected to increase to a set-point of cellular volume in post-mitotic cells, and as research shows that the transcriptome and proteome of *Saccharomyces cerevisiae* scales sublinearly with increasing ploidy, nuclear scaling in multinucleated OCs conforms with mitotic cells through the limited components model. Overall, the sublinear scaling of OC nuclei sheds light on the subcellular changes that pOCs must undergo following fusion to form their highly specialized cell type in the dynamic human body.

ABSTRACT #43:

Role of P2 receptors in nucleotide-induced calcium signalling in murine appendicular and axial tenocyte cultures – implications for tendon mechanotransduction

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Background: To adapt to the repeated mechanical load produced by movement, tenocytes, the cells within tendon, translate mechanical signals into biological responses – a process known as mechanotransduction. Previous literature has shown that mechanical loading of tendons alters intracellular signalling and matrix synthesis; however, the mechanisms mediating this process remain to be fully elucidated. Mechanical stimuli induce the release of nucleotides (and other soluble factors) into the extracellular space that then signal through P2X and P2Y nucleotide receptors expressed by target cells. Previous research has revealed P2 receptors to be essential for mechanotransduction in musculoskeletal tissues such as bone; however, the expression and role of P2 receptors in tendon is unknown.

Hypothesis: Murine tenocytes express P2 receptors that signal through intracellular calcium to modulate gene expression in response to exogenous nucleotides.

Experimental Approach: Primary tenocytes were isolated from appendicular tendons (i.e., Achilles, patellar) and axial tendons (i.e., tail) of 1-2-month-old wildtype C57BL/6N mice and grown in 2-D culture. To investigate P2 receptor expression, RNA was extracted from Achilles, patellar, and tail tenocyte cultures and qPCR was performed using gene-specific primers. To investigate nucleotide-induced calcium signalling, 2-D tenocyte cultures were loaded with the calcium-sensitive dye Fura-2-AM, subjected to live-cell calcium imaging using fluorescence microscopy, and then treated with exogenous nucleotides.

Results: Gene expression analysis of 2-D primary Achilles, patellar, and tail tenocyte cultures showed similar receptor profiles, with P2X4, $P2Y_2$, $P2Y_6$, $P2Y_{12}$ and $P2Y_{13}$ being most abundantly expressed. In this regard, treatment with 100 µM ATP and 100 µM UTP elicited transient intracellular calcium signalling compared to vehicle in fura-2-AM-loaded Achilles, patellar, and tail tenocyte cultures. As $P2Y_2$ is the only ATP- and UTP-sensitive receptor, we further hypothesized that $P2Y_2$ mediates nucleotide-induced calcium signalling in these tenocyte cultures. Indeed, we observed marked cross-desensitization of secondary calcium signalling when 100 µM ATP was given before 100 µM UTP and vice versa – this is consistent with the agonist specificity of $P2Y_2$.

Significance and Future Directions: These results reveal that axial and appendicular tenocytes express various P2Y and P2X receptors. Moreover, intracellular calcium signalling elicited by treatment with exogenous ATP or UTP is likely mediated through $P2Y_2$. Future studies are exploring the changes in mechanosensitive and tendon-specific gene expression following treatment with nucleotides or in response to dynamic mechanical loading ex vivo. Taken together, the results of this study will improve our understanding of tendon mechanobiology.

ABSTRACT #44:

Fate and function of mesenchymal progenitors in musculoskeletal regeneration

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In many adult tissues resident stem cells, such as *Pax7*+ satellite cells in skeletal muscle, regenerate parenchymal elements following damage. However, essentially all tissues also contain multiple types of mesenchymal progenitors (MPs) whose function and fate during regeneration are unclear. Here, we have used lineage tracing with a new Cre line to identify quiescent MPs across multiple tissues and through the course of a musculoskeletal injury model to interrogate any contribution therein. TdTomato expression in MPs was induced in 8-week-old mice with tamoxifen (TAM). An intramuscular injection of notexin into the tibialis anterior was used to induce myofibre hypercontraction which leads to myofiber injury and consequent myotendinous junction damage. Tissue samples from various time points after injury were dissociated, enriched for tdTomato positive MPs by FACS and lysates were generated for population or single cell RNA-seq analysis. Single cell RNA-seq analysis of MP populations in skeletal muscle demonstrated the existence of 3 predominant MP subpopulations with distinct function(s) and lineage potential. Time-resolved RNA-seq analyses revealed that these cells coordinate multiple aspects of the regenerative process by activating diverse stage-specific transcriptional programs that modulate the microenvironment. Following muscle regeneration, labelled MPs generated progeny that were found contributing to several mesenchymal structures. An abundance of tdTomato positive cells were observed forming a basket-like myotendinous junction (MTJ) and co-expressing the MTJ specific collagen 22a1 (*Col22a1*). Within the tendon proper, tdTomato positive, scleraxis GFP (ScxGFP) positive tenocytes were observed among ScxGFP positive tenocytes near regenerated MTJs. A negligible contribution of MPs to myofibres or endothelium was observed. These results reveal that in addition to fibroadipoprogenitor (FAPs), a characterized pro-regenerative cell population that undergoes transient expansion and fibrogenic phenotype following injury, there are specialized muscle resident MP subpopulations that are capable of self-renewal and contribution to tenogenic and myotenogenic structures. All populations underwent transient modification to their gene expression profile after injury, however unlike FAP progeny, tenocyte (TP) and “myotenocyte” (MTP) progenitor derivatives persisted after regeneration. The addition of a scleraxis (*Scx*) reporter allele to identify tenocytes by GFP expression confirmed that a subpopulation of tdTomato positive cells represent TPs. Our findings identify several novel MP subpopulations that exhibit transient and enduring roles in musculoskeletal regeneration. The combination of these genetic tools allows for detailed analyses and manipulation of adult tenogenic and myotenogenic progenitor biology *in vivo*. This research was supported by grants from the Canadian Institutes of Health.

Connective Tissue Repair, Regeneration & Bioengineering

ABSTRACT #45:

The Yin and Yang of myofibroblast activation: matrix production or contraction?

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Background: The irreversible hypertrophic scarring resulting from imperfect wound healing of large area skin wounds, such as burns, is a major clinical burden. This fibrotic process is characterized by excessive collagen secretion and contraction by myofibroblasts (MFs). The stiffness of fibrotic scars compared to soft surrounding extracellular matrix (ECM) of normal tissue and the presence of pro-fibrotic factors like TGF-β1 co-activate fibroblasts to become highly contractile MFs by neo-expression of a-smooth muscle actin (a-SMA). Although most definitions of the MF stipulate that it performs collagen secretion and contractile functions simultaneously (hence the name), we propose that both functions can be uncoupled. In fact, depending on the activation state and environment, ECM production and contraction may become mutually exclusive functions to allow efficient tissue repair.

Hypothesis and Objective: Collagen production and contraction are sequential MF activation states that are differentially controlled by substrate mechanics and pro-fibrotic TGF-β1. We aim to show how TGF-β1 controls MF activation states depending on mechanical environment.

Methods: Subcutaneous fibroblasts were isolated from transgenic dual reporter mice expressing green fluorescent protein (GFP) under control of the collagen type I promoter (Col1α) and red fluorescent protein (RFP) under α-SMA-promoter control. Cells were always cultured on normal skin-soft (E modulus = 0.2 kPa) or scar-stiff (70 kPa) polymer substrates for different times with or without TGF-β1. Ratios of α-SMA and Col1α reporter activities were determined using live videomicroscopy and flow cytometry. Cell contraction was simultaneously assessed using elastomer substrates that exhibit visible 'wrinkling' upon cell force transmission. Expression of ECM and contractile proteins was analyzed by Western blotting, immunofluorescence, and qRT-PCR. Gene expression profiles were established using bulk RNA sequencing.

Results: Fibroblasts freshly isolated from dual reporter mice exhibited low activity of the Col1α and no activity of α-SMA promoter. Both increased over days after seeding and with higher passage numbers. Col1α signal increased with faster rate and reached an earlier plateau than that of α-SMA. The promoter activity patterns before reaching maximum were similar yet accelerated in fibroblasts cultured on stiff versus soft substrates and upon treatment with TGF-β1 in both conditions. However, treatment with TGF-β1 at maximal promoter activities resulted in further enhanced α-SMA but reduced Col1α-promoter activity.

Conclusion: MF contractile and ECM-producing states are stimulated by stiff environment *in vitro* and *in vivo*. Addition of TGF-b1 exacerbates contraction but reduces ECM

production. Thus, MFs adapt ECM production and contraction activities to the environmental needs.

ABSTRACT #46:

Mechanical stress reduces global chromatin condensation and enhances DNA methylation in mesenchymal stromal cells

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Background: Patient-derived mesenchymal stromal cells (MSC) repair severe skin wounds in the short term. However, acquisition of a myofibroblast (MF) phenotype during cell expansion on conventional stiff surfaces or in the wound site results in scarring rather than regeneration. We have published that culturing MSC in skin-soft conditions prevents MF activation and preserves MSC regenerative potential even after transfer to stiff environment or hypertrophic wounds. It is elusive how mechanical environment establishes a persistent regenerative capacity or MF activation of MSC. Our preliminary results indicate that mechanically stiff environment results in reduced chromatin condensation and enhanced global DNA methylation in MSC, suggesting a causal relationship.

Hypothesis: Mechanically induced chromatin stretching renders DNA more accessible for the methylation of genes that characterize a persistent MF phenotype.

Methods: To test if mechanical stress increases chromatin accessibility, we used a variety of methods that induce acute mechanical stress. MSC pre-cultured on skin-soft environment were (1) treated with cell contraction agonists, (2) strained for up to 35% on stretch membranes and (3) grown on small (low stress) and large (high stress) micropatterned islands. After 2 h exposure to altered stress conditions, we analyzed chromatin condensation using DAPI staining and DNA methylation using methylated DNA-specific antibodies in confocal microscopy.

Results: MSC grown on stiff culture substrates exhibit lower chromatin condensation but higher DNA methylation levels than MSC grown on skin-soft substrates. Exposing soft-grown MSC to high mechanical stress reduces chromatin condensation and augments global DNA methylation levels within 2 h. Such acutely induced chromatin decondensation and DNA methylation persist for 24 h. However, the high DNA methylation levels established in MSC exposed for 3 weeks to stiff environment is preserved even after enhancing chromatin condensation by extracellular and intracellular relaxation.

Conclusion: Physical cues regulate global DNA methylation levels in MSC through chromatin de-condensation. Significance: This project establishes epigenetic changes that are associated with MF activation in MSC which could be targeted in the development of fibrosis therapy.

ABSTRACT #51:

Cold plasma-based redox therapy for bone tumor growth control and bacteria inactivation

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Incidence of cancer is on the rise in Canada, and metastasis is often associated with lowered life expectancy. Bone, especially the spine, is the common site of metastasis for breast, lung and prostate cancers. Treatments for these tumors rely on heavy doses of chemotherapeutic agents and invasive surgical procedures. To fully remove metastatic lesions, surgical procedures need to extend onto healthy cell tissue to reduce the probability of remaining malignant cells. This difficult procedure often requires bone reconstruction and graft, but also leaves a high risk of open wound infection. Cold plasma therapy is a new type of therapy which could greatly assist surgical intervention by delivering locally and non-invasively highly reactive oxygen and nitrogen species. This technology operates at temperatures lower than 40 °C and can selectively modify the apoptosis dynamics of the tumors by interfering with the cell-to-cell communication pathway. Similarly, the induced high voltage properties create conditions where bacteria undergo electroporation, which enhance the antimicrobial properties of the redox treatment. While research show promising results in the domain, the reaction mechanism between plasma and the tissue, and proper treatment dosage to reach hormesis between healthy and cancer cells are still yet to be defined. Therefore, we propose to build and characterize a plasma-bio interaction platform which will combine bioprinted tissue model to an automated cold plasma source. We suggest that this platform allow us to investigate the spread of breast-to-bone type of bone cancer metastasis as well as inactivate bacteria responsible for bone infections through a tailored cold plasma treatment. Results show that media treated with plasma for 5 minutes and added to a A1G7 cell-laden hydrogel in 3D culture have strong antitumoral effects. Dose responses of direct plasma treatment on 2D cultures show a selective effect of the plasma on MDA-MB-231 cancer cells with an IC50 of 42 seconds compared to 98.98 seconds for hbmMSCs healthy cells. A direct plasma treatment method is used on *E. Coli*. in suspension in PBS for different time of treatment. A 2.5 minutes treatment at 0.5 cm of distance have shown a significant decrease in the bacteria activity. Colorimetric assays have also confirmed that long-lived species (H_2O_2 and NO_2-) can be tailored through the energy, the distance and the duration of treatment. These results show promising antitumoral and antibacterial perspectives for a tailored and less invasive therapy.

ABSTRACT #52:

A biocompatible and biodegradable hydrogel for an infection-free wound and recovery

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Purpose: Infection remains a global challenge. While care and treatments for trauma and injuries are improving survival rates, infection-based complications remain a significant cause of morbidity. As bacteria form biofilms at the wound site, infection becomes significantly more difficult to treat, increasing both healing time and cost. Conventional care involves both surgical debridement combined with a course of antibiotics to prevent infection. However, the misuse and overuse of antibiotics are causing over 50% of pathogenic bacteria to become resistant to standard antibiotics. Here, we designed a biocompatible and bioresorbable hydrogel free of antibiotics to heal infected wounds while reducing the dependence on antibiotics. We explored the benefits and intrinsic anti-microbial of synthesized borophene nanosheets inside an injectable and thermo-responsive hydrogel based purely on silk-fibroin.

Methods: Silk-fibroin solution was prepared following Rockwood protocol, while borophene following Ranjan protocol. Physicochemical properties were characterized using ultra-bright TEM, SEM, XRD, TGA, FTIR, and compressive mechanical testing. Borophene release and ROS (oxygen species) generation were measured. Biocompatibility was assessed over 72hrs *in-vitro* using Human Dermal Fibroblasts (HDF). Bacteria viability was measured over 5 days using *S. Aureus* and *P. aeruginosa*.

Results: TEM imaging demonstrated synthesis of thin borophene sheets with mean lateral size of 350nm. Physicochemical characterization confirmed homogenous distribution of borophene within hydrogels. HDF cell cultures demonstrated viability significantly over 70%. Unlike its effect on regular cells, borophene incorporated hydrogels had significant toxicity and lower bacteria viability on *S. Aureus* and *P. aeruginosa* than silk-fibroin alone.

Conclusion: With the release of ROS, our results demonstrate the effect and utility of borophene's intrinsic antibacterial properties in a biocompatible and biodegradable hydrogel. In addition to depriving bacteria of required electrons within their respiratory chain's and disrupting bacteria membranes, we believe these hydrogels can promote DNA and RNA damage, and protein dysfunction to kill bacteria.

ABSTRACT #53:

Targeting bone healing with a novel bioresorbable borophene-monetite implant

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Purpose: Alternative synthetic and osteoinductive calcium-phosphate bioceramic implants, like monetite, have been used to enhance bone healing. Reinforcing bioceramic implants with 2D nanosheets have further improved bone engineering potential and mechanical strength. Ultra-thin and large lateral dimensions of 2D nanostructures create larger surface areas with higher electron mobility and catalytic capabilities aiding in the adhesion, growth, proliferation, and differentiation of osteoblasts. Borophene is a novel, thin, biocompatible, biodegradable, and graphene-like 2D nanosheet of boron atoms, with desirable properties including thermal and electrical conductivity, strong mechanical resistance, and flexibility. Boron is an essential compound in humans, shown to modulate the Wnt/β-catenin signaling pathway and critically involved in bone healing and skeletal metabolism. Modulating this pathway remains a promising target to accelerate bone healing. We hypothesize a biodegradable borophene-monetite implant increases osteoblast proliferation and differentiation to accelerate bone repair.

Methods: Monetite was prepared from monocalcium-phosphate-monohydrate and β-tricalcium-phosphate, while borophene was mechanically exfoliatiated. Physicochemical properties were characterized using ultra-bright TEM, SEM, XRD, TGA, and compressive mechanical testing. Viability and ALP differentiation were measured over 21 days under *in-vitro* pre-osteoblastic MC3T3-E1 cell cultures. Gene expression analysis were performed for select osteoblastic markers (osteocalcin, osteopontin, DMP1, Runx2, osterix, Col1a1).

Results: TEM and SEM imaging demonstrated synthesis of thin borophene sheets with mean lateral size of 350nm. Physicochemical characterization confirmed homogenous distribution of borophene within monetite scaffolds with enhanced mechanical integrity without affecting monetite crystallinity. MC3T3-E1 cells cultures demonstrated cell viability over 70%, increased ALP differentiation, and increased gene expression of osteoblastic markers.

Conclusion: Our results demonstrate that a biodegradable implant combining monetite and borophene as synthetic alternatives may promote osteogenesis through the Wnt/β-catenin pathway. With indispensable ions and beneficial structural properties, borophene remains a favorable candidate to reinforce bioceramic implants as an accessible, less invasive, and cost-effective treatment modality compared to harvesting autografts.

ABSTRACT #55:

Development of a novel inorganic polyphosphate-releasing thermoresponsive hydrogel aimed towards improving fracture outcomes.

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Bone fractures are among the most common musculoskeletal injuries. They severely disrupt an individual's quality of life and increase healthcare costs. Furthermore, 5-10% of patients experience complications such as delayed unions and non-unions, and subsequently may require invasive revision surgeries which are not always successful. Immune cells such as macrophages, neutrophils, and mast cells play a crucial role in fracture healing, and their dysregulation can lead to impaired healing. Mast cell granules have the broadest range of stored growth factors and play an essential role in bone healing as shown by our team. Inorganic polyphosphates (polyP) are ubiquitously found in nature and modulate various biological functions in a dose and chain-length-dependent manner. PolyPs have also been shown to modulate immune function by closely regulating the functions of macrophages, monocytes, and mast cells. At the fracture site, polyPs released by activated platelets play a role in recruiting immune cells and induce the degranulation of mast cells by interacting with P2Y1 receptors. Accordingly, we developed a polyP-loaded thermoresponsive poloxamer-based hydrogel system to be implanted at the site of fracture to enhance the recruitment and degranulation of mast cells, and other immune cells, and thereby increase the release of pro-osteogenic growth factors and cytokines. Our hydrogel formulation shows a sol-to-gel transition time of approximately 60 secs. at 37°C and sustains a polyP concentration of 64.5 mM during the rapid-release phase, and 38.2 mM during extended-release. The maintained concentration is directly proportional to the initial polyP doping. Approximately 50% of initially loaded polyP is released after 5 days. Approximately 92.5% of cells adhere to the polyP-doped hydrogel after a 24-hour period. Temperature sweeps show an increasing elastic behaviour with increasing polyP doping, and strain sweeps show the formulations maintain structural strength beyond 40% strain. The desired therapeutic effect of polyP pertains to the inflammatory phase of fracture healing (days 1-5); as such, the fast release of polyP should be concluded before day 5, which corresponds to the release profile of our formulation. Finally, after the exhaustion of released polyP, the remaining hydrogel at the site will act as a bio-scaffold allowing cells to migrate and enhance the development of the fracture callus. Ultimately, this study will generate a novel drug delivery system augmented with a bioactive compound, modulating the body's immune system to enhance bone healing, improve clinical outcomes, and reduce clinical strain.

ABSTRACT #56:

Mechanical control of extracellular vesicle formation by mesenchymal stromal cells

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Rationale: Mesenchymal stromal cells (MSC) have vast potential as cell therapies to repair hypertrophic scars due to their availability from donors, their capacity to regenerate damaged tissues, and immune toleration by the host. However, expansion on conventionally stiff cell culture surfaces mechanically activates MSC into scar-forming myofibroblasts. Myofibroblast activation associates with loss of regenerative potential, reducing the effectiveness of MSC in therapies. We found that growing human umbilical cord perivascular MSC on 'skin-soft' (E modulus=5 kPa) silicone substrates suppresses myofibroblast activation, compared to MSC grown on 'scar-stiff' (100 kPa) substrates. Transplanted onto hypertrophic rat skin wounds, 'skin-soft'-grown MSC suppressed whereas 'scar-stiff'-grown MSC fostered scarring after 9 d. Because grafted human MSC were not detectable in rat wounds 4 d post-wounding, we set out to study how mechanical environment controls MSC secretion of soluble factors, with a focus on extracellular vesicles (EV).

Objective: To identify EV mediators produced of soft-grown MSC that suppress scarring.

Hypothesis: Mechanical environment of MSC controls the biogenesis and content of extracellular vesicles.

Methods: Human umbilical cord MSC were directly isolated on 'skin-soft' and 'scar-stiff' substrates. Conditioned media was collected and fractionated into soluble cytokines and EVs, using phase separation assays. EV fractions (microvesicles and exosomes) were analyzed for surface markers (e.g., CD9, CD63, CD81) and size distributions using nano-particle tracking analysis, flow cytometry, Western blotting, sub-diffraction microscopy, and atomic force microscopy. MicroRNAs were quantified using nano-string analysis as signal-carrying entities in EV. All data were normalized to the numbers of MSC producing the respective factors and subjected to subsequent signalling pathway analysis.

Results: Flow cytometry, Western blotting and protein content quantification reveal higher production of cytokines and exosomes by soft-grown versus stiff-grown MSC. The ratios between exosomes and microvesicles in the EV fractions are affected by the mechanical environment of the producing MSC. Expression patterns of soluble cytokines and proteins contained in the EV fractions substantially differed between stiff- and soft-grown MSC. Likewise, EV produced by either soft-or stiff-grown MSC contained microRNA sets characteristic for the respective mechanical condition. In general, factors that are unique in the secretomes obtained from soft- versus stiff-grown MSC are indicative of inflammation- and scar-suppression.

Conclusion: Transplantation of MSC expanded in a tissue-soft mechanical environment is a promising approach to improve the healing of large area wounds such as burns. However, for specific therapeutic applications, delivery of their active trophic factors identified in our study may prove beneficial and more effective.

ABSTRACT #57:

Assessing the effect of design parameters on 3D-printed scaffolds for bone tissue repair

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Current materials used to fill bone defects (ceramics, cement) either lack strength or do not induce bone repair. The use of biodegradable polymers such as PLA may promote patient healing by stimulating the production of new bone in parallel with a controlled degradation of the scaffold. This project aims to determine the design parameters maximizing scaffold mechanical performance in such materials.

Starting from a base cylindrical model of 10 mm height and of outer and inner diameters of 10 and 4 mm, respectively, 27 scaffolds were designed. Three design parameters were investigated: pore distribution (crosswise, lengthwise, and eccentric), pore shape (triangular, circular, and square), and pore size (surface area of 0.25 mm^2, 0.5625 mm^2, and 1 mm^2). Using the finite element approach, a compressive displacement (0.05 mm/s up to 15% strain) was simulated on the models and the resulting scaffold stiffnesses (MPa) were compared. The models presenting good mechanical behaviors were further printed along two orientations: 0◦ (cylinder sitting on its base) and 90◦ (cylinder laying on its side). A total of n = 5 specimens were printed with PLA for each of the retained models and experimentally tested using a mechanical testing machine with the same compression parameters. Rigidity and yield strength were evaluated from the experimental curves.

Both numerically and experimentally, the highest rigidity was found in the model with circular pore shape, crosswise pore distribution, small pore size (surface area of 0.25 mm^2), and a 90◦ printing orientation. Its average rigidity reached 961 ± 32 MPa from the mechanical testing and 797 MPa from the simulation, with a yield strength of 42 ± 1.5 MPa. The same model with a printing orientation of 0◦ resulted in an average rigidity of 515 ± 7 MPa and a yield strength of 32 ± 1.6 MPa.

Printing orientation and pore size were found to be the most influential design parameters on rigidity. Following these results, further research is currently underway to optimize scaffolds design and choice of materials for soft tissues, with a focus on ligament and tendon repair.

ABSTRACT #58:

Collagen hybridizing peptide induce in vitro collagen fibril growth

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Collagen is a critical protein involved in tissue repair and regeneration, and promoting collagen synthesis and assembly has been the focus of new therapeutic methods. Collagen hydrogel is widely used in tissue engineering to represent the extracellular matrix architecture; however, collagen hydrogel fibril density is significantly lower than that of native tissue. The plastic compression method irreversibly removes over 90% of the water from collagen hydrogel, producing a thin collagen membrane with fibril density similar to native tissue. A solid collagen material is constructed by treating the collagen membrane with lactic acid to degrade the fully formed collagen fibrils and air-dried onto a glass slide. The resulting solid collagen material showing no collagen fibrils and D-banding patterns imaged by atomic force microscope (AFM) confirms the complete degradation of collagen fibrils.

The collagen deposition is treated with collagen hybridizing peptide (CHP) for 24hrs, and results show networks of collagen fibril growth induced by CHP. CHP is a molecular probe that binds to unwound collagen triple helix by mimicking the Gly-Pro-Hyp amino acid sequence of the native collagen. With a fluorophore attached to the end, CHP can detect damaged collagen in fibrosis tissue and aging skin. CHP-induced collagen growth exhibits a signature bundle-like structure. The core area contains densely packed aligned fibrils with indistinguishable edges, while the tail area contains numerous smaller fibrils joining together from different directions. All CHP-induced fibrils have the same 67nm D-banding pattern as native collagen, as confirmed by AFM imaging, and the fibril size ranges from 205nm to over 2µm in width. 24hr PBS control is included and showed no CHP-like growth. However, increasing the PBS incubation time to 2 weeks shows a similar bundle-like structure, resembling the CHP-induced collagen growth.

Based on the findings, it can be inferred that collagen fibril growth in a high-density environment is a spontaneous process independent of cellular or enzymatic activities. The highly aligned core area of the collagen bundle suggested that collagen fibril alignment can be independent of cross-linking in high-density conditions. This work has demonstrated that CHP can induce fibril growth, suggesting it as a promising agent to accelerate wound healing and tissue regeneration. Further work is needed to understand the mechanism of CHP-induced fibril growth and evaluate the effect of collagen fibril growth in a high-density environment under the influence of cellular regulation.

ABSTRACT #60:

Acute contact with macrophages induces lasting myofibroblast activation through mechanical stress-dependent YAP signalling

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Background: Myofibroblast (MF) activation is a key event leading to the loss of tissue architecture and function in fibrotic organs. MFs contract collagen matrix into stiff scar tissue, enhanced by the neo-expression and incorporation of alpha-smooth muscle actin (α-SMA) into stress fibers. We published that prolonged (days) direct contact between fibroblasts and pro-fibrotic macrophages (Mϕ) promotes and sustains MF activation. Unpublished data show that acute contact with Mϕ (minutes) induces contractile bursts in fibroblasts. How such Mϕ-induced acute stress responses lead to enhanced α-SMA expression and MF activation is unknown. Transcription factor Yes-Associated Protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ) translocate into the nucleus of fibroblasts under mechanical stress where they drive expression of pro-fibrotic genes, including α-SMA. Consequently, YAP/TAZ are central regulators of fibroblast-to-MF activation in fibrosis.

Hypothesis: Acute contact with Mϕ induces α-SMA expression and MF activation in fibroblasts in a YAP/TAZ-dependent mechanical stress response.

Methods: Mouse fibroblasts expressing Red Florescent Protein (RFP) under α-SMA promoter control were transfected with a TAZ-citrine construct to visualize TAZ localization and α-SMA promoter activity together in real time. Fibroblasts were grown on glass bottom dishes with an elastomer surface providing the softness of normal tissue (modulus=0.2 kPa) for an initially relaxed state. Mϕ were then added under videomicroscopy for up to 48 h. Nuclear versus cytosolic TAZ ratios and changes in RFP intensity were quantified by image analysis. In parallel, fibroblasts seeded onto soft substrates were assessed for levels of p-YAP (inactive), total YAP, and α-SMA using Western blotting and confocal immunofluorescence microscopy. YAP/TAZ activity was confirmed by blotting and performing qRT-PCR of other transcription control targets including CCN2.

Results: Fibroblasts grown on soft substrates exhibited low nuclear/cytosol TAZ (live microscopy) and YAP (fixed samples) ratios over a 4 h observation time. In response to acute contact with Mϕ (2 h), fibroblast levels of nuclear TAZ were higher compared to controls at all time points. Fibroblasts in acute contact with Mϕ exhibited a 1.5-fold increase in nuclear YAP and a 2-fold decrease in p-YAP levels, compared to control fibroblasts. α-SMA-RFP promoter activity was enhanced in fibroblasts upon Mϕ contact as compared to control fibroblasts. Inhibition of YAP function with verteporfin and relaxation of fibroblasts with cytoskeletal drugs prevented MF activation despite Mϕ contact.

Conclusion: Acute contact with Mϕ induces mechanical stress in fibroblasts, leading to YAP/TAZ nuclear translocation as a first step in the activation of α-SMA-positive MFs.

ABSTRACT #61:

Establishment and evaluation of an ex vivo osteochondral-synovim model for assessing psoriatic arthritis therapies.

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Background: The absence of a cure for PsA and limited studies surrounding its models increases the need to develop a reliable *ex vivo* model. We have developed a high-throughput *ex vivo* PsA model that captures the multi-tissue interactions omitted in most studies. Osteoarthritis (OA) and PsA share common features. Our lab has developed an OA co-culture model of cartilage and synovium tissues to evaluate injectable therapies. This OA co-culture system is modified to include PsA synovial fluid (SF) and osteochondral grafts, incorporating bone, bone and cartilage interphase. This more complex model provides multiplexed readouts of cartilage, synovium, bone gene expression and protease activity, and secreted factors.

Hypothesis: Adding psoriatic arthritis (PsA) synovial fluid SF to Osteoarthritis (OA) explant cartilage-bone and synovium increases the inflammation, degradation, and erosion markers in explant tissues compared to the addition of OA SF.

Aim 1: Establish a local end-stage PsA-like model based on a human OA model.
Aim 2: Validation of the proposed model by adding dexamethasone (DEX).

Materials & Methods: To develop the *ex vivo* model, synovial fluid (SF) is obtained from PsA patients, and tissues are obtained from end-stage OA knee joints following total knee replacement surgery. The groups in this study contain cartilage-bone and synovium and will be referred to as COCUL. The following groups are included: one negative control containing COCUL+ medium, one positive control containing COCUL+ pro-inflammatory cytokines and two treatments, COCUL+ PsA SF, COCUL+OA SF. To validate our model, in addition to having the above conditions, we have added COCUL+ PsA SF + DEX and COCUL+ OA SF+ DEX treatments. Dexamethasone is a known anti-inflammatory drug used to validate the proposed model.

Readouts investigated: Gene expression on cartilage-bone and synovium explant tissues, histology of cartilage-bone and synovium, ELISA and multiplex on the secreted factors in the conditioned medium, endogenous MMP3 activity in the conditioned medium.

Results: There has been an upregulation in the expression of genes associated with inflammation and catabolism in the positive control and COCUL+PsA SF groups in cartilage-bone and synovium explant tissues after the co-culture. Also, the addition of DEX to the COCUL+PsA SF and COCUL+OA SF groups has demonstrated a rescue effect in cartilage-bone and synovium explants and downregulated the genes associated with inflammation and degradation. There was also an increase in the endogenous MMP3 activity in the conditioned medium when PsA SF was added to the COCUL compared to when OA SF was added.

ABSTRACT #64:

Chitosan/platelet-rich plasma implants in a large arthroscopic model of meniscus repair

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Meniscal tears are the most common knee condition, with an annual incidence of 66 per 100,000 which corresponds to approximately 25,000 new cases/year in Canada. Although various repair techniques exist, most meniscal tears are deemed irreparable and, meniscectomy is commonly performed, which increases the risk of developing osteoarthritis. There remains an important need to develop therapies to enhance meniscus repair. Ortho-R, a freeze-dried chitosan-based formulation reconstituted in autologous platelet-rich plasma has shown promise in pre-clinical studies to enhance tissue repair in orthopedic indications, and is currently undergoing clinical trial for rotator cuff repair.

The main objectives of this project are to 1) Develop a fully arthroscopic model of meniscus repair in the sheep. 2) Test the effectiveness of Ortho-R as an augmentation strategy for meniscus repair in this novel minimally invasive model.

Osteotomy pins were inserted into the medial tibia and condyle and a distractor was used to obtain good visualisation. A Nanoscope was inserted through the medial portal and a 5 cm cannula through the lateral portal for passing instruments. Unilateral radial lesions were created with Nanoscissors or Nanobiters in the medial menisci of 22 skeletally mature ewes. Tears were sutured using an inside-out technique. To our knowledge, arthroscopic meniscal repair has not been attempted in sheep before. Two groups received an Ortho-R injection into the sutured lesions (0.5 or 1 mL doses, n = 6/group). In addition, one group was injected with 1 mL autologous PRP, while the control group was only sutured (n = 5/group). Two animals, one each from groups 1 and 2 were sacrificed at 1 day post-surgery. Ortho-R was resident in the meniscal tears and, as expected induced polymorphonuclear cell recruitment. The remaining animals will be sacrificed at 6-months post-operative. Indentation tests will be performed on menisci and femoral and tibial articular surfaces with the Biomomentum multiaxial mechanical MACH-1 tester (instantaneous modulus, maximum forces, and thicknesses); followed by the use of the Arthro-BST probe (Quantitative Parameters). Mappings of the repaired and contra-lateral joints will be generated and the data will be statistically compared. These results will be correlated with structural/histological and biochemical properties of the different tissues.

This study will investigate whether Ortho-R has the potential to become a new treatment for meniscal tears, eventually providing a long-term solution to Canadians suffering from this common condition whose medical needs remain unmet.

ABSTRACT #67:

Protein phosphatase 6 is a new regulator of GEF-H1/Rho signaling in kidney tubular cells

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Rho family small GTPases play a vital role in the structural and functional integrity of many tissues, including epithelial cells. Accordingly, RhoA dysregulation is implicated in a broad range of pathological processes including maladaptive tissue repair and fibrosis. Kidney fibrosis is the outcome of chronic kidney disease, which in turn is caused by common conditions such as diabetes and hypertension. Injury to kidney tubular epithelial cells have emerged as an important initiator of the development of kidney disease. Specifically, injury induces a RhoA-dependent genetic reprogramming of tubules, leading to the release of fibrogenic mediators, that in turn cause the release of extracellular matrix from interstitial cells. In previous studies we identified the guanine nucleotide exchange factor, GEF-H1 (ArhGEF2) as a central activator of pro-fibrotic tubular RhoA signaling. GEF-H1 is activated and upregulated by inflammatory and pro-fibrotic input. Its activation involves unbinding from inhibitor proteins at the microtubules or tight junctions, a process controlled by phosphorylation. Although GEF-H1 contains multiple phosphorylation sites targeted by a broad array of kinases, its control by phosphatases is less well known. In this study we demonstrate a novel interaction between GEF-H1 and PP6, an evolutionary conserved and ubiquitously expressed Ser/Thr phosphoprotein phosphatase implicated in inflammation and cancer. Using LLC-PK_1 tubular cells we found that components of the PP6 heterotrimer, including the catalytic domain (PP6C) and ankyrin repeat domain containing subunits ANKRD52 and 28 co-immunoprecipitated with GEF-H1. Silencing of either of these subunits elevated levels of phospho-S885 GEF-H1 and induced GEF-H1-dependent RhoA activation. PP6C-depletion-induced GEF-H1 and RhoA activation were prevented by inhibition of Mark2/Par1b, a kinase that has been implicated in GEF-H1 regulation. To further explore the possible effects of PP6 on cell functions relevant to tissue repair, we used a live imaging migration assay. Silencing PP6C slowed epithelial migration, and reduced stress fiber-associated phospho-myosin. In contrast, peripheral phospho-myosin was increased following PP6 depletion. These findings suggest that PP6 regulates cell contractility and migration in tubular cells. Finally, we found that the expression of PP6C was reduced in a kidney fibrosis mouse model. Taken together, our findings identify PP6 as a new negative regulator of the GEF-H1/RhoA pathway. Downregulation of PP6 during fibrogenesis might contribute to

dysregulation of GEF-H1/RhoA signaling and could promote a maladaptive repair and ultimately the development of kidney fibrosis.

ABSTRACT #83:

Combined transcriptome and epigenome profiling reveal regulators of dermal fibroblast state switching

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Fibroblasts, the major cell population in connective tissue, are the guardians of the extracellular matrix homeostasis. In adult tissue they exist in a quiescent state that can persist for years, but upon tissue injury, they become rapidly activated and re-enter the cell cycle, followed by deposition and reorganisation of the dermal matrix. The fundamental mechanism by which fibroblasts are able to maintain quiescence and be primed for prompt activation is currently unknown. Deregulations of this process in the skin and other organs is associated with several pathologies including fibrosis or chronic wounds. Notably, a rapid fibroblast state switching is also observed during skin development, where a tightly controlled change from highly proliferative to quiescent promotes extracellular matrix deposition and maturation of the dermal architecture. Understanding the shared mechanisms and differences between development and wound healing will help in the development of tissue regenerative therapies.

Using dermal development as a model for fibroblast state switching, we integrated several single cell RNA-seq and ATAC-seq datasets of whole skin during embryonic and postnatal growth. Our novel integrated multiomics single cell dataset is able to recapitulate skin developmental changes observed in vivo and reveal the major regulatory networks, including AP1, EGF and TGFβ signalling. We identified the AP1 member cJun as the key regulator of fibroblast state switching in skin development and wound repair. Mechanistically, in quiescent adult fibroblasts cJun is highly transcribed, but the protein is constantly degraded, priming fibroblasts for rapid activation when needed. Upon injury cJun becomes stabilised by post-transcriptional modification via a switch in EGF and TGFβ signalling. Accumulation of cJun leads to a change in AP1 dimer composition at the chromatin level, inhibiting fibroblast quiescence and promoting proliferation and activation. While in normal wounds cJun accumulation is inhibited during the advanced (remodelling) wounding phase, chronic wounds and fibrotic disease conditions show a continuous and pathological cJun stabilisation which can be targeted through pharmacological intervention.

ABSTRACT #85:

A novel and bioresorbable hematene-doped monetite scaffold for bone repair

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Three-dimensional scaffolds have developed as some of the most advanced and effective bone tissue engineering approaches that can be constructed using biofactors, cells, and highly selective materials. With advancing nanotechnology, nanomaterial lattices are now being incorporated into highly porous bioceramics with tremendous success in optimizing mechanical and biological performances. Graphene, a 2D atomic layer of carbon is an example of a well-publicized material that has improved the strength, functionality, and osteogenic potency of a variety of scaffold constructs. Other strategies to accelerate bone healing have emphasized materials such as iron oxide nanoparticles. Excitingly, Hematene, a novel ultrathin 2D nanosheet of iron oxide has been developed in our laboratory that has introduced an unconventional route to 2D material discovery. While iron oxide nanoparticles have long-established their photothermal and regenerative abilities, bioresorbable hematene exhibits utterly distinct optical, magnetic, electrical, and mechanical properties attributed to its dimension. In this work, we displayed the simplicity of obtaining ultrathin mono- and bi-layer hematene nanosheets (~150nm) through ultrasonic exfoliation and their decoration over monetite implants to facilitate bone repair. Hematene-loaded scaffolds exhibited an 18% increase in compressive strength compared to untreated scaffolds with good thermal stability and degradation profiles. The proliferative activity of MC3T3-E1 cells was enhanced by 24% when cocultured with hematene-treated scaffolds with good cell infiltration and adhesion. Furthermore, hematene loading significantly improved the osteogenic potency of monetite scaffolds with enhanced bone biomarker profiles with osteoinductive agents. For the first time, these findings uncover the potential of hematene derivatives as the next big scaffold candidate for bone tissue regeneration. The unique magnetic, thermal, and electrochemical properties of hematene derivatives offers encouraging avenues for further therapeutic discovery.

ABSTRACT #87:

Latent Transforming Growth Factor Binding Protein – 2 is Important for Stiffness-Induced Cardiac Fibroblasts Activation in Tissue Culture Plates

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Introduction: Cardiac Fibroblasts (Cfb) are the primary cell type involved in extracellular matrix protein deposition, leading to pathological remodelling of the heart. Latent Transforming Growth Factor Binding Protein – 2 (LTBP2) is a protein released by fibroblasts as part of the fibrotic response, with a suggested role in the progression of fibrosis. While numerous stimuli differentiate Cfb, tissue stiffness is one of the driving factors leading to the progression of fibrosis. In this study, we hypothesize that LTBP2 plays an important role in stiffness-induced Cfb differentiation

Methods: We assessed primary Cfb's response to plating conditions by plating them in 5, 20, and 70 Kpa silicone substrate (Sylgard), or non-coated plastic tissue culture plates (TCP) (Corning). Using qRT-PCR, the extent of Cfb activation was assessed by expression of differentiation marker genes such as *COL1A1*, *CTHRC1*, *LTBP2*, and *TBHS4* and compared to fluorescence-activated cell sorted non-plated Cfbs controls. Using the stiffest culturing condition we compared the differentiation of Wt to LTBP2 KO Cfb.

Results: After 1 week of platting, expression of differentiation markers such as *COL1A1*, *THBS4*, *LTBP2*, and *CTHRC1* all increased with increasing stiffness of culturing conditions with TCP being the greatest. Furthermore, LTBP2 KO Cfbs had lower expression of the same markers plated in the TCP condition compared to WT Cfbs (p-values < 0.03).

Conclusion: Our findings suggest that LTBP2 plays a key role in stiffness-induced Cfbs differentiation. As tissue stiffness in heart failure is one of the main drivers of fibrosis, LTBP2 may be a potential marker for affecting the extent of pathological fibrosis.

ABSTRACT #90:

Unlocking the power of macrophages through dectin-1: Novel yeast beta-glucan targets disease-driven phenotypes in lung fibrosis and cancer

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Background: Macrophages play a crucial role in the immune system but can promote disease progression in certain diseases like lung fibrosis and cancer. We identified Dectin-1, a macrophage membrane receptor, as upregulated in both murine and human models. Yeast beta-glucans (YBGs) are well-known macrophage immunomodulator that binds to Dectin-1. However, variability in extraction and drying methods limits their potential as a therapeutic. We collaborated with experts to identify a novel YBG that binds specifically to Dectin-1, and we investigated the potential of this YBG to result in a more effective immunomodulatory response and promote anti-fibrotic potential.

Methods: We compared our novel YBG to commercially available YBGs in terms of size, morphology, density, porosity, and Dectin-1 activation. To investigate the ability of YBG to modulate macrophages, we used human monocyte-derived THP1 macrophages, murine bone marrow-derived macrophages (BMDMs), and ex vivo murine precision-cut lung slices (PCLS). THP1s, BMDMs, and PCLS were polarized into pro-inflammatory M1- or pro-fibrotic M2-like macrophages or remained untreated (M0) and stimulated with 1-1000 ug/mL of YBG for 24-96 hours. We assessed the activation status of pro-fibrotic macrophages by measuring arginase activity in murine BMDM and PCLS, and CCL18 production in THP1s. Furthermore, we used formalin-fixed, paraffin-embedded PCLS transferred to a tissue microarray to perform immunohistochemical analysis for visualizing macrophage phenotype and quantifying Arginase-1. Additionally, we assessed TNFα and nitrite production as indicators of M1 induction. To ensure that YBG did not cause cytotoxicity, we measured cell viability by monitoring LDH release in the supernatant.

Results: Our YBG demonstrate favorable size and porosity characteristics for inhalation and macrophage uptake. Treatment with YBG prevented M2 polarization in both THP1s and BMDMs, as evidenced by decreased CCL18 production and arginase activity, respectively. Furthermore, our YBG induced a shift from M2 to M1 phenotype in profibrotic macrophages, as demonstrated by increased TNFα and Nitrite production. These effects were consistent in PCLS, where YBG treatment reduced arginase activity and Arginase-1 expression. Minimal toxicity was observed at all time-points, with a slight increase at 96 hours.

Conclusion/Significance: This study demonstrated that our novel YBG can reduce and reprogram profibrotic macrophages into anti-fibrotic macrophages. Our YBG shows promise as a therapeutic agent to be delivered directly to fibrotic regions and has the potential to be used as a standalone anti-fibrotic treatment for the lungs, with its high porosity offering the possibility for drug loading to further enhance its therapeutic potential.

ABSTRACT #91:

Evaluation of the immuno-tactic effects of inorganic polyphosphates as a therapeutic target for early fracture healing

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Fractures are among the most prevalent musculoskeletal injuries. In the years 2015-2016 alone, there were a total of 130,000 fractures in Canada, which are associated with significant morbidity and healthcare expenses. Approximately 5-10% of fracture patients will incur fracture complications such as non- and mal- unions. These complications typically require invasive revision surgeries; however, they are not always successful. The incidence of fractures associated with metabolic disorders, which carry a substantially increased risk of developing complications, has increased with the ageing of Canada's population. During the initial inflammatory phase of fracture healing, immune cells such macrophages, neutrophils and mast cells play a vital role in orchestrating the molecular stimuli to initiate bone healing. Mast cells, in particular, play a crucial role in bone healing as shown by our team. Activated platelets at the fracture site release tremendous quantities of inorganic polyphosphates (polyPs) which play a role in initiating blood coagulation and modulating immune cell function. By interacting with P2Y1 receptors, polyP can induce the degranulation of mast cells; however, its role in the recruitment of mast remains undefined. To assess the immuno-attractant properties of polyP, we used a chemotaxis microfluidic chip with an AI-powered single-cell tracking software. Several polyP-45 (chain length 45 PO_4 units) doses were tested for their chemotactic potential with RBL-2H3 cells, a mast cell analog. RBL-2H3 cells exhibited dose-dependent chemotaxis in the direction of a polyP gradient. Moreover, these same cells displayed an increase in average speed following polyP exposure. The next steps will include studying the maturation and degranulation profiles of these cells in response to polyP treatment. In addition, the same chemotaxis assay will be applied to J774A.1 cells to study the macrophage response. To the best of our knowledge, we have shown for the first time that polyP-45 has the potential to attract mast cells. The dysregulation of inflammatory processes in metabolic disorders has been shown to contribute to the development of fracture complications. The broadest range of pro-osteogenic growth factors and cytokines are found in mast cells. Taken together, therapeutically targeting mast cells using polyP may enhance the recruitment and degranulation of mast cells, resulting in an increase in the release of pro-osteogenic growth factors and cytokines at the fracture site. This finding may aid in developing immuno-modulatory therapeutic strategies to enhance fracture outcomes and reduce clinical strain.

Machine Learning, AI, Computational Biology

ABSTRACT #84:

Male and female-derived cardiac fibroblasts show distinct subpopulation abundances following pressure overload injury

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Cardiac fibrosis is a major risk factor for cardiovascular disease, leading to impaired electrical conduction and reduced ventricular compliance in the heart. While biological sex has emerged as an important factor in the development of cardiac fibrosis, its effects on cardiac fibroblasts (CFs) and their response to pathophysiologic stimuli are not fully understood. Based on reports that females are more resistant to the development of fibrosis, we hypothesize that female CFs display reduced proliferation and differentiation into injury-induced subpopulations under pathophysiologic conditions. Trans-aortic constriction and pulmonary artery banding surgeries were performed in separate cohorts of mice to model pressure overload injury in the left ventricle (LV) and right ventricle (RV) respectively. CFs were then isolated from male and female hearts after two weeks of pressure-overload stimulus, and analyzed using single-cell RNA-sequencing. Sham samples from both the LV and RV showed a larger abundance of Postn-High CFs in females compared to males. However, in pressure overload conditions, males developed a larger population of Postn-High and Thbs4+ CFs in both ventricles. Comparisons with published datasets suggest that Postn-High CFs are primed for differentiation following injury, while Thbs4+ CFs are a pro-fibrotic, injury-induced population. These findings suggest that male CFs start in a less fibrotic state, but have a stronger response to pressure overload injury. Furthermore, the abundance of Igfbp3-High CFs is greater in the RV of females in both sham and injury conditions, but shows no differences in the LV. Though Igfbp3-High CFs have not previously been described, analysis of top marker genes, including Igfbp3, Fgl2, and Tbx20, suggests a protective role in cardiac injury. These findings demonstrate that male and female-derived CFs display differences in injury-relevant subpopulations that may be explain sex discrepancies in the development of fibrosis.

Other

ABSTRACT #70:

Adjuvant therapy using senolytic drugs to prevent breast-to-bone metastasis

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INTRODUCTION: Chemotherapeutic treatment of breast cancer with Doxorubicin can induce tumor and stromal cell senescence leading to therapy resistance. Senescence-associated secretory phenotype (SASP) promotes the secretion of pro-inflammatory and tumorigenic factors causing systemic inflammation. Combined, this can result in immune suppression, tumor growth, and the secondary spread of cancer. Our objective is to target senescent and cancerous cells using a combination of chemotherapeutic and senolytic drugs. Removing senescent cells may reduce systemic inflammation, improve therapeutic efficacy, and prevent metastasis, thus, allowing for physiological cell regeneration and tissue repair.

METHODS: We analyzed chemotherapy-induced senescence using immunohistochemistry (IHC) stain $p16^{INK4}$ in breast cancer (MDA-MB-231), IRM-90 fibroblasts, osteoblast, and spine metastatic cells in monolayer. We then measured the effect of combined Doxorubicin with senolytics (RG-7112, o-Vanillin) regarding spheroid viability and growth using a 3D co-culture bone-like tumor microenvironment model *in vitro*. IHC stains KI-67 and $p16^{INK4}$ were performed for evaluation of proliferation and senescence along with fluorescence imaging and AlamarBlue assays for spheroid size, outgrowth, and cell viability. Cytokine Array assays will assess SASP release.

RESULTS: Cell lines, osteoblasts, and spine metastatic cells secondary to breast cancer showed increased senescence following treatment with Doxorubicin. Combining therapeutics resulted in a reduction in the size, outgrowth, and viability of spheroids.

DISCUSSION: Chemotherapeutic treatment of patients with breast cancer may be optimized by adding senolytic drugs. The above results demonstrate *in vitro* reduction of tumor size, inflammation, and potential for skeletal metastasis. In turn, this may enhance normal cell regeneration and tissue repair in patients suffering from solid tumor cancers.

ABSTRACT #71:

A 3D culture model of intestinal cells and fibroblasts as a screening platform for therapeutics in IBD

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BACKGROUND: Inflammatory bowel disease (IBD) is a chronic disease, that affects patient survival and Quality of Life. Although there are classic treatment options and newer therapeutics, we don't know in advance which therapeutic agent is appropriate for each patient. In addition, some patients don't respond to the existing therapy, indicating a need for improved therapies and personalized medicine.

METHODS: We first made a 3D single-cell culture of HT-29 cells and IRM-90 cells in Alginate-Gelatin Hydrogel. Then, we made a 3D co-culture model of the above cell lines seeded in Alginate-Gelatin Hydrogel. We tested the model using the Alamar Blue Assay, Viability/Cytotoxicity Assay, and Hoechst Assay. We also treated the gut model with Lipopolysaccharide (LPS) and measured the cytokine (TNFα and IL-1β) expression. We used three groups of 3D co-culture model. In group 1, LPS was mixed with the hydrogel. In group 2, LPS was added to the medium. Group 3 was not treated with LPS, and it was the control group. Finally, we added dexamethasone to the LPS-treated co-culture model, and we assessed the response to anti-inflammatory factors by measuring the cytokine (TNFα) level. There were 3 groups of 3D co-culture model as well. In group 1, both LPS and dexamethasone were mixed with the hydrogel. In group 2, dexamethasone was added to the LPS-containing medium. Group 3 was the control group, where neither LPS nor dexamethasone was added.

RESULTS: Our results showed that the 3D model with a single cell line as well as the co-culture had high metabolic activity, high percentages of viability, and increased cell proliferation. Concerning the inflammatory response, higher TNFa, and IL-1β cytokine levels were found in the LPS-treated cells compared to controls. The highest TNFα concentration was found in the 3D model treated with LPS-containing medium, whereas IL-1β highest levels were measured when LPS was mixed with the hydrogel. Finally, the results of dexamethasone treatment, showed greater decrease in TNFα levels when dexamethasone was added to the media.

CONCLUSIONS: The results showed that the 3D co-culture model is maybe a good candidate for tissue modeling and inflammatory response. Also, treating the 3D model with LPS leads to increased levels of IL-1β and TNFα compared to control. Finally, dexamethasone treatment along with or after LPS treatment decreased cytokine levels compared to the LPS-treatment group. More studies are needed to generate a 3D culture model of IBD patient-derived intestinal cells and fibroblasts.

ABSTRACT #72:

To understand the dichotomy between synovial resident macrophages and infiltrating monocytes in osteoarthritis progression

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Osteoarthritis (OA) is a degenerative disease in diarthrodial joints and the most widespread joint disease affecting more than 4 million Canadians. Monocytes and macrophages, a mixed population termed MΦs, are the key contributors to OA pathogenesis, and studies showed the correlation between the activated MΦs levels with OA severity. While the elevated presence of MΦs expressing CCR2 (a key MΦ chemokine receptor for CCL2) in OA vs. non-OA joint makes the $CCR2^{+}$MΦs a promising candidate to modulate OA progression, the global knock-out of CCR2 resulted in contradictory outcomes in animal OA models. This is because both infiltrating monocytes and tissue-resident macrophages express CCR2 and contribute to OA pathogenesis, although their homeostatic vs. pro-inflammatory functionality during OA progression is not entirely understood. There is a gap in understanding the phenotypic and functional differences between tissue-resident macrophages vs. infiltrating monocytes in OA. To address this, we will use a conditional CCR2 transgenic mouse model that enables fate mapping of tissue-resident macrophages vs. infiltrating monocytes to analyze their differential contributions to OA pathogenesis. **Methods:** We will backcross tamoxifen (TAM)-inducible $CCR2^{CreER}$ mice with transgenic $Rosa26^{td}$ mice to selectively label CCR2-expressing cells with tdTomato upon TAM pulses. Mice with experimentally induced OA will receive TAM chow for 14 days. The retention of tdTomato in tissue MΦs will be studied using flow cytometric and immunofluorescence (IF) analyses and be compared with that of non-OA mice to study their contributions to OA pathogenesis. We will also conduct exploratory evaluations of signaling pathways by IF to investigate the signaling pathway activation in $CCR2^{+/neg}$$tdTomato^{+/neg}$ subsets during OA progression. **Results:** Our preliminary data in non-OA mice showed that $CCR2^{+}$ synovial resident macrophages were labeled with the tdTomato and retained 93% after 4 weeks, as there is not much macrophage turnover in homeostatic conditions. $CCR2^{+}$ peripheral monocytes lost the tdTomato label after 2 weeks. We anticipate that in OA mice, many of the tdTomato-labeled $CCR2^{+}$ macrophages will lose their label but remain $CCR2^{+}$, suggestive of synovial OA macrophage replacement with unlabeled $CCR2^{+}$ monocytes due to OA injury. We will use the results of this work in our future investigations to selectively ablate different populations of macrophage/monocytes using a transgenic mouse model with a TAM-inducible diphtheria toxin receptor to verify their individual roles in OA progression. **Conclusions:** This study will help understand the differential contributions of tissue macrophages vs. infiltrating monocytes, enabling a more nuanced understanding of MΦ pathogenesis in OA.

Skin and Matrix Biology

ABSTRACT #74:

Differential interaction of LOXL1 variants linked to pseudoexfoliation syndrome with elastic tissue matrix proteins

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Introduction: Pseudoexfoliation syndrome (XFS) is an age-related systemic disorder involving excessive production and accumulation of abnormal elastic fibers. Manifested prominently in the eye, XFS pathology is linked to *LOXL1* variants, a gene encoding elastic fiber cross-linking enzyme lysyl oxidase-like 1. Triple LOXL1 single nucleotide polymorphisms (SNPs; denoted as nucleotide triplets below) leading to amino acid substitutions at p.141, p.153 and p.407 are associated with XFS, but their contribution to disease severity varies among populations.

Methods: LOXL1 variants, wild-type GGA identified risk factor in German population, TGA risk factor in Japanese population, and German (GAA) and Japanese (GAT) protective variants, were recombinantly produced by HEK293-EBNA cells and purified. We hypothesize that variability between populations is linked to how different variants interact with matrix proteins leading to deposition of various degree of abnormal elastic material. Binding characteristics and affinity of LOXL1 variants to fibrillin-1, tropoelastin and fibronectin as well as crucial accessory elastogenic proteins LTBP4-L, LTBP4-S, fibulin-4, fibulin-5 and MFAP4 were determined by surface plasmon resonance spectroscopy (SPR).

Results: German protective GAA binds fibulin-4 and MFAP4 with high affinity (K_D=2.7-4.5 nM) stronger than German risk GGA. Japanese protective GAT binds fibulin-4, tropoelastin, N terminus fibrillin 1 and LTBP4L with high affinity (K_D=0.7-3.8nM). Japanese risk TGA bind only to fibulin-5 and tropoelastin with moderate affinity. Binding to LTBP4-S is weak (0.3-2.4 uM) for any variant. Japanese protective GAT binds much stronger to tropoelastin (K_D=3.5 nM) than German protective GAA (K_D=78 nM). German protective GAA binds weaker to tropoelastin and N terminus fibrillin1 than German risk GGA. No weaker binding to any protein risk vs protective was found in Japanese. German variants bind MFAP4, but Japanese not. All variants bind LTBP4-L with high affinity (K_D=2.2-3.8 nM), except TGA that does not bind. All LOXL1 variants bind fibulin-5 with moderate affinity (K_D=95-207 nM). No variant binds to fibronectin or C terminus fibrillin-1.

Conclusion: SNPs significantly change LOXL1 binding behaviour to matrix proteins as assessed by SPR. Changes are due to the nature of amino acid residues substituted and combination at the triple locations, contributing to the enhancement or inhibition of the binding sites. These data bring new important mechanistic insights at the protein level in understanding why in some populations the disease risk is elevated, while in others not.

Presenting Author: Valentin Nelea
ABSTRACT #75:

MFAP4 forms calcium-dependent tetramers of homodimers which bind some, but not all, extracellular matrix proteins in a calcium-dependent manner

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Elastic fibers confer elasticity to tissues such as blood vessels and skin. Elastic fiber assembly and function are dependent on elastogenic accessory proteins, including microfibrillar-associated protein 4 (MFAP4). MFAP4 is a 36-kDa glycoprotein consisting of a N-terminal signal peptide followed by a recognition site for integrin receptors, and a C-terminal fibrinogen-related domain (FReD) which constitutes the bulk of MFAP4. FReD-containing proteins are named after fibrinogen, the precursor of fibrin, where the FReD of fibrinogen is crucial for fibrin clot formation. Indeed, MFAP4 binds to tropoelastin, the precursor of mature elastin which assembles into fibers on the extracellular surface of cells. Although MFAP4 co-localises with fibrillin-containing microfibrils and is abundant within microfibril-rich tissues, both the oligomeric state of MFAP4 and the molecular mechanism of MFAP4 interactions with elastogenic proteins are unclear.

We used cryo-electron microscopy and single particle analysis to resolve the structure of purified human MFAP4. In the presence of calcium, we found that MFAP4 assembles as a tetramer of homodimers resulting in an octamer, where a pair of homodimers constitute either the top or bottom halves of each tetramer, and that each of the homodimer is linked together by an inter-molecular disulfide bond. Using our cryo-EM map, we have built an atomic model of MFAP4 and describe the intermolecular interactions which mediate MFAP4 self-interactions. Our model suggests that several salt-bridge interactions are important for interactions within and between homodimers forming the top and bottom halves of the tetramer. Interactions between the top and bottom halves of the tetramer occur near opposing putative calcium binding sites of MFAP4. In the absence of calcium, MFAP4 dissociates to dimers of homodimers (tetramers), which resemble the top/bottom halves of the homodimer tetramer. We further investigated MFAP4 interactions with elastogenic proteins using surface plasmon resonance spectroscopy (SPR) to determine binding affinities. We determined two very high affinity binding sites for MFAP4 on fibrillin-1 (K_D=1-3 nM), one on the N-terminus and one in the center. Interactions with other elastogenic proteins mentioned below showed K_D values of 20-60 nM. MFAP4 interaction with fibrillin-1, fibulin-4, fibulin-5, tropoelastin as well as MFAP4 self-interaction is calcium-dependent, whereas binding to LTPBP4L/S is calcium independent. This suggests that MFAP4 has multiple surfaces for interactions with elastogenic proteins and some interactions might depend upon the higher-order assembly of MFAP4. From these findings, we propose a molecular model for MFAP4-elastogenic protein interactions.

ABSTRACT #76:

Integrin α7β1 represses differentiation of intestinal absorptive cells

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The differentiation of intestinal epithelial cells is a highly controlled process taking place along the crypt-villus axis of the intestinal epithelium. The stem cell population located in the lower third of the crypt gives rise to absorptive cell precursors. These precursors undergo multiple cycles of amplification in the transit amplifying (TA) zone found in the middle third of the crypt before they reach the upper third, where they undergo terminal differentiation. Various epigenetic mechanisms and signaling events are known to interrupt the differentiation of absorptive cells in the TA zone, enabling the cell population to expand. Interestingly, recent studies from our group have shown that intestinal cell interactions with α5-containing laminins may be involved. In the present study, we investigate the involvement of integrin α7β1 as a potential mediator of α5-containing laminins on this transient repression of intestinal absorptive cell terminal differentiation. Using the intestinal epithelial cell model Caco-2/15, known to spontaneously initiate a process of differentiation upon reaching confluence, integrin α7 subunit expression was knocked down using a CRISPR/Cas9 strategy. Abolition of the α7 integrin subunit resulted in a significant increase in the level of the differentiation and polarization markers as well as the morphological features of early 3 day post-confluent cells. Activities of focal adhesion kinase (tyr397) and Src kinase (tyr416) were both reduced in α7-knockdown cells and three major intestinal pro-differentiation factors CDX2, HNFα1 and HNF4α were found to be overexpressed. Moreover, the activity of polycomb repressive complex-2, evaluated by measuring the levels of tri-methylated lysine 27 in histone H3 which inhibits intestinal absorptive cell terminal differentiation, was found to be repressed in α7-knockdown cells. On the other hand, the ablation of α7 had no significant effect on Caco-2/15 cell proliferation. In summary, the findings suggest that integrin α7β1 has a significant inhibitory effect on the final differentiation of absorptive cells and suggest that the laminin-α7β1 integrin interaction occurring in the transit amplifying zone of the adult intestine is implicated in the transient halting of absorptive cell terminal differentiation.

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Stems Cells and Therapy

ABSTRACT #79:

Mechanical priming on skin-soft elastomer substrates improves the wound healing potential of human mesenchymal stromal cells

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Rationale: Hypertrophic scars impose a significant burden on the quality of life of burn patients and on health care costs. Therapy with regenerative mesenchymal stromal cells (MSCs) is one approach to accelerate the healing of severe burns. To produce MSCs in sufficient numbers for therapy, cell culture expansion following a biopsy is essential. However, culture on stiff surfaces of conventional tissue culture plastic dishes or bioreactor beads gradually induces scar-promoting cell features at the cost of reduced regenerative potential. The fibrogenic conversion of mesenchymal cells is called myofibroblast activation. We published that prolonged culture on skin-soft silicone elastomer substrates suppresses rat MSC fibrogenesis observed on stiff culture surfaces. Delivery of soft-primed rat bone marrow-derived MSCs improved the wound healing in a rat model of hypertrophic skin scarring whereas stiff-primed MSC induced scar features. How mechanical priming affects MSC contributions to wound healing is unknown.

Objective: To uncover the mechanisms and features mediating anti-scarring actions of soft-primed human MSCs in a rat transplantation model.

Hypothesis: Expansion on skin-soft culture substrates generates MSCs with secretory profiles that support scarless wound healing after therapeutic delivery.

Methods: Human umbilical cord-derived MSCs were isolated onto and mechanically primed on skin-soft and scar-stiff culture substrates for three culture passages (4 weeks). Prior to therapeutic use, mechanically primed MSCs were profiled for fibrotic activation, secreted factors, differentiation, and regeneration potential. MSCs were then transplanted onto rat full-thickness skin wounds, splinted with a plastic frame to induce hypertrophic scar features in rodents. Wound tissue was assessed after 2-9 days for MSC survival after grafting using human-specific antibodies, fibrogenesis using molecular markers of myofibroblast activation, and effect on different inflammatory wound cell populations using flow cytometry and immunohistochemistry.

Results: Soft-primed MSCs retained expression of stem cell markers CD44+/CD90+, multi-lineage differentiation potential, and low expression of the myofibroblast marker α-SMA. Transplanted soft-primed MSCs grafted for 4 days in the wound granulation tissue that was characterized by earlier recruitment of CD68+ macrophages and enhanced vascularization. The amounts of host scar myofibroblasts and mature collagen matrix were lower than in wounds receiving stiff-primed MSCs. Cytokine analysis revealed profound effects of mechanical priming on the MSC secretome, including pro- and anti-inflammatory cytokines.

Conclusion: Soft priming generates human MSCs that suppress scarring and improve wound healing by enhancing macrophage recruitment, likely through trophic effects.

ABSTRACT #82:

Cryopreserved Hair Follicles: An Abundant and Accessible Source for Autologous Mesenchymal Stromal Cells in Cell Replacement Therapies

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Personalized stem cell therapies hold immense potential in repairing and regenerating damaged tissue. However, challenges regarding sourcing and expanding a suitable cell source, have limited their widespread use. To address these obstacles, attention has turned to the human hair follicle as a source of multiple cell types that can be promptly utilized in cell therapies. Hair follicles can be collected non-invasively, yielding abundant and viable cells at lower costs than traditional harvesting methods. The outer root sheath of the hair follicle contains keratinocytes, and the dermal papilla contains mesenchymal stromal cells (MSCs), both of which can be harnessed in multiple regenerative therapies. MSCs exhibit paracrine effects via exosomes which can facilitate wound healing without the risks of tumor formation or immunogenicity. To demonstrate the capabilities of the cell itself, hair follicles were collected and cryopreserved from multiple participants and their respective MSCs were cultured. The isolated cells were characterized using flow cytometry, immunohistochemistry, and through the assessment of multi-lineage differentiation potential. The characterized hair follicle-derived MSCs will subsequently be transplanted into a mouse cartilage injury model to evaluate the specific therapeutic efficacy of these cells. In summary, hair follicle-derived MSCs offer an accessible and scalable solution for cell replacement therapies, holding the potential to revolutionize the field of regenerative medicine.

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