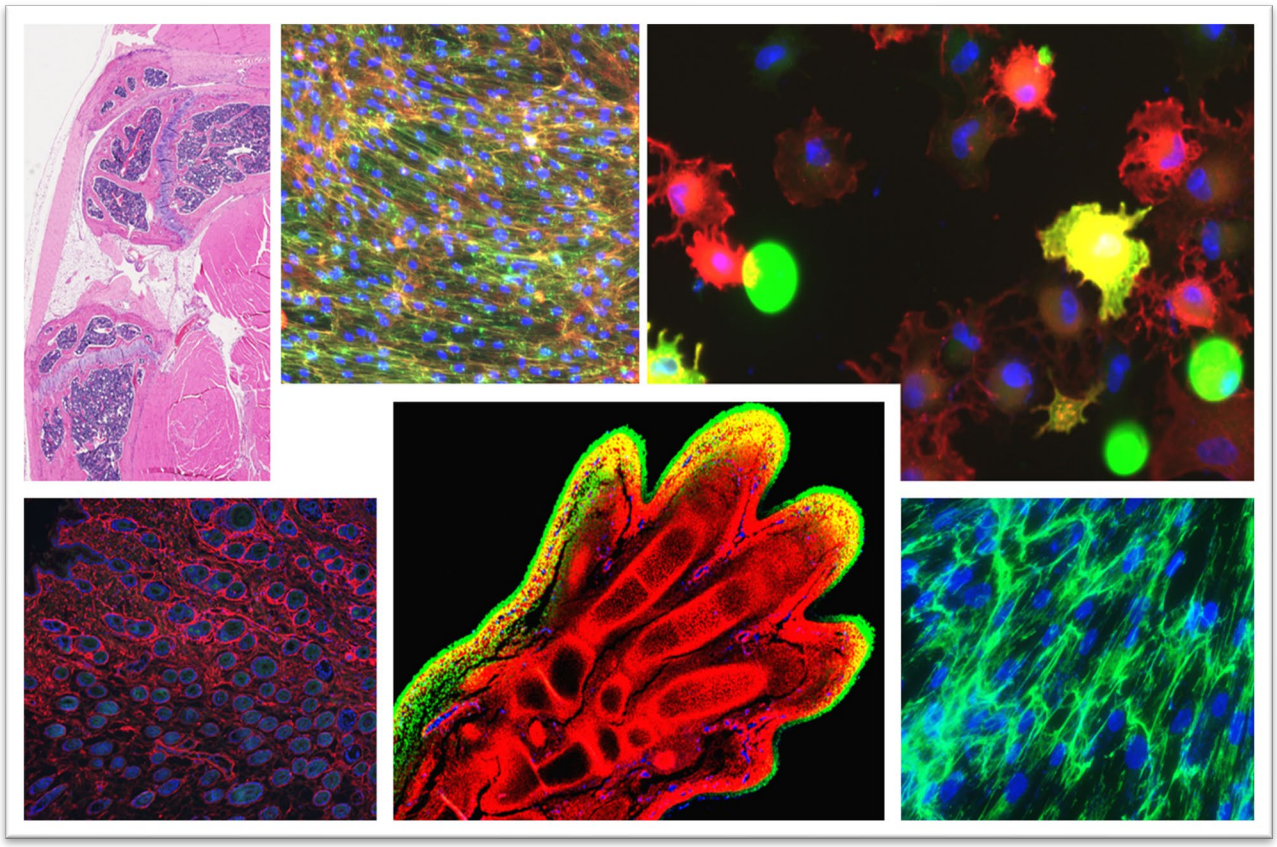
Canadian Connective Tissue Society

28th Annual Canadian Connective Tissue Conference

May 13-15th, 2024

Western University
London, ON



Message from Organizers

On behalf of the Canadian Connective Tissue Society, we're happy to invite you to the 28th Annual Canadian Connective Tissue Conference (CCTC), which will take place in London, ON from May 13th to 15th, 2024!

The Scientific Program will foster knowledge exchange of research aimed at understanding the scientific and clinical aspects of diseases that affect connective tissues such as bone, cartilage, tendon, muscle, skin alone or together at anatomical sites such as synovial joints. In keeping with the traditions valued by the CCTC, this event is focused on our young and emerging scientists, with most of our podium presentations being given by trainees as well as all the registered scientific posters. In addition, a subset of trainees has been selected as Session Chairs, providing another opportunity for our early-career scientists to refine their scientific communication skill sets. We encourage all to attend both the oral and poster sessions, where all will benefit from opportunities to network with colleagues, clinicians, and scientists in the field of connective tissue research.

This year's conference will begin on Monday, May 13th with an Equity, Diversity, and Inclusion (EDI) Workshop Day that focuses on EDI-D implementation in team building and research. This year, we will open the 2024 CCTC on the evening of May 13th with the 2024 Suzanne Bernier Memorial Lecture in Skeletal Biology followed by a Welcome Reception held in Delaware Hall. A Gala Dinner will take place on May 14th and will also be held at Delaware Hall. The conference will conclude with awards for Best Poster and Podium Presentations, as well as our two new awards established in 2023, namely one for research that incorporates EDI principles and one for research that incorporates Patient Engagement.

Please join us in London from May 13-15th for a great conference that will highlight all the amazing milestones achieved in connective tissue research in Canada over the past year!

With warm regards,

Matthew W. Grol

Assistant Professor
Western University
Chair of CCTC 2024

Message from the President

Dear Friends, Colleagues, and Trainees,

It is with the greatest of pleasure that I welcome everyone to our 28th Annual Canadian Connective Tissue Conference!

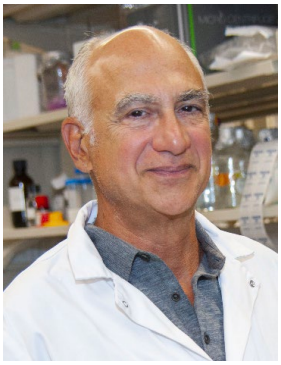
Our mandate is to foster the careers of young Canadian scientists by providing trainees an opportunity to present their work and chair scientific sessions. Equally important is to provide a forum that will facilitate trainees to form a network of colleagues that can be relied upon during their future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

I have attended about 21 of the 27 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 dependent 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!

With warm regards,



Morris F. Manolson

CCTS, President

Organizing Committee for the 28th Canadian Connective Tissue Conference

Prof. Dieter Reinhardt
McGill University

Neha Dinesh, *PhD Candidate*
McGill University

Maya Ezzo, *PhD Candidate*
University of Toronto

Dr. Shelby Oke, *Research Associate*
Western University

Abstract Reviewers

We would like to extend our sincere thanks to our panel of abstract reviewers who took time out of their busy schedules to evaluate all the submitted abstracts for CCTC 2024.

Dr. Marc Gynpas, University of Toronto

Dr. Silvia Penuela, Western University

Dr. Lidan You, Queens University

Dr. Lisa Hoffman, Lawson Health Research Institute

Dr. Dieter Reinhardt, McGill University

Dr. Marc Lavertu, Polytechnique Montréal

Dr. Cheryle Séguin, Western University

Dr. Eli Sone, University of Toronto

Dr. Amanda Ali, Henry Ford Health

Dr. Rahul Gawri, McGill University

Dr. Borris Hinz, University of Toronto

Dr. Carl Richards, McMaster University

Dr. Derek Rosenzweig, McGill University

Dr. Tom Appleton, Western University

Dr. Katherine Willmore, Western University

Dr. Emily Lalone, Western University

Dr. Frank Beier, Western University

Dr. Shelby Oke, Western University

Dr. Doug Hamilton, Western University

Dr. Adele Changoor, University of Toronto

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GROL LAB

CCTS TRAVEL AWARD WINNERS

Awarded to the highly ranked three abstracts submitted to the CCTC 2024 meeting.
(Value of $500 each)

Tarek Klaylat
Gawri Lab
McGill University

Fiona Milano
Lavertu Lab
Polytechnique Montréal

Neha Dinesh
Reinhardt Lab
McGill University

CCTS INTERNTAIONAL TRAINEE TRAVEL AWARD WINNERS

Awarded to the top two applicants in the CCTS International Trainee Travel Award Program to support their participation in an international meeting or conference outside of Canada in the field of connective tissue research.
(Value of $1,000 each)

Anca Maglaviceanu
Kapoor Lab
University of Toronto

Megan Vierhout
Ask Lab
McMaster University

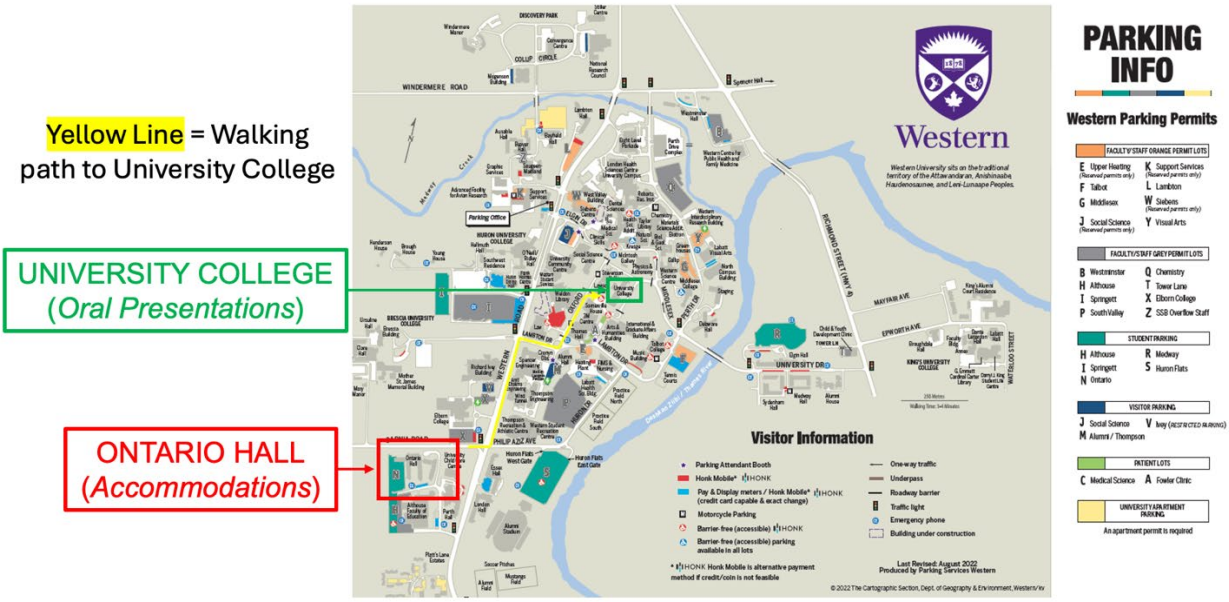
CONFERENCE OVERVIEW

SCIENTIFIC SESSIONS

All oral presentations from Session 1-9 of the Scientific Program as well as the Workshop will be held in Conron Hall, located in the University College building in Room 3110.

All oral presenters are requested to bring their presentation on a USB key, ready to be uploaded at 8:30 a.m. the morning of their session. If unable to provide the presentation on a USB key, please email it directly to Dr. Grol at mgrol2@uwo.ca.

The map to Conron Hall, located in the University College building, Room 3110, is below:



A link to a higher magnification map of the Western University campus can be found at this link: https://www.uwo.ca/parking/find/pdf/Parking_September_2021.pdf

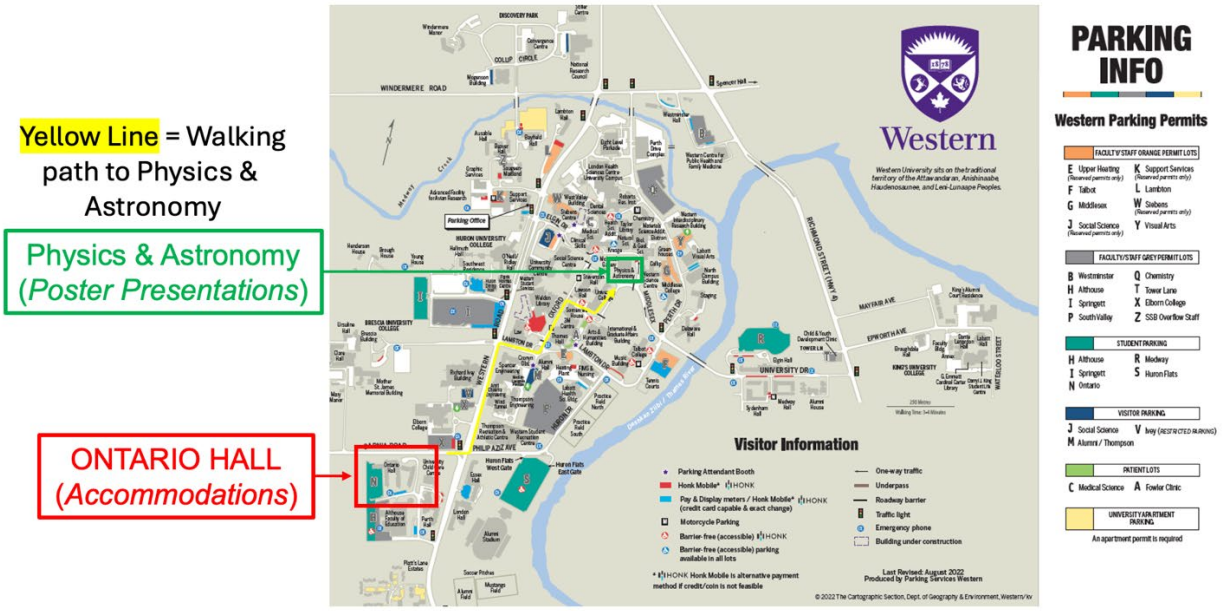
Registration and Poster Sessions

Registration and all poster presentations will be held in the Atrium of the Physics and Astronomy Building, located just next to University College.

Posters should be a maximum of 4 feet × 4 feet to ensure that all posters can be accommodated on the provided poster boards. Poster judging will be held on Monday, May 13th and Tuesday, May 14th. Posters can be taken down on Wednesday, May 15th (after 1 p.m. and before 2:30 p.m.).

Poster presentation times will be made available on Friday, May 10th.

The map to Physics and Astronomy is below:



A link to a higher magnification map of the Western University campus can be found at this link: https://www.uwo.ca/parking/find/pdf/Parking_September_2021.pdf

Dr. Suzanne Bernier Memorial Lecture in Skeletal Biology, Welcome Reception, and Gala

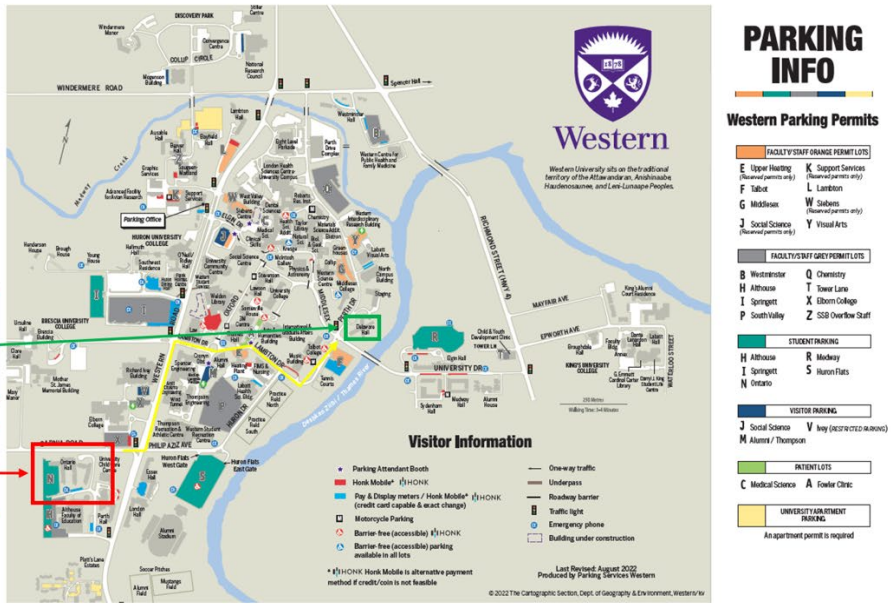
The Bernier Memorial Lecture, Welcome Reception, and Gala will be held in Dining Room of Delaware Hall.

The map to Delaware Hall is below:

Yellow Line = Walking path to Delaware Hall

Delaware Hall (*Welcome Reception & Gala*)

ONTARIO HALL (*Accommodations*)



A link to a higher magnification map of the Western University campus can be found at this link: https://www.uwo.ca/parking/find/pdf/Parking_September_2021.pdf

SCIENTIFIC PROGRAM

Day 1	**Monday, May 13th – Workshop Day** Physics & Astronomy Building – Atrium (Registration and Poster Presentation) University College, Room 3110 (Workshops and Oral Presentations) University of Western Ontario 1151 Richmond Street London, ON N6A 5C1
7:30 a.m. – 8:30 a.m.	Registration Opens & Continental Breakfast Physics & Astronomy Building – Atrium
9:00 a.m. – 9:15 a.m.	**Welcome Remarks** Dr. Matthew W. Grol (CCTC 2024 Chair) University College, Room 3110
9:15 a.m. – 10:45 a.m.	**WORKSHOP SESSION I: Establishing and Maintaining a Diverse and Representative Research Team** Dr. Imogen Coe, Toronto Metropolitan University
10:45 a.m. – 11:00 a.m.	Coffee Break
11:00 a.m. – 12:30 p.m.	**WORKSHOP SESSION IIA: Meaningful Implementation of EDI Principles in Research Design and Execution** Dr. Alex Levine and Mariam Hayward, Western University
12:30 p.m. – 2:15 p.m.	**Lunch and Poster Judging – Session 1** Physics & Astronomy Building – Atrium
2:30 p.m. – 3:30 p.m.	**WORKSHOP SESSION IIB: Discussion Period** Dr. Alex Levine and Mariam Hayward, Western University
4:30 p.m. – 7:00 p.m.	**Suzanne Bernier Memorial Lecture & Welcome Reception** Delaware Hall, Dining Room/Patio University of Western Ontario
5:00 p.m. – 5:10 p.m.	Welcome & Introduction to the Bernier Memorial Lecture – Dr.

	Cheryle Séguin
5:10 p.m. – 5:20 p.m.	Bernier Memorial Award in Skeletal Biology – Garth Blackler (*Appleton Lab*)
5:30 p.m. – 6:30 p.m.	Introduction to Guest Speaker by Dr. Cheryle Séguin Lecture – Dr. Alayna E. Loiselle, University of Rochester Medical Center
Day 2	**Tuesday, May 14th – Scientific Program** Physics & Astronomy Building – Atrium (Registration and Poster Presentation) University College, Room 3110 (Workshops and Oral Presentations) University of Western Ontario 1151 Richmond Street London, ON N6A 5C1
7:30 a.m. – 8:30 a.m.	Continental Breakfast Physics & Astronomy Building – Atrium
8:30 a.m. – 10:00 a.m.	**SESSION 1: Cell Biology of Connective Tissues** Session Chairs: Peter Suderman & Garth Blackler
8:30 a.m. – 9:00 a.m.	Keynote Speaker: Dr. Roshni Rainbow, Queens University
9:00 a.m. – 9:15 a.m.	Local activation of perivascular stromal cells by vascular endothelial cells – Elham Karimizadeh *(Hinz Lab)*
9:15 a.m. – 9:30 a.m.	How do fibronectin mutations cause spondylometaphyseal dysplasia? – Neha Dinesh *(Reinhardt Lab)*
9:30 a.m. – 9:45 a.m.	Sall1: a novel mechanosensitive nuclear factor regulating myofibroblast activation? – Xinying Guo *(Hinz Lab)*
9:45 a.m. – 10:00 a.m.	Searching for a “slice” of fresh air: using precision-cut lung slices to combat profibrotic macrophages – Megan Vierhout *(Ask Lab)*
10:00 a.m. – 10:15 a.m.	Coffee Break
10:15 a.m. – 11:45 a.m.	**SESSION 2: Western’s Bone and Joint Institute Special Session** Session Chairs: Tarek Klaylat & Jeffrey Hutchinson
10:15 a.m. – 10:40 a.m.	Keynote Speaker: Dr. Assaf Kadar, Western University

10:40 a.m. – 11:05 a.m.	Keynote Speaker: Dr. Geoffrey Ng, Western University
11:05 a.m. – 11:20 a.m.	Pain and synovial fibroblast subsets in osteoarthritis – Garth Blackler *(Appleton Lab)*
11:20 a.m. – 11:35 a.m.	Autoimmune responses correlate with synovial joint pathology in collagen-induced arthritis – Jaspreet Kaur *(Barra Lab)*
11:35 a.m. – 11:50 a.m.	Harnessing the extracellular matrix to design cell-instructive pro-angiogenic cell delivery platforms – Connor Gillis *(Flynn Lab)*
11:50 p.m. – 1:30 p.m.	**Lunch and Poster Judging – Session 2**
1:30 p.m. – 2:45 p.m.	**SESSION 3: Muscle and Tendon Biology** Session Chairs: Elham Karimizadeh & Jaspreet Kaur
1:30 p.m. – 2:00 p.m.	Keynote Speaker: Dr. R. Wilder Scott, Sunnybrook Research Institute
2:00 p.m. – 2:15 p.m.	Differentiating human bone marrow and adipose-derived stem cells towards ligamentogenic lineage using physiological oxygen tensions for tissue engineering applications – Tarek Klaylat *(Gawri Lab)*
2:15 p.m. – 2:30 p.m.	Freeze-dried chitosan-thrombin-platelet-rich plasma (CS-FIIa-PRP) implants improve supraspinatus tendon repair in a transosseous rotator cuff repair model in the rabbit – Fiona Milano *(Lavertu Lab)*
2:30 p.m. – 2:45 p.m.	P2 receptors mediate nucleotide-induced calcium signalling and gene expression changes in tenocytes: implications for tendon mechano-transduction – Ryan Armstrong *(Grol Lab)*
2:45 p.m. – 3:00 p.m.	Coffee Break
3:00 p.m. – 4:30 p.m.	**SESSION 4: Adipose Tissue and Inflammation** Session Chairs: Ermina Hadzic & Dr. Eli Sone
3:00 p.m. – 3:30 p.m.	Keynote Speaker: Dr. Lauren Flynn, Western University
3:30 p.m. – 3:45 p.m.	Investigating the crosstalk between osteoarthritis synovial fibroblasts and monocyte/macrophages – Mozhgan Rasti *(Viswanathan Lab)*
3:45 p.m. – 4:00 p.m.	Investigating oxidative stress in human chronic wounds – Dylan Tinney *(Hamilton Lab)*
4:00 p.m. – 4:15 p.m.	Lack of fibrilin-1 in maturing adipocytes leads to reduced body weight and perturbs metabolic homeostasis – Iram Fatima Siddiqui *(Reinhardt Lab)*

4:15 p.m. – 4:30 p.m.	Human decellularized adipose tissue hydrogels as a novel culture and delivery platform for endothelial colony forming cell-mediated limb revascularization – Agnes Terek *(Hess Lab)*
4:30 p.m. – 6:00 p.m.	**SESSION 5: Mechanobiology in Skeletal Development and Disease** Session Chairs: Megan Vierhout & Fiona Milano
4:30 p.m. – 5:00 p.m.	Keynote Speaker: Dr. Bettina Willie, McGill University
5:00 p.m. – 5:15 p.m.	Mechanical loading of osteocytes via oscillatory fluid flow regulates prostate cancer cell extravasation to bone *in vitro* – Kimberly Seaman *(You Lab)*
5:15 p.m. – 5:30 p.m.	Acute contact with profibrotic macrophages initiates myofibroblast activation in soft environment through αvβ3 integrin mediated activation of Piezo1 – Maya Ezzo *(Hinz Lab)*
5:30 p.m. – 5:45 p.m.	Mechanical memory of myofibroblast features in mesenchymal stromal cells via GATA-Sall regulatory networks – Fereshteh Younesi *(Hinz Lab)*
5:45 p.m. – 6:00 p.m.	Effects of low-magnitude high-frequency vibration on prostate cancer progression and bone metastasis – Amel Sassi *(You Lab)*
6:30 p.m. – 9:30 p.m.	**GALA DINNER** Delaware Hall, Dining Room/Patio University of Western Ontario
Day 3	**Wednesday, May 15th – Scientific Program** Physics & Astronomy Building – Atrium (Registration and Poster Presentation) University College, Room 3110 (Workshops and Oral Presentations) University of Western Ontario 1151 Richmond Street London, ON N6A 5C1
7:30 a.m. – 8:30 a.m.	Continental Breakfast Physics & Astronomy Building – Atrium
8:30 a.m. –10:15 a.m.	**SESSION 6: Stem Cells in Development, Disease and Therapy** Session Chairs: Anca Maglaviceanu & Ryan Armstrong
8:30 a.m. – 9:00 a.m.	Keynote Speaker: Dr. Florian Bentzinger, Sherbrooke University

9:00 a.m. – 9:15 a.m.	Matrix production or contraction: the myofibroblast eternal decision – Raquel Benitez *(Hinz Lab)*
9:15 a.m. – 9:30 a.m.	Delivery of adipose-derived stromal cells within bioengineered granulation tissue substitutes to enhance skin regeneration – Baasil Afzal *(Flynn Lab)*
9:30 a.m. – 9:45 a.m.	Interplay of matrix and solution molecules in biomimetic collagen mineralization – Dr. Eli Sone
9:45 a.m. – 10:00 a.m.	Exploring the long-term effects of a novel media formulation on chondrocyte viability and cartilage quality in stored osteochondral allografts – Sarah Aloi *(Changoor Lab)*
10:00 a.m. – 10:15 a.m.	Coffee Break
10:15 a.m. – 12:00 p.m.	**SESSION 7: Intervertebral Disc Development and Disease** Session Chairs: Fereshteh Younesi & Neha Dinesh
10:15 a.m. – 10:45 a.m.	Keynote Speaker: Dr. Chan Gao, McGill University
10:45 a.m. – 11:00 a.m.	Regulation of precursor osteoclast proliferation in adolescent idiopathic scoliosis: insights into chondrocyte-osteoclast crosstalk via the TLR-M-CSF axis – Kai Sheng *(Haglund Lab)*
11:00 a.m. – 11:15 a.m.	Investigating the role of sex hormones in the intervertebral disc – Jeffrey Hutchinson *(Séguin Lab)*
11:15 a.m. – 11:30 a.m.	Regenerative approaches to treat disc degeneration and low back pain – Saber Ghazizadeh Darband *(Hagland Lab)*
11:30 a.m. – 11:45 a.m.	Developing a mineralized collagen-containing hydrogel for repairing bone – Zi Xuan Zhang *(Sone Lab)*
11:30 a.m. – 1:00 p.m.	**Lunch and Poster Judging – Session 3**
11:30 a.m. – 1:00 p.m.	CCTS Board Meeting (CCTS Board Members only)
1:00 p.m. – 2:15 p.m.	**SESSION 8: Unraveling Musculoskeletal Development and Disease through Multi-Omics Approaches** Session Chairs: Kimberly Seaman and Saber Ghazizadeh Darband
1:00 p.m. – 1:30 p.m.	Keynote Speaker: Dr. Tom Appleton, Western University
1:30 p.m. – 1:45 p.m.	Cell and transcriptomic diversity of infrapatellar fat pad during knee osteoarthritis – Hayley Peters *(Kapoor Lab)*

1:45 p.m. – 2:00 p.m.	Sequencing identifies microRNAs that distinguish early osteoarthritis and early rheumatoid arthritis – Madhu Baghel *(Ali Lab)*
2:00 p.m. – 2:15 p.m.	Spatial transcriptomics of joint space-interfacing tissues using a pre-clinical mouse model of osteoarthritis – Teodora Tockovska *(Kapoor Lab)*
2:15 p.m. – 2:30 p.m.	Single cell RNA sequencing analysis of human osteoarthritis synovial tissue in response to PPAR delta agonist – Margaret Man-Ger Sun *(Appleton Lab)*
2:30 p.m. – 2:45 p.m.	Coffee Break
2:45 p.m. – 5:00 p.m.	**SESSION 9: Synovial Joint Development and Disease** Session Chairs: Raquel Benitez & Teodora Tockovska
2:45 p.m. – 3:15 p.m.	Keynote Speaker: Dr. Sowmya Viswanathan, University Health Network
3:15 p.m. – 3:30 p.m.	Oral delivery of delta-9-tetrahydrocannabinol provides symptom and disease modification in mouse models of knee osteoarthritis – Anca Maglaviceanu *(Kapoor Lab)*
3:30 p.m. – 3:45 p.m.	An atlas of the synovium in knee osteoarthritis: fibroblast activation defines disease progression – Kabriya Thavaratnam *(Kapoor Lab)*
3:45 p.m. – 4:00 p.m.	Investigating the use of intra-articular injections of GSK3787 for osteoarthritis – Ermina Hadzic *(Beier Lab)*
4:00 p.m. – 4:15 p.m.	Characterizing PANX mutants and identifying their implications in erosive osteoarthritis – Justin Tang *(Penuela Lab)*
4:15 p.m. – 4:30 p.m.	Non-invasive electroarthrography to monitor cartilage in an equine model of early osteoarthritis – Peter Suderman *(Changoor Lab)*
4:30 p.m. – 4:45 p.m.	Lysophosphatidylcholine and microvascular dysfunction in knee osteoarthritis – Hanyu Jiang *(Appleton Lab)*
4:45 p.m. – 5:00 p.m.	Chitosan/platelet-rich plasma implants in an ovine arthroscopic model of meniscus repair – Margaux Delvaux
5:00 p.m. – 6:00 p.m.	**General Assembly and Awards Presentation**

GUEST SPEAKERS: WORKSHOPS

Dr. Imogen R. Coe, Toronto Metropolitan University

Dr. Imogen R. Coe is a professor of Chemistry and Biology at Toronto Metropolitan University (TMU) and an affiliate scientist at St. Michael's Hospital in Toronto. She is an active researcher and former academic leader, being the founding dean of the Faculty of Science at TMU. Dr. Coe is also an award-winning scholar-activist in Canada with respect to the integration of principles of inclusion, diversity, equity and accessibility (IDEA) into research cultures in science.

Dr. Alex Levine, Western University

Alex is the Equity, Diversity, Inclusion and Decolonization Research Specialist, and part of the Inclusive Research Excellence and Impact Team at Western Research. Her academic background is in Psychology, Cognitive Neuroscience and Neuroimaging, which she completed in the UK, at Nottingham and York. Since joining Western University as a Postdoctoral researcher in 2017, she has taken on roles that advance equity, inclusion, and diversity within the research landscape. Driven by the goal of supporting the integration of EDID principles throughout academia, Alex works towards challenging conventional research norms by centering historically marginalized perspectives.

Mariam Hayward, Western University

Mariam is the inaugural Director, Inclusive Research Excellence and Impact at Western Research, Western University where she leads alongside a team of dedicated professionals the institutional strategy for knowledge mobilization/translation, equity, diversity, inclusion & decolonization, research assessment & impact, and Indigenous research. With a background in mixed-methods evaluation and community-based participatory research with Indigenous communities, Mariam also supports program evaluation and partnered research. Outside of Western, Mariam has worked with the Office of the Auditor General, Health Canada, Diabetes Canada, and the BC Provincial Diabetes Strategy.

Dr. Suzanne Bernier Memorial Lecture in Skeletal Biology & Welcome Reception

Dining Room & Patio, Delaware Hall
Western University
1151 Richmond Street
London, Ontario, Canada
N6A 5B9

Speaker: Dr. Alayna Loiselle, University of Rochester Medical Center

Dr. Loiselle received her Bachelor's Degree in Biology from Niagara University and completed her PhD in Pathology at the University of Rochester Medical Center under the supervision of Dr. Regis O'Keefe. Her PhD research established the first murine model of flexor tendon healing and demonstrated that bone marrow derived cells promoted scar-mediated healing via expression of matrix metalloproteinase 9 (MMP9). From there she joined the lab of Dr. Hank Donahue at Penn State College of Medicine and focused on the role of the gap junction protein Connexin 43 in bone regeneration and utilized a nanotopographic resurfacing technique of donor bone to enhance allograft healing. In 2013, Dr. Loiselle founded her own lab as an Assistant Professor at the University of Rochester. Dr. Loiselle's research program focuses on identifying novel therapeutic strategies for flexor tendon healing using a combination of mouse genetics, surgical and metabolic models of injury, and complex multiomics approaches. Dr. Loiselle has received numerous awards in recognition of her important discoveries and their impacts for tendon biology and disease, including the 2014 Goldner Pioneer Award from the American Society for Surgery of the Hand and the 2024 Orthopaedic Research Society's Adele L. Boseky Award, PhD Award in recognition of her contributions to mentorship. Dr. Loiselle has published over 50 peer-review scientific manuscripts along with 5 book chapters, and her laboratory is currently funded by several NIH/ NIAMS operating grants.

Bernier Memorial Award in Skeletal Biology – Graduate Student Recipient: Garth Blackler, Western University (*Appleton Lab*)

CCTS SCIENTIFIC AWARDS

Dining Room & Patio, Delaware Hall
Western University
1151 Richmond Street
London, Ontario N6A 5B9

Lifetime Achievement Award – Dr. Marc D. Grynpas, PhD

Dr. Grynpas received his undergraduate degree in Physics from the Free University of Brussels and completed his PhD in Crystallography and Biophysics at the University of London where he studied the structure of bone. His postdoctoral work at Queen Mary College examined the relation between bone structure and its mechanical properties, and his work with Dr. Melvin Glimcher at Children's Hospital in Boston examined the nature of bone mineral. Dr. Grynpas is currently a professor in the Department of Laboratory Medicine and Pathobiology and a member of the Institute for Biomaterial and Biomedical Engineering at the University of Toronto. He is also a Senior Scientist at the Lunenfeld-Tanenbaum Research Institute of Sinai Health System and the Director of the Bone and Mineral Research Group at U of T. Dr. Grynpas's current research focuses on 1) understanding the pathophysiology of osteoporosis and osteoarthritis, 2) the effects of drugs and trace elements on bone quality, and 3) the determinants of bone fragility. Dr. Grynpas is also involved in several collaborative endeavors focused on novel approaches to skeletal tissue engineering and research on the effects of metabolic syndrome on the skeleton.

Robin Poole Investigator Award – Dr. Cheryle A Séguin, PhD

Dr. Séguin obtained her undergraduate degree from the University of Western Ontario. Working in the lab of Dr. Suzanne Bernier at Western, Dr. Séguin completed her MSc thesis examining the mechanisms governing the suppressive effects of TNF-alpha on link protein and type II collagen expression in chondrocytes. Dr. Séguin went on to receive her PhD from the University of Toronto under the supervision of Dr. Rita Kandel where she applied cell biology and tissue engineering principles to the study of the intervertebral disc. She then completed a postdoctoral fellowship under the supervision of Dr. Janet Rossant at SickKids Hospital where she developed novel strategies and protocols to generate endoderm progenitors from human embryonic stem cells and was instrumental in the establishment of the Ontario Human Induced Pluripotent Stem Cell (iPSC) Facility. Dr. Séguin is currently a Professor and Acting Chair of the Department of Physiology and Pharmacology at the University of Western Ontario. Her world-renowned research program uses mouse genetics, multi-omics strategies, and novel in vitro systems to elucidate the pathways that regulate the fate and function of cells of the intervertebral disc in health and disease. Dr. Séguin has been recognized by the Canadian Arthritis Network Scholar Award (2009-2013), the CIHR New Investigator Award (2014-2019), a Faculty Scholar Award from the Schulich School of Medicine & Dentistry at Western (2020-2022), and an Arthritis Society Stars Career Development Award (2020-2025).

KEYNOTE SPEAKERS FOR SCIENTIFIC SESSIONS

Session 1 Speaker: Dr. Roshni Rainbow, Queens University

Dr. Roshni Rainbow joined Queen's University in July 2018 as an assistant professor in the Department of Mechanical and Materials Engineering. She is establishing a research program in regenerative engineering using a morphogenesis-drive paradigm that leverages developmental biology, mechanobiology, and tissue engineering. Dr. Rainbow received a BS in Biomedical Engineering from Boston University and MS in Biotechnology Engineering from Tufts University. After serving as a Process Development Engineer for Nephros Therapeutics (Lincoln, RI), she completed her PhD in Biomedical Engineering at Brown University within the Artificial Organs Lab and where she held a US Department of Veteran's Affairs Predoctoral Associated Health Rehabilitation Research Fellowship. She continued her training at Tufts University School of Medicine in musculoskeletal developmental biology and received a fellowship in academic research and teaching from the US National Institute of Health and US National Institute of General Medical Science. Prior to her current role with the Department of Mechanical and Materials Engineering, Dr. Rainbow completed additional postdoctoral training in the Department of Chemical Engineering.

Session 2 Speaker: Dr. Assaf Kadar, Western University

Dr. Assaf Kadar graduated from the Hebrew University School of Medicine and completed his Orthopedic Residency at the Orthopedic Division at Tel Aviv Medical Center. Dr. Kadar went on to complete three clinical fellowships at the Tel Aviv Medical Center's Hand and Elbow Unit, the Yale Department of Orthopaedics' Trauma Section, and the Hand and Upper Limb Centre in London, ON. As part of his ongoing research endeavors, Dr. Kadar recently completed a research fellowship at the Mayo Clinic he developed a new animal model for flexor tendon injury funded by several grants including the Mayo Clinic Bio-discovery Grant. Dr. Kadar is currently an Assistant Professor and physician leader of the world-renowned upper extremity biomechanics lab of the Roth | McFarlane Hand and Upper Limb Centre at St. Joseph's Health Care London, where his research focuses on novel imaging approaches for hand injuries. Dr. Kadar has published over 60 peer-reviewed manuscripts, 2 book chapter and has given numerous presentations at international orthopedic and hand surgery conferences. He was also recently appointed deputy editor of the Journal of the American Association of Orthopedic Surgeons (JAAOS Global). Dr. Kadar's research program is funded by various grants, including a Catalyst Grant from Western's Bone and Joint Institute.

Session 2 Speaker: Dr. Geoffrey Ng, Western University

Dr. Geoffrey Ng is a Scientist at the Robarts Research Institute and Assistant Professor in Medical Biophysics, Medical Imaging, and Surgery at Western University (London, Canada). He specializes in orthopaedic biomechanics focusing on the effects of musculoskeletal hip-pelvis structure and function on joint loading mechanics. His multidisciplinary research combines novel in vivo (imaging, gait, functional metrics) and in silico methods (musculoskeletal and finite element simulations) with in vitro testing platforms (physical joint loading and injury simulations) to characterize optimal joint loading mechanics and improve diagnosis, treatment, and injury prevention strategies. Graduating as Valedictorian, Dr Ng received his PhD in Mechanical Engineering from the University of Ottawa (Ottawa, Canada) and then completed his postdoctoral program at Imperial College London (London, UK). He recently received the Carroll A. Laurin Award for Clinical Research Excellence (Canadian Orthopaedic Foundation, 2023), Excellence in Basic Science Research Award (Arthroscopy, 2021), the coveted New Investigator Recognition Award (Orthopaedic Research Society, 2019), and was co-recipient of the prestigious Kappa Delta Award (American Academy of Orthopaedic Surgeons).

Session 3 Speaker: Dr. R. Wilder Scott, Sunnybrook Research Institute

Dr. R. Wilder Scott is a Scientist at the Sunnybrook Research Institute Holland Bone and Joint Program and assistant professor in the Department of Laboratory Medicine and Pathobiology at the University of Toronto. He completed his post-doctoral training at the University of British Columbia School of Biomedical Engineering in Vancouver. There, he characterized a cell of origin for synovial sarcoma and thesarcomagenic transformation. His current research is focused around mesenchymal cell biology and spans a variety of contexts from embryonic development to the, thus far inevitable, degeneration associated with aging. Dr. Scott is particularly interested in the fate and function of adult mesenchymal progenitor cells in normal musculoskeletal tissue renewal but also their contribution to regeneration following injury. In this context, he recently identified a unique progenitor subset responsible for regenerating the myotendinous junction.

Session 4 Speaker: Dr. Lauren Flynn, Western University

Dr. Lauren Flynn holds a joint appointment as a Professor in the Departments of Chemical & Biochemical Engineering and Anatomy & Cell Biology at The University of Western Ontario. Following her undergraduate degree in the Engineering Science program at the University of Toronto, Dr. Flynn completed her Ph.D. in the Department of Chemical Engineering & Applied Chemistry and the Institute of Biomaterials & Biomedical Engineering (IBBME) in Toronto, investigating the design and characterization of naturally-derived bioscaffolds for adipose tissue engineering applications. In 2007, she joined Queen's University as an Assistant Professor and was subsequently recruited to Western in 2014. Her team is focused on the development of cell-based regenerative therapies incorporating bioscaffolds derived from the extracellular matrix (ECM) for applications in soft connective tissue regeneration, wound healing, and therapeutic angiogenesis. Her interdisciplinary research program involves numerous collaborations with biologists, imaging scientists, engineers, and clinicians, and has been supported by funding from the CIHR, NSERC, Stem Cell Network, Heart & Stroke Foundation, and OIRM. Dr. Flynn was elected to the Royal Society of Canada's College of New Scholars, Artists and Scientists in 2019, and she was the Co-Director of the multi-institutional CONNECT! NSERC CREATE Training Program in Soft Connective Tissue Regeneration.

Session 5 Speaker: Dr. Bettina Willie, McGill University

Dr. Bettina Willie is a Professor and the Associate Dean of Research and Graduate Education in the Faculty of Dental Medicine and Oral Health Sciences at McGill University. She is an Associate Member in the Departments of Biomedical Engineering, Surgery, and Pediatric Surgery. She is also an investigator at Shriners Hospitals for Children-Canada. Dr. Willie earned a doctoral degree in Bioengineering from the University of Utah and performed postdoctoral training at the University of Ulm and the Hospital for Special Surgery. She led a research group at Charité – Universitätsmedizin Berlin for eight years prior to her joining McGill and Shriners in 2015. Her research program uses multidisciplinary approaches, including state-of-the-art high resolution imaging technologies, to study the causes and improve the detection and treatment of skeletal fragility, especially in rare bone disorders. She is particularly interested in understanding how the local mechanical environment influences bone adaptation and regeneration.

Session 6 Speaker: Dr. Florian Bentzinger, Université de Sherbrooke

Dr. Florian Bentzinger received his M.Sc. and Ph.D. degree from the University of Basel in Switzerland. Following his postdoctoral studies at the Ottawa Hospital Research Institute in Canada, Dr. Bentzinger moved back to Switzerland and joined the Nestlé Institute of Health Sciences. In 2016, he was appointed at the Université de Sherbrooke in Canada where he investigates the microenvironmental regulation of skeletal muscle stem cells in health and disease. Dr. Bentzinger has published over 30 peer-review scientific manuscripts and several patents approved and pending, and his laboratory is currently funded by CIHR, NSERC, and the Canadian Stem Cell Network.

Session 7 Speaker: Dr. Chan Gao, McGill University

Dr. Chan Gao received his M.D. from Peking University in 2003, and his Ph.D. from McGill University in 2014, which focused on the preclinical assessment of novel strategies to enhance bone regeneration. Dr. Gao went on to complete a Postdoctoral Fellowship in the Bone Engineering Lab at McGill in 2015, residency training in Physical Medicine and Rehabilitation at Vanderbilt University Medical Center in 2019, and a Clinical Fellowship in Primary Care Sports Medicine and Interventional Spine at Icahn School of Medicine at Mount Sinai. In 2020, Dr. Gao joined McGill and the MUHC as an Assistant Professor and Physician in the Department of Medicine, Division of Physical Medicine & Rehabilitation. His lab currently investigates the pathogenesis of neurogenic heterotopic ossification (NHO) and neuropathic pain associated with spinal cord injury with the goal to provide new knowledge for development of early diagnostic tools and innovative treatments for patients with spinal cord injury / disease. Dr. Gao holds funding from AO Spine North America, North American Spine Society, and Le Fonds de recherche du Québec – Sante (FRQS). Dr. Gao's work has been published in over 20 peer-reviewed manuscripts as well as 7 book chapters.

Session 8 Speaker: Dr. Tom Appleton, Western University

Tom is passionate about research, communication, and clinical care in rheumatology. He is a clinician-scientist at the University of Western Ontario and was appointed chief of rheumatology at St. Joseph's Health Care London in 2020. Tom founded the Western Ontario Registry for Early Osteoarthritis (WOREO) Knee Study, the Synovial Translational Biology Laboratory at Western, and established single cell RNA sequencing and molecular spatial profiling in the London and Western biomedical research community. Discovery is at the core of Tom's research program studying inflammation resolution, focusing on clinical and molecular mechanisms in chronic arthritis. His team was the first to discover that macrophage dysfunction underpins non-resolving inflammation in patients suffering from osteoarthritis. Tom is a past director of the Ontario Rheumatology Association (ORA) and past chair of the Canadian Rheumatology Association Scientific Committee. He now serves as advisor to Arthritis Society Canada, the Western Bone and Joint Institute, and chair of the ORA Informatics Committee.

Session 9 Speaker: Dr. Sowmya Viswanathan, University Health Network

Dr. Viswanathan is a Scientist at the Osteoarthritis Program, Division of Orthopedic Surgery, Schroeder Arthritis Institute, and the Krembil Research Institute (University Health Network) and an Associate Professor at the Institute of Biomedical Engineering and Department of Medicine, University of Toronto. She is co-Director of the Schroeder Arthritis Advanced Therapeutic Centre. Her research is focused on developing novel cellular and immunotherapies to target osteoarthritis (OA), including i) using proprietary enhanced mesenchymal stromal cells (MSCs), and ii) reprograming monocytes/macrophages using small molecules and gene edited iPSCs. Dr. Viswanathan is a co-Principal Investigator of a recently completed trial using autologous MSCs to treat osteoarthritis patients, a North American first. Dr. Viswanathan's publication was cited as the most downloaded paper in Stem Cells Translational Medicine in 2019 and garnered The Arthritis Society's (TAS) Top 10 Research Advances in 2019. Dr. Viswanathan serves on several committees at the International Society for Cell and Gene Therapy (ISCT) including as chair of the MSC scientific committee and the North American Legal and Regulatory Affairs Committee. She is Associate Editor of Cytotherapy, the official journal for ISCT. She is North American VP Elect (2024-2026) at ISCT and serves on the Strategic Advisory Council. She is co-chair of the ISCT Annual 2024 meeting (Vancouver).

SESSION 1: Abstracts for Oral Presentations

Local activation of perivascular stromal cells by vascular endothelial cells

Elham KARIMIZADEH[1], Boris HINZ[1,2]

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

Background: Vascular endothelial cells (ECs) are first responders launching organ repair following injury, infection, and inflammation. When these conditions endure, life-threatening fibrosis develops. Perivascular mesenchymal stromal cells (MSCs) in proximity to ECs provide structural support in normal vessels and are activated into myofibroblasts upon injury, which is best studied in lung fibrosis. Central for myofibroblast activation in all organs is the locally confined extracellular activation of profibrotic TGF-β1. Whether ECs directly activate MFs and if TGF-β1 is involved in the process is unknown.

Hypothesis: Perivascular MSCs and vascular ECs establish a niche of active TGF-β1 in fibrosis.

Objective: To investigate the crosstalk between ECs and MSCs in the perivascular fibrotic niche.

Methods: To evaluate the potential of ECs to produce and present latent TGF-β1, we analyzed six independently published datasets collectively comprising 123 single-cell RNA-sequencing (scRNA-seq) samples extracted from normal (n=58) and fibrotic (n=65) lung tissues. Data integration and clustering were conducted using Harmony and Seurat, respectively. To investigate if ECs activate MSCs and if myofibroblast activation is locally confined, we immunostained co-cultures of primary ECs and MSCs for the EC marker CD31 and the myofibroblast marker α-SMA. Expression of molecular components of TGF-β1 presentation and activation were additionally assessed.

Results: In silico analysis revealed that blood vascular ECs most highly express LRRC32 of all TGF-β1 presenting proteins and compared to other cell types in lung tissues. In co-culture monolayers, CD31-positive ECs segregate from MSCs to form linear 'capillary-like' structures surrounded by MSCs. We consider that ECs at the edges of these structures represent an in vitro state of 'vessel-injury' due to the lack of contact with other ECs. MSCs in proximity to such peripheral ECs express higher levels of α-SMA than MSCs further away or MSC monocultures. These results suggest that contact with ECs at free 'vessel' edges locally activates MSCs into myofibroblasts.

Significance: Deciphering perivascular MSC-EC interactions and their perturbation can help to design targeted therapies for vascular disorders and fibrosis.

Abstract #: 17
Presenting Author: Elham Karimizadeh
Research Theme: Cell Biology of Connective Tissues
Preferred Presentation: Either Oral or Poster
Presenter Category: Postdoctoral Fellow

How do fibronectin mutations cause spondylometaphyseal dysplasia?

Neha DINESH[1], Justine ROUSSEAU[2], Deane MOSHER[3], Mike STRAUSS[1], Jeannie MUI[4], Philippe CAM PEAU[2], Dieter REINHARDT[1]

[1]McGill University; [2]Centre de Recherche CHU Ste-Justine; [3]University of Wisconsin Madison WI USA; [4]Facility for Electron Microscopy Research of McGill University

Aim: Fibronectin (FN) is an extracellular matrix glycoprotein indispensable for the development and function of major vertebrate organ systems. Autosomal dominant FN mutations cause corner fracture-type spondylometaphyseal dysplasia (SMDCF). However, the molecular pathomechanisms underlying this pathology are not known.

Methods and results: To determine the consequence of FN mutations in SMDCF, we have developed an SMDCF cell culture model with induced pluripotent stem cells (iPSCs) derived from patient skin fibroblasts (FN mutations p.Cys123Arg and p.Cys231Trp) and an unaffected control. These iPSCs were differentiated into mesenchymal stem cells (MSC) and chondrocytes. Mechanistic analyses by immunoblotting and immunolabelling experiments revealed that FN mutations significantly impaired FN secretion from MSCs, leading to the accumulation of FN within the cells and a notable reduction of FN levels in the extracellular matrix. This finding was further corroborated by the analysis of plasma samples from SMDCF patients, which also exhibited reduced circulating FN levels. Ultrastructural analysis using transmission electron microscopy unveiled the presence of large vesicular structures in FN mutant MSCs, covered with ribosomes and closely associated with the endoplasmic reticulum (ER). The vesicles were identified to transition into lysosomes that contained the accumulated FN and persisted within the cytosol, contributing to elevated cellular stress markers and structural alterations in mitochondria. Moreover, we assessed the impact of these cellular changes on chondrogenesis by examining the condensation of mesenchymal stem cells under chondrogenic stimuli. FN mutant stem cells displayed impaired mesenchymal condensation, reduced expression of chondrogenic markers, and reduced cell proliferation. Bulk RNA sequencing identified significant downregulation of more than 31 chondrogenic regulators and alterations in FN splice variants, specifically under chondrogenic conditions. Notably, FN mutant cells also exhibited markedly reduced expression of transforming growth factor beta-1 (TGFβ1), a crucial chondrogenic factor essential for mesenchymal condensation. Impaired stem cell condensation and chondrogenesis associated with SMDCF-causing FN mutations could be partially rescued by exogenous supplementation of FN or TGFβ1. This rescue was evident through improved MSC condensation and increased chondrogenic marker expression in FN mutant cells upon treatment with FN or TGFβ1.

Conclusion: Taken together, our findings provide a comprehensive understanding of the cellular consequences of FN mutations in SMDCF, elucidating the molecular pathogenic pathways leading to altered chondrogenesis. Additionally, our results highlight the potential therapeutic benefits of exogenous FN or TGFβ1 supplementation in addressing the cellular defects associated with FN mutations in SMDCF.

Abstract #: 18
Presenting Author: Neha Dinesh
Research Theme: Cell Biology of Connective Tissues
Preferred Presentation: Oral
Presenter Category: Postdoctoral Fellow

Sall1: a novel mechanosensitive nuclear factor regulating myofibroblast activation?

Xinying GUO[1,2], Fereshteh YOUNESI[1,2], Boris HINZ[1,2]

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Background: Myofibroblasts play a crucial role in wound healing by producing and contracting extracellular matrix (ECM) into scar tissue. Persistent and/or dysregulated myofibroblast activities result in excessive scarring and pathological fibrosis. Myofibroblast are activated from resident fibroblasts by injury signals such as TGF-β1 and changes in mechanical environment (i.e., increasing tissue stiffness). Understanding the mechanisms of myofibroblast activation and persistence is crucial for developing anti-fibrotic therapies.

Rationale: Our lab established silicone culture substrates to imitate the mechanical microenvironment of healthy and fibrotic tissues. Various fibroblastic cells, including mesenchymal stromal cells (MSCs) adopt the contractile myofibroblast phenotype when cultured on fibrosis-stiff but not on normal-tissue soft substrates. Our RNA and ATAC-sequencing studies revealed significantly different gene expression profiles between MSCs isolated from rat bone marrow and grown on soft and stiff substrates. Computational analysis revealed binding motifs for spalt-like transcription factor 1 (Sall1) in the promoters of differentially regulated genes. Because Sall1 is also highly expressed in soft but not stiff environment , we investigate its putative function as a suppressor of myofibroblast activation and novel mechanosensitive transcription factor.

Methods & Results: We cultured human umbilical cord-derived MSCs on normal-tissue soft (E modulus=1 kPa) and fibrosis-stiff (100 kPa) substrates to first validate our previous results obtained with rat MSCs. Sall1 expression was assessed from passage 3 to 7 by qPCR, immunoblotting and immunostaining. MSC activation was determined by measuring expression levels of the myofibroblast marker α-SMA and ECM proteins, such as collagen and fibronectin. Inversely correlated with the low expression levels of α-SMA and less myofibroblast activation, Sall1 transcript levels were high in soft-grown and low in stiff-grown MSCs. To assess the function of Sall1, we depleted Sall1by siRNA knockdown in soft- and overexpression in stiff-grown MSCs. Our preliminary results indicate that Sall1 depletion associated with increased myofibroblast activation of soft-grown MSCs.

Significance: This work will provide insights into tissue stiffness-driven myofibroblast activation in fibrosis and the development of anti-fibrotic therapies.

Abstract #: 19
Presenting Author: Xinying Guo
Research Theme: Stem Cells and Therapy
Preferred Presentation: Either Oral or Poster
Presenter Category: Postdoctoral Fellow

Searching for a "slice" of fresh air: using precision-cut lung slices to combat profibrotic macrophages

Megan VIERHOUT[1], Pareesa ALI[1], Vaishnavi KUMARAN[1], Safaa NAIEL[1], Anmar AYOUB[1], Joanna KASI NSKA[1], Takuma ISSHIKI[1], Martin KOLB*[1], Kjetil ASK*[1]

[1]Department of Medicine, Firestone Institute for Respiratory Health, McMaster University and The Research Institute of St. Joe's Hamilton, Hamilton, ON, Canada

Background/Objectives: The lungs are a breathtaking organ, containing over 20 cell types in an elaborate landscape. As such, recapitulating the lung environment in a physiologically relevant manner is a long-standing challenge faced by scientists. Many traditional systems for respiratory research attempt to mimic interactive cellular processes, but lack spatial complexity and fall short of modelling the lung. Precision-cut lung slices (PCLS) are living tissue slices created from whole lung that are maintained ex vivo. PCLS contain all lung resident cells and connective tissue components, and preserve their native interactions and arrangements.
Macrophages are the most common lung immune cells. Alternatively activated macrophages (AAM) have been linked to pulmonary fibrosis and other lung diseases, thus warranting further investigation into these cells in the lung. Here, we aimed to explore the induction of macrophage alternative activation in PCLS, and ultimately, determine if a PCLS system could constitute a valid model to study lung AAM.

Methods: PCLS were generated from murine lungs. Slices were cultured ex vivo and stimulated with a polarization cocktail (IL-4+IL-6+IL-13). Timecourse studies were conducted to evaluate activation status of macrophages in PCLS. Multiple markers of AAM were assessed in tissue homogenates and secretions. Immunohistochemical (IHC) and multiplex staining were used to visualize AAM in the tissue and determine specific activation phenotype. Viability and structural integrity of PCLS were evaluated with WST-1 and H&E histology, respectively. Additionally, macrophage depletion studies using liposomal clodronate were conducted to investigate macrophage-specific responses.

Results: PCLS remained viable and structurally intact. Following cocktail treatment, AAM markers were elevated throughout the timecourse. Specifically, Arg1, MRC1, and Chil3 mRNA levels were increased in stimulated PCLS homogenates at all timepoints, as well as Arginase-1 enzyme activity ($p<0.05$). CD206 and Arginase-1 stained macrophages were visualized in the alveoli and interstitium, and overall elevated in the tissue ($p<0.05$). In PCLS secretions, YM1 and CCL17 levels increasingly rose throughout the timecourse ($p<0.05$). Additionally, increased markers of myofibroblasts and extracellular matrix components, including aSMA IHC staining and ACTA2 and FN1 expression, were observed at later timepoints (48 and 72 hours). Following clodronate-mediated depletion, Arginase-1 levels were significantly reduced, demonstrating contribution of macrophages to observed responses.

Conclusions: Our results support the validity of studying AAM in the ex vivo lung using PCLS, with potential applications to fibrosis and other lung diseases. Compared to traditional systems, this involves a more translational approach to study AAM mechanisms and treatment strategies to disrupt their disease-contributing state.

Abstract #: 32
Presenting Author: Megan Vierhout
Research Theme: Cell Biology of Connective Tissues
Preferred Presentation: Oral
Presenter Category: PhD Student

SESSION 2: Abstracts for Oral Presentations

Pain and synovial fibroblast subsets in osteoarthritis

Garth BLACKLER[1], Holly PHILPOTT[1], Joseph KLAPAK[1], Easton FARRELL[2], Tristan MAERZ[2], Cherlye S ÉGUIN[1], C. Thomas APPLETON[1]

[1]Western University; [2]University of Michigan

Background: Normal synovial tissue function is critical for joint health. Fibroblasts that line the synovium (lining fibroblasts) produce hyaluronan and lubricin, which are necessary for resisting mechanical compression and supporting joint lubrication. Fibroblast-macrophage communication plays a central role in wound healing and is likely involved in defining synovial fibroblast subsets including lining fibroblasts but the mechanisms governing synovial macrophage-fibroblast communication are not well defined. Synovial fibroblast subsets are altered by osteoarthritis (OA), but the role of fibroblast subsets in mediating OA pain remains unclear. This study aimed to identify the association of synovial fibroblasts with pain and the mechanisms driving lining fibroblast phenotype.

Methods: A human knee OA synovial tissue single-cell RNA-sequencing dataset was analyzed to identify synovial fibroblast subsets associated with worse pain, fibroblast differentiation trajectories and regulatory transcription factors, and cellular communication. Mineralization is associated with worse pain in OA; therefore, we tested the ability of OA synovial fibroblasts to stimulate mineralization through exposure to mineralization media with or without synovial fluid (to mimic the harsh joint environment) and TGFβ1 (a pleotropic growth factor increased in OA).

Results: Lining fibroblasts were strikingly reduced in patients experiencing worse pain, whereas LRRC15+, senescent-like, and sub-lining progenitor fibroblasts were enriched. Trajectory analysis suggested that sub-lining progenitors differentiate through an LRRC15+ subset (mediated by FOXO1) to either a lining (mediated by ETS2) or senescent-like lining (mediated by CEBPβ) terminal state. Pathway responsive gene enrichment analysis suggested that progenitor phenotype may be associated with PI3K and TGFβ signaling while lining may be associated with MAPK, VEGF, and EGFR signaling. Cell-cell communication analysis identified that crosstalk between macrophages and fibroblasts is reduced in patients with worse OA pain. Compared to sub-lining fibroblasts, macrophage-to-lining fibroblast communication was associated with enriched cell adhesion (ITGB2-ICAM2 and SELPLG-SELP), anti-inflammatory (GAS6-AXL), and wound healing (GRN-SORT1) ligand-receptor interactions, suggesting a key role for macrophages in sustaining a lining fibroblast phenotype. LRRC15+ fibroblasts express the pro-mineralization gene AMTN while lining fibroblasts express mineralization inhibitors, VWC2 and FAM20A. When exposed to TGFβ1 and synovial fluid, synovial fibroblasts from 3 of 6 patients produced mineral.

Conclusions: The loss of synovial lining fibroblasts is associated with worse OA outcomes including worse pain and mineralization. Macrophage-to-fibroblast communication may represent key mechanisms required for the maintenance of lining fibroblasts. Understanding the mechanisms that lead to and maintain synovial lining fibroblasts may help identify novel avenues to improve patient outcomes and joint health in OA.

Abstract #: 15
Presenting Author: Garth Blackler
Research Theme: Cell Biology of Connective Tissues
Preferred Presentation: Oral
Presenter Category: MSc Student

Autoimmune responses correlate with synovial joint pathology in collagen-induced arthritis

Jaspreet KAUR[1], Sofya ULANOVA[1], Ewa CAIRNS[1], Lillian BARRA[1]

[1]Western University

The Collagen-Induced Arthritis (CIA) mouse model is frequently used to study Rheumatoid Arthritis (RA), an autoimmune disease that primarily affects synovial joints. CIA mice develop similar joint pathology as RA patients, including joint pain, swelling, and joint damage. In RA, these disease manifestations are driven by aberrant immune responses that target modified joint proteins containing citrulline (CitP) or homocitrulline (HomoCitP). These responses include T cell proliferation and RA-specific autoantibodies production. However, it remains unclear whether similar immune responses develop in CIA.

We determined if RA-specific immune responses develop in CIA, and whether they relate to joint pathology. DBA/1J mice were injected with type II collagen (CIA; N = 17) or PBS (Negative Control; N = 6) and sacrificed day 49 post primary immunization, corresponding with peak arthritis. Pain-like behaviour was assessed using the von Frey method and joints were imaged using MicroCT. Splenocyte proliferation following antigen stimulation was evaluated using flow cytometry and serum antibodies were measured via ELISA.

CIA mice, but not PBS mice, developed arthritis, with symptoms such as joint swelling and a reduced pain withdrawal threshold. Imaging showed a decrease in bone mineral content and density, indicative of osteopenia, as well as bony erosions in CIA joints. CD4+ T cell proliferation was higher in CIA vs. PBS mice when stimulated with collagen ($p = 0.0200$) or HomoCitP ($p = 0.0457$), but not CitP. In CIA, T cell responses to collagen and HomoCitP correlated very strongly with lower bone mineral density ($R2 = 0.9$). CIA mice developed antibodies against collagen; however, RA-specific antibodies were not higher compared to controls.

These data suggest that T cell responses to HomoCitP may be related to joint destruction in the CIA model. As similar responses occur in human RA, CIA mice may be useful in exploring RA disease pathogenesis.

Abstract #: 71
Presenting Author: Jaspreet Kaur
Research Theme: Cell Biology of Connective Tissues
Preferred Presentation: Oral
Presenter Category: PhD Student

Harnessing the extracellular matrix to design cell-instructive pro-angiogenic cell delivery platforms

Connor GILLIS[1], Samantha FLOOD[1], Agnes TEREK[1], John WALKER[1], Paula FOSTER[1], David HESS[1], L auren FLYNN[1]

[1]Western University

Introduction: Currently, a major barrier in tissue engineering and regenerative medicine is the inability to form stable perfused blood vessel networks to support new tissue development. Cell therapies involving the localized delivery of pro-angiogenic cell populations have shown promise in inducing new blood vessel formation but are limited by poor cell retention and survival following transplantation. For this reason, there is a need for new strategies to augment the localized retention, survival, and pro-angiogenic functionality of therapeutic cell populations. Recently, extracellular matrix-derived decellularized adipose tissue (DAT) hydrogels have been developed that are injectable and enable rapid in situ cell encapsulation by gelling at body temperature. These platforms represent a promising strategy for the regeneration of stable perfused blood vessels to support new tissue development.

Methods: Human adipose tissue was collected with informed consent (HSREB 105426), decellularized and digested with pepsin or papain to form the DAT “pre-gels” that gel via pH neutralization and raising the temperature to 37°C. The pre-gels can be combined with micronized DAT particles to form composite DAT hydrogels. Hydrogel physical parameters are being compared through rheological testing and SEM imaging. The cell supportive nature of each hydrogel formulation is being tested in vitro by encapsulating human adipose-derived stromal cells (ASCs) and assessing cell viability, proliferation, and matrix remodeling over time. The ability of the gels to direct human monocyte polarization is also being assessed through flow cytometry. Finally, the efficacy of the hydrogel delivery systems is being assessed in vivo using a femoral artery ligation-induced critical limb ischemia model in NOD/SCID mice.

Results: Thermosensitive DAT hydrogel platforms were successfully established that enable rapid in situ gelation. The hydrogels support the viability of encapsulated human ASCs and monocytes in vitro and demonstrate a cell-instructive capacity to direct encapsulated human monocytes towards a pro-regenerative macrophage phenotype. Preliminary results suggest that co-delivery of monocytes and ASCs within the DAT hydrogels can enhance limb perfusion in the murine critical limb ischemia model. Additionally, preliminary cell tracking studies using magnetic particle imaging (MPI) support that the DAT hydrogels can promote localized cell retention following intramuscular injection relative to saline delivery.

Conclusion: Novel injectable DAT hydrogels capable of delivering regenerative cell types were successfully established. The new cell delivery platforms support ASC and monocyte viability and represent a promising strategy for harnessing the pro-angiogenic capacity of regenerative cell types for the regeneration of stable perfused blood vessels to support new tissue development.

Abstract #: 29
Presenting Author: Connor Gillis
Research Theme: Adipose Tissues and Blood Vessels
Preferred Presentation: Either Oral or Poster
Presenter Category: PhD Student

SESSION 3: Abstracts for Oral Presentations

Differentiating human bone marrow and adipose-derived stem cells towards ligamentogenic lineage using physiological oxygen tensions for tissue engineering applications

Tarek KLAYLAT[1,2], Peter MOUNSEF[1,2], Paul MARTINEAU[1,2], Derek ROSENZWEIG[1,2], Rahul GAWRI[1,2]

[1]McGill University; [2]McGill University Health Center

INTRODUCTION: Ligament injuries are common musculoskeletal injuries. Due to biomechanics and poor blood supply, ligament tissues do not heal properly after injury. Ligament reconstruction surgery using auto/allografts suffers from high failure, complication, and revision rates. Implantable cell-laden bioengineered ligament grafts constitute a potential alternative solution but still face the challenge of non-integration at the graft site. The optimal source of cells and culture conditions for bioengineering ligament grafts remain a challenge. Bone marrow (hBM-MSCs) and adipose-derived stem cells (hAD-MSCs) have been used for ligament bioengineering with limited success. Ligament tissue and the articular joint space have lower oxygen tensions than arterial and venous levels which is critical for optimal tissue physiology and bioengineered graft integration as the grafts grown in standard non-physiological conditions face “adverse conditions” upon implantation. In this study, we hypothesized that culturing hBM-MSCs and hAD-MSCs under near physiological oxygen tensions would enhance their differentiation into the ligamentogenic lineage for use in ligament tissue engineering.

METHODS: hBM-MSCs and hAD-MSCs (RoosterBio) were cultured for 10 days at 37℃ in DMEM supplemented with TGF-β, bFGF, and L-ascorbate under three oxygen tensions: atmospheric and standard cell culture (20% O2, S), intravenous (5% O2, IV), and intraarticular (2% O2, IA) oxygen tensions. Cell-seeded culture plates were placed in a standard incubator with 5% CO2 at 37℃ for atmospheric oxygen tension. For IV and IA, hypoxia incubator chambers (StemCell Technologies) were sealed and flushed with 5% O2 and 2% O2 gas premixes respectively and placed inside an incubator at 37℃ with media changes every 3 days, after which they were resealed and reflushed. After 10 days, cell proliferation, viability, and metabolic activity were assessed, and qPCR was performed to evaluate the gene expression of ligament markers.

RESULTS: Our data show that IV does not affect the viability of hBM-MSCs and hAD-MSCs, whereas IA slightly decreases the viability of hBM-MSCs. Cell growth remained unaffected under IV and IA. A significant increase in the metabolic activity of hBM-MSCs was observed under IA and IV and hAD-MSCs under IV. Finally, qPCR results indicate higher levels of collagen type-1, tenascin C, and scleraxis expression in hBM-MSCs under IA.

CONCLUSIONS: These findings suggest that hypoxic culture conditions enhance the ligamentization potential of hBM-MSCs cultured in media with TGF- β and bFGF. Optimizing culture conditions for differentiating hBM-MSCs and hAD-MSCs into the ligamentogenic lineage would allow us to use these cells in bioengineered ligament grafts for ligament reconstruction.

Abstract #: 35
Presenting Author: Tarek Klaylat
Research Theme: Connective Tissue Repair, Regeneration, Bioengineering
Preferred Presentation: Either Oral or Poster
Presenter Category: PhD Student

Freeze-dried chitosan-thrombin-platelet-rich plasma (CS-FIIa-PRP) implants improve supraspinatus tendon repair in a transosseous rotator cuff repair model in the rabbit

Fiona MILANO[1], Anik CHEVRIER[1], Gregory DE CRESCENZO[1], Marc LAVERTU[1]

[1]Polytechnique Montreal

Introduction: With 11 million Canadian affected annually, rotator cuff tears stand as the most prevalent shoulder condition prompting medical intervention. Failure rates as high as 60% have been reported after surgery, indicating the need for improved treatments. Ortho-R, a hybrid biomaterial developed at Polytechnique Montreal, is composed of lyophilized chitosan (CS) that is solubilized in platelet-rich-plasma (PRP). It solidifies in situ and shows potential to improve surgical repair for a variety of orthopedic conditions, the more advanced being the rotator cuff tear. Following demands from orthopedic surgeons using Ortho-R, a new formulation of the biomaterial has been developed, incorporating thrombin (FIIa) to shorten its solidification time and therefore ease its application in wet arthroscopy (CS-FIIa-PRP formulation). This formulation allows a fourfold decrease in the time required to reach the gelation point. The main hypothesis of the present study was that this formulation is effective to improve supraspinatus tendon surgical repair in a New Zealand White rabbit model.

Method: Complete tears of the supraspinatus tendon were created bilaterally, then repaired using transosseous sutures. On the treated shoulder, CS-FIIa-PRP implants were injected both in the transosseous tunnels and on the tendon at the repaired site. Animals were euthanized at day 14 (n=6), day 28 (n=6) and day 84 (n=6).

Results: Mechanical testing showed similar results for control and treated side at day 14 (20% average increase in dynamic modulus for treated side, not significant) but improved mechanical properties for treated side at day 28 (98% average increase in dynamic modulus, p value < 0.05). This difference diminished after 84 days (39% average increase in dynamic modulus, not significant), suggesting that the action of the implant is predominantly exerted during the initial stages of repair. This early improvement is further shown by an 18% decrease in tendon cross-sectional area of the repair tissue between day 14 and 28 for treated shoulder, while the control shoulder shows a constant area, greater than that of intact specimens. Treatment also inhibited the development of heterotopic ossification in the tendon, which is a phenomenon observed both at 28 days and 84 days for the suture only controls. Furthermore, no deleterious effect was observed in the rabbit after implantation.

Conclusion: These results suggest that the addition of thrombin to Ortho-R implants allows to decrease their coagulation time while maintaining their efficacy, therefore fulfilling orthopedic surgeons demands. Histological and micro-CT analysis is ongoing.

Abstract #: 55
Presenting Author: Fiona Milano
Research Theme: Connective Tissue Repair, Regeneration, Bioengineering
Preferred Presentation: Oral
Presenter Category: PhD Student

P2 receptors mediate nucleotide-induced calcium signalling and gene expression changes in tenocytes – implications for tendon mechanotransduction

Ryan ARMSTRONG[1], Mayeesha KHAN[1], Matthew GROL[1]

[1]Western University

INTRODUCTION: To adapt to repeated mechanical load produced by movement, tenocytes (tendon cells) translate mechanical signals into biological responses – a process known as mechanotransduction. Previous literature indicates that mechanical loading of tendons alters intracellular signalling and extracellular matrix synthesis; however, the mechanisms remain to be fully elucidated. Mechanical stimuli induce the release of nucleotides into the extracellular space that then activate P2X and P2Y receptors expressed by target cells. P2 receptors are essential for mechanotransduction in musculoskeletal tissues, such as bone; however, the expression and role of P2 receptors in tendon are unknown.

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

METHODS: Primary tenocytes isolated from the Achilles, patellar, and tail tendons of 1-2-month-old wildtype C57BL/6N mice were grown in 2-D culture. P2 receptor expression was assessed via qPCR on RNA extracted from these tenocyte cultures. Nucleotide-induced calcium signalling was investigated by loading 2-D tenocyte cultures with Fura-2-AM (calcium dye), adding exogenous nucleotide treatment and monitoring changes in calcium using live-cell fluorescence microscopy. In addition, the contribution of $P2Y_2$ was investigated using a $P2Y_2$-specific antagonist (AR-C 118925XX). The effect of nucleotide-induced calcium signalling on tenocyte gene expression was evaluated by treating tail tenocyte cultures with exogenous nucleotide and performing qPCR.

RESULTS: Gene expression analysis of 2-D primary Achilles, patellar, and tail tenocyte cultures showed predominant expression of P2X4, $P2Y_2$, $P2Y_6$, $P2Y_{12}$ and $P2Y_{13}$. In this regard, ATP or UTP treatments elicited transient intracellular calcium signalling compared to Veh in tenocyte cultures. As $P2Y_2$ is the only receptor expressed by these cells sensitive equally to both ATP and UTP, we further hypothesized that $P2Y_2$ mediates nucleotide-induced calcium signalling in these cultures. Indeed, we observed cross-desensitization of secondary calcium signalling between ATP and UTP as well as an attenuated intracellular calcium response with a $P2Y_2$-specific antagonist. Finally, treatment of tail tenocyte cultures with ATP acutely increased gene expression of interleukin-6 (*Il6*) and prostaglandin E2 (*Pgts2*), and increased expression of Scleraxis (*Scx*) – the tenocyte master transcription factor – at later time points.

SIGNIFICANCE: These results reveal that tenocytes express multiple P2X and P2Y receptors with $P2Y_2$ likely mediating intracellular calcium signalling elicited by exogenous ATP or UTP treatment. Additionally, exogenous ATP drove tenogenic gene expression. Future studies will investigate the role of P2 receptors in the response of tendons to mechanical loading *ex vivo* and *in vivo*.

Abstract #: 62
Presenting Author: Ryan Armstrong
Research Theme: Cell Biology of Connective Tissues
Preferred Presentation: Either Oral or Poster
Presenter Category: MSc Student

SESSION 4: Abstracts for Oral Presentations

Investigating the crosstalk between osteoarthritis synovial fibroblasts and monocyte/macrophages

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Background: Osteoarthritis (OA) is the most prevalent chronic joint disease with limited palliative treatments. Our lab has shown CD14+CD16+ intermediate monocytes-macrophages subset in the joint correlates inversely with OA pain, symptoms, and stiffness. Within OA synovium tissue, there is an accumulation of infiltrated peripheral monocytes and differentiated macrophage subsets, which undergo modulation by cues secreted by activated fibroblasts and through cellular interactions. These cells play a pivotal role in fueling joint inflammation and subsequent destruction. Our Western blot analyses of OA synovium tissues have underscored an upregulation in p-JNK/JNK levels, particularly in mid and late-stage OA patients compared to non-OA tissues, indicating an augmentation in proinflammatory MAP kinase signaling within OA synovium tissue. This study aims to explore the reciprocal effects of OA fibroblast cells on healthy peripheral monocytes/macrophages.

Method: Fibroblast cells were isolated from the synovium tissue of end-stage OA patients. Monocytes were subjected to differentiation into macrophages by M-CSF for five days, followed by a two-day polarization phase into M1, M2a, and M2c macrophages. Monocytes/macrophages were co-cultured with OA fibroblast cells for 72 hours. Surface markers of monocytes/macrophages were assessed through flow cytometry, while protein expression within OA fibroblast cells was analyzed via Western blotting.

Results: Co-culturing healthy peripheral monocytes with isolated OA fibroblasts led to a significant increase in the CD14+CD16+ intermediate population, accompanied by heightened expressions of CD163 and CD206. Furthermore, upon examining the impact of OA fibroblast co-culture on differentiated monocytes into macrophages, it was observed that OA fibroblasts decreased the expression of CD163 and CD206 while elevating the levels of pro-inflammatory markers such as HLA-DR and CD86 specifically on M2a macrophages, with no discernible change noted in M1 and M2C macrophages. CCL2 receptor (CCR2) and Lyve markers were also increased by OA fibroblast on M2a macrophages. Additionally, OA fibroblasts heightened the expression of the phagocytosis marker, MerTK on polarized macrophages compared to non-polarized macrophages. Furthermore, the addition of conditioned media from co-cultures of OA fibroblasts with macrophages to OA fibroblasts resulted in a significant increase in the expression of α-Smooth muscle actin and Collagen II proteins compared to OA fibroblasts cultured alone. In summary, our findings delineate distinct effects of OA fibroblasts on monocytes and macrophages, highlighting their role in promoting differentiation markers (CD163 and CD206) on monocytes, decreasing pro-resolving characteristics of M2a macrophages, while simultaneously augmenting their phagocytic capabilities and CCR2 markers. Also, macrophages induced the expression of myofibroblast proteins in OA fibroblasts.

Abstract #: 59
Presenting Author: mozhgan rasti
Research Theme: Cell Biology of Connective Tissues
Preferred Presentation: Poster
Presenter Category: Other

Investigating oxidative stress in human chronic wounds

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Dermal wound healing is an essential process that allows skin to maintain its ability protect against environmental stressors. Impairment of the healing process, associated with co-morbidities including diabetes mellitus, critical limb ischemia, and immobility, leads to the development of chronic wounds, classified as diabetic, venous, and pressure ulcers. Standard treatment for these wounds in the early stages is mostly limited to debridement and appropriate dressings of the wound to maintain moisture and offload pressure, while at end-stage amputation becomes necessary if possible. Overall, many experimental therapies targeting different aspects of wound chronicity have been unsuccessful at promoting wound closure leaving chronic wounds in a perpetual pro-inflammatory state. Many of the molecular mechanisms responsible for the chronic inflammation and subsequent impairment of healing processes are still not well understood. Although oxidative stress and hypoxia are considered a central part of the pathology, an analysis of human chronic wound tissue with respect to cellular markers has not yet been performed.
We hypothesized that the transcription factor, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) (pro-oxidant) would be upregulated and active in keratinocytes on the edge of non-healing wounds, that oxidative damage will be detected in keratinocytes and surrounding matrix through the presence of 8-hydroxy-2'-deoxyguanosine (8-OHG) (nucleic acids), 3-nitrotyrosine (3-NT) (proteins), and 4-hydroxynoneal (4-HNE) (lipids), and that the transcription factor nuclear factor erythroid 2–related factor 2 (Nrf2) (anti-oxidant) would not be active in keratinocytes on the edge of non-healing wounds. Additionally, we expect the predicted effects to be more severe in end-stage amputation tissue compared to early-stage debridement tissue.
Using antibodies specific to phosphorylated (activated) NF-kB, phosphorylated (activated) Nrf2, 8-OHG, 3-NT, and 4-HNE, immunohistochemistry was performed on samples of end stage human chronic wounds from lower limb amputations and debridement tissue from early presentation human chronic wounds. Activation of NF-kB and Nrf2, and accumulation of 8-OHG were determined based on nuclear localization, while 3-NT and 4-HNE were evaluated based on staining of intracellular components and the extracellular matrix.
Amputation (n=10) and debridement (n=5) samples did show evidence of pro-oxidant signaling and accumulation of oxidative damage in the epidermis and dermis. However, evidence of anti-oxidant signaling was also detected in these samples, raising further questions.
These observations suggest that dermal wound chronicity could be associated with the accumulation of oxidative damage in the tissue, as well as keratinocytes receiving multiple signals from antagonistic pathways resulting in impaired cellular functions necessary for wound closure.

Abstract #: 46
Presenting Author: Dylan Tinney
Research Theme: Skin and Matrix Biology
Preferred Presentation: Either Oral or Poster
Presenter Category: PhD Student

Lack of fibrilin-1 in maturing adipocytes leads to reduced body weight and perturbs metabolic homeostasis

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

[1]McGill University

INTRODUCTION: Fibrillin-1 is a 350 kDa extracellular protein that promotes elastic fiber formation and intersects with basement membranes in tissues such as adipose tissue. Mutations in fibrillin-1 lead to Marfan syndrome with either a lipodystrophic or overweight phenotypic appearance. Molecular mechanisms underlying this dichotomous role of fibrillin-1 remain elusive.

METHODS AND RESULTS: To investigate the role of fibrillin-1 in late adipogenesis, we have developed a novel adipose tissue-specific fibrillin-1 knockout mouse model deleting fibrillin-1 in maturing adipocytes (Fbn1-AKO) using the Adiponectin-Cre driver. Fbn1-AKO mice demonstrated a ~25% reduction in overall body weight at 30 weeks compared to controls. DEXA analysis showed a significant reduction in fat mass and percentage, whereas the lean mass was unaffected. The weight of metabolic organs such as liver, spleen, and brown adipose tissue remained unchanged between the control and Fbn1-AKO mice. Gene expression levels of adipogenic markers such as Adipoq, Cebpa, and Pparg and metabolic markers such as Glut4 and Fabp4 were significantly downregulated in the Fbn1-AKO mice. However, Fbn1-AKO mice showed no difference in the gene expression levels of inflammatory markers such as Mcp1, Tnfa, and Il1b. Immunofluorescence staining for the CD68 marker showed the absence of macrophages in the adipose tissue of Fbn1-AKO mice. H&E staining revealed that the white adipose tissue harvested from Fbn1-AKO mice had small-sized adipocytes (hypotrophy), whereas the morphology of brown adipocytes remained unaffected. Strikingly, these mice displayed severe insulin resistance, whereas the glucose metabolism was not affected. Serum lipid analysis showed that these mice had severe dyslipidemia marked by increased triglyceride and high-density lipoprotein levels. To explore the role of fibrillin-1 in the early phase of adipogenesis, we used primary mesenchymal stem cells from bone marrow (BM-MSCs) and adipose tissue (ASCs) harvested from wild-type mice and differentiated them in the presence of recombinant fibrillin-1. Surprisingly, the exogenous addition of a fibrillin-1 sub-fragment containing an integrin-binding RGD motif significantly reduced adipocyte differentiation of BM-MSCs and ASCs and gene expression levels of Adipoq, Cebpa, Pparg and Glut4. Contrary, a subfragment with an inactive RGA motif did not affect adipocyte differentiation.

CONCLUSION: In summary, the data suggest a biphasic role of fibrillin-1 in the early and late phases of adipogenesis, consistent with the dichotomous role of fibrillin-1 in Marfan syndrome with respect to adipose tissue. The data suggest an inhibitory RGD-mediated role of fibrillin-1 early in adipocyte differentiation, whereas it promotes metabolic homeostasis of mature adipocytes in fully developed adipose tissue.

Abstract #: 56
Presenting Author: Iram Fatima Siddiqui
Research Theme: Adipose Tissues and Blood Vessels
Preferred Presentation: Oral
Presenter Category: PhD Student

Human decellularized adipose tissue hydrogels as a novel culture and delivery platform for endothelial colony forming cell-mediated limb revascularization

John WALKER[2], Gillian BELL[1,2], John RONALD[1,2], Lauren FLYNN[1,2], David HESS[1,2]

[1]Robarts Research Institute; [2]Western University

Introduction: Critical limb ischemia (CLI) is the most severe form of peripheral artery disease and is characterized by resting pain and non-healing ulcers. With few effective treatment options, cell therapy to recover perfusion in ischemic limbs is under intense investigation. Human endothelial colony forming cells (hECFC) are proliferative progenitor cells with vessel formative function. However, revascularization after intramuscular (i.m.)-administration of hECFC has shown to be limited by poor survival and engraftment. To address this unmet clinical need, we are developing hydrogels derived from human decellularized adipose tissue (DAT) as a clinically relevant injectable bioscaffold with the goal of improving hECFC survival and function.

Hypotheses: Culture within DAT hydrogels will promote hECFC viability, proliferation and maintenance of a primitive pro-angiogenic phenotype. Furthermore, delivery within DAT hydrogels will improve hECFC engraftment and retention and accelerate the recovery of perfusion in immunodeficient NOD/SCID mice with femoral artery ligation-induced unilateral hind limb ischemia.

Methods: Human adipose tissue was decellularized and enzymatically digested to generate the thermally cross-linkable DAT pre-gels. hECFC isolated from umbilical cord blood were encapsulated in the DAT hydrogels, or OptiColTM human collagen type I or GeltrexTM murine basement membrane extract hydrogels as comparators. Flow cytometry was used to assess cell viability, proliferation, and primitive/mature marker expression after 1 and 6 days in culture. For the in vivo studies, hECFCs were transduced using lentiviral vectors to co-express firefly luciferase (fluc2) and tdTomato to enable longitudinal cell tracking through bioluminescence imaging. Mice were treated with a low-(2.5×105) or high-dose (1×106) of hECFCs i.m.-injected in the DAT hydrogels or saline, with control mice treated with cell-free DAT hydrogels or saline alone.

Results: Culture in DAT hydrogels showed comparable results to culture in OptiColTM human collagen type I hydrogels, which both demonstrated enhanced hECFC viability compared to culture in GeltrexTM basement membrane extract hydrogels ($p<0.05$). Lentiviral transduction allowed for stable expression of fluc2 and tdTomato ($p<0.0001$), with no impact on cell viability or proliferation. After transplantation, bioluminescence imaging demonstrated improved local viable hECFC retention in the mice treated with the low-dose hECFC delivered in DAT, with retention for up to 5 weeks post-delivery. Laser Doppler Perfusion Imaging performed weekly showed similar recovery of perfusion across all treatment groups.

Conclusion: These ongoing experiments will broaden our understanding of the effects of tissue-specific DAT hydrogels on hECFC function and advance the development of cell therapies using allogeneic hECFC for the treatment of CLI.

Abstract #: 31
Presenting Author: Agnes Terek
Research Theme: Adipose Tissues and Blood Vessels
Preferred Presentation: Either Oral or Poster
Presenter Category: MSc Student

SESSION 5: Abstracts for Oral Presentations

Mechanical loading of osteocytes via oscillatory fluid flow regulates prostate cancer cell extravasation to bone in vitro

Kimberly SEAMAN[1], Chun-Yu LIN[1], Xin SONG[1], Amel SASSI[1], William W. DU[2], Burton YANG[1,2], Yu SUN[1], Lidan YOU[1,3]

[1]University of Toronto; [2]Sunnybrook Research Institute; [3]Queen's University

Background: Bone metastasis occurs in the vast majority of advanced-stage prostate cancer patients. Exercise has been shown to attenuate metastatic tumour progression and preserve bone structure in recent in vivo studies. As the major mechanosensors and regulators of the bone microenvironment, the role of osteocytes under mechanical loading warrants further investigation. Previous in vitro studies have indicated that direct prostate cancer-osteocyte interactions in loading conditions promote cancer cell growth and migration. However, these findings are likely more reflective of late-stage colonization when the secondary tumour is in much closer proximity to the bone matrix. Therefore, the aim of this study is to elucidate the role of osteocytes during the initial stages of prostate cancer bone metastasis, specifically when cancer cells extravasate through the endothelial barrier before colonizing bone tissue. We hypothesize that osteocytes under mechanical loading may play an indirect role in early-stage prostate cancer bone metastasis by signalling to endothelial cells in the metastatic bone niche.

Methods: Both conditioned media and microfluidic approaches were used to investigate the mechanical loading of osteocytes on prostate cancer cell extravasation. Oscillatory fluid flow (OFF) was applied to osteocytes at a frequency of 1 Hz and a peak shear stress of 1 Pa for two hours in all experiments to simulate physiologically relevant flow experienced by osteocytes in vivo. Conditioned media collected from static or OFF-stimulated MLO-Y4 osteocyte-like cells were used to assess PC-3 prostate cancer cell adhesion and trans-endothelial migration. A well-established microfluidic tissue model capable of applying mechanical stimuli to osteocytes was used to assess PC-3 extravasation towards MLO-Y4 cells and primary osteocytes through a lumen lined with human umbilical vein endothelial cells (HUVECs).

Results: Adhesion and trans-endothelial migration assays indicated that mechanical loading of osteocytes reduced PC-3 adhesion to HUVEC monolayers and trans-endothelial migration compared to static controls. Moreover, mechanical loading of MLO-Y4 cells and primary osteocytes extracted from 2-month-old male mice reduced both the extravasation distance and rate of PC-3 cancer cells in the microfluidic device. Application of a neutralizing vascular cellular adhesion molecule 1 antibody to HUVECs abolished the difference in PC-3 adhesion and extravasation rate between static and OFF-stimulated groups.

Conclusions: Taken together, these findings will provide more information on the role of mechanical loading of osteocytes during the initial stages of prostate cancer bone metastasis, and aid in the development of osteocyte-targeted cancer mechanotherapies to improve patient outcomes.

Abstract #: 1
Presenting Author: Kimberly Seaman
Research Theme: Bone and Developmental Biology
Preferred Presentation: Oral
Presenter Category: PhD Student

Acute contact with profibrotic macrophages initiates myofibroblast activation in soft environment through αvβ3 integrin mediated activation of Piezo1

Maya EZZO[1,2], Katrin SPINDLER[2], Jun Bo WANG[1], Dahea LEE[2], Gilbert PECORARO[1], Justin COWEN[1], Pardis PAKSHIR[1], Boris HINZ[1,2]

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

The excessive scarring of organs after injury is called fibrosis and causes >40% of disease-related deaths worldwide. In a misguided attempt to repair, activated fibroblasts drive the destruction of organ architecture and function in fibrosis by accumulating and contracting collagen extracellular matrix into a stiff scar. The resulting stiff scar matrix in turn enhances fibroblast contraction, which raises the intriguing question of how this positive feedback loop is started. We show that direct contact with macrophages, in polarization states characteristic of the remodelling phase of tissue repair, triggers acute fibroblast contraction. The contractile response depends on αvβ3 integrin expression on the macrophage surface and the presence of Piezo1 stretch-activated channels in the fibroblast. The touch of a macrophage elevates cytosolic calcium in the fibroblast, followed by translocation of nuclear transcription factors NFAT1, YAP, and TAZ, all shown to drive the expression of genes associated with fibroblast activation and fibrosis. Intriguingly, macrophages induce stress responses in fibroblasts in soft environments that alone suppress spontaneous and/or profibrotic cytokine-induced fibroblast activation. Thus, we propose that acute contact with suitable, i.e., αvβ3 integrin expressing macrophages can mechanically kick-start fibroblast contraction, collagen remodelling, and activation in an otherwise non-permissive soft environment. The molecular components mediating macrophage-fibroblast mechanotransduction, like αvβ3 integrin, Piezo1, and NFAT are potential intervention targets for anti-fibrosis strategies.

Abstract #: 2
Presenting Author: Maya Ezzo
Research Theme: Cell Biology of Connective Tissues
Preferred Presentation: Poster
Presenter Category: PhD Student

Mechanical memory of myofibroblast features in mesenchymal stromal cells via GATA-Sall regulatory networks

Fereshteh Sasdat YOUNESI[1, 2], Andrew MILLER[3], Dr. Thomas H. BARKER[3], Dr. Boris HINZ[1, 2]

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

Background: Mesenchymal Stromal Cells (MSC) are used in cell therapy to reduce scarring following burn injuries. Autologous MSC is 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 a '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 for transcriptional factors (TFs). Hypothesis: Mechanical cues alter accessible regions for transcription regulators leading to the higher expression of pro-fibrotic genes and lower expression of regenerative genes in MSC. Objective: To enhance MSC regeneration potential by erasing mechanical MF memory.

Methods: MSC were cultured on skin-soft and scar-stiff substrates for 3 weeks (soft/stiff-primed MSCs), followed by switching to the respective other substrate MSCs with a mechanical memory. Mechanically primed MSC and MSCs with mechanical memory were assessed for transcription profiles by RNA-seq and for chromatin accessibility by Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq). We performed enrichment analysis of known TF binding motifs for differentially accessible regions and annotated differential accessible regions to differentially expressed genes. To explore the role of predicted TFs in MF activation, we assessed MF phenotypes of MSCs after a knockdown by measuring contraction capacity and the expression of profibrotic genes using Immunofluorescence and qPCR.

Results: Stiff-priming increased the transcription of MF and osteogenic genes and decreased the transcription of regeneration-associated genes in MSC, compared to soft-priming. Stiff-priming also induced and memorized the chromatin accessibility of regions predicted to bind mechanosensitive transcription factors, such as the GATA family. Also, stiff-priming reduced the chromatin accessibility and expression of TFs regulating MSC stemness, such as the Sall family. Silencing of the Sall family upregulated the GATA family and enhanced the myofibroblast activation (the increased number of alpha-smooth muscle actin (α-SMA) organization in stress fibres), contraction, expression pro-fibrotic genes. Conversely, knockdown of GATA TFs in stiff-primed MSCs restored reduced MF features after switching to soft environment, indicating GATA6 preserve MF memory of MSC.

Conclusions: The mechanically induced regulatory networks of Sall and GATA can be promising targets to consolidate regenerative features in MSC.

Abstract #: 11
Presenting Author: Fereshteh Younesi
Research Theme: Connective Tissue Repair, Regeneration, Bioengineering
Preferred Presentation: Either Oral or Poster
Presenter Category: PhD Student

Effects of low-magnitude high-frequency vibration on prostate cancer progression and bone metastasis

Amel SASSI[1], Kimberly SEAMAN[1], Xin SONG[1], Yu SUN[1], Lidan YOU[1,2]

[1]University of Toronto; [2]Queen's University

INTRODUCTION: Prostate cancer preferentially metastasizes to bone, which typically leads to bone pain and fractures. Notably, 80% of men who die from prostate cancer exhibit signs of bone metastases. To mitigate these effects, exercise is often recommended to cancer patients due to the beneficial effects on bone remodeling. However, physical activity may be challenging for elderly or bedridden patients. As such, vibration has emerged as a safe, effective, and easy to perform alternative therapy. Specifically, low magnitude high frequency (LMHF) has been shown to activate osteocytes and thereby reduce breast cancer cell migration. Nevertheless, the effects of vibration on prostate cancer progression and extravasation remains to be elucidated. We hypothesize that LMHF vibration (0.3 g, 60 Hz, 1h/day) will decrease prostate cancer cell extravasation through the activation of osteocytes and LMHF will directly influence prostate cancer cell growth.

METHODS: The bone-metastasis-on-a-chip model was fabricated using photolithography methods and each device is composed of a PDMS chip plasma bonded to a glass slide. MLO-Y4 cells (osteocyte-like cells) were seeded into the osteocyte channel. HUVECs (endothelial cells) and PC3s (prostate cancer cells) were seeded into the lumen channel. Microfluidic devices were placed on a custom-made vibration platform for 1 hour every day for 3 consecutive days. Extravasation distance for each of the side channels was quantified. To further examine how vibration stimulated osteocytes regulate PC3 extravasation, we examined the effects of PC3 adhesion onto HUVECs treated with either static or vibration-stimulated MLO-Y4 condition media (CM). Additionally, to assess the effects of vibration on prostate cancer cells, an apoptosis assay, viability, and colony formation assay was carried out.

RESULTS: LMHF vibration (0.3 g, 60 Hz, 1h/day for 3 days) significantly reduced extravasation distance by approximately 42.8%. We also observed that 29% more PC3 cells remained adhered to HUVECs in static MLO-Y4 CM when compared to vibration MLO-Y4 CM. Furthermore, a significant reduction in colony formation was observed following vibration treatment. Specifically, a significant reduction in cell growth and no changes in viability or apoptosis were determined.

DISCUSSION: Results suggest that LMHF vibration may be effective at reducing the incidence of prostate cancer bone metastases through a reduction in extravasation and colony formation. Specifically, the reduction in extravasation may be due to soluble factors secreted by vibration stimulated osteocytes that decrease the adhesion of prostate cancer cells to the endothelial monolayer. These findings highlight the transformative potential of LMHF vibration in cancer care.

Abstract #: 5
Presenting Author: Amel Sassi
Research Theme: Bone and Developmental Biology
Preferred Presentation: Oral
Presenter Category: MSc Student

SESSION 6: Abstracts for Oral Presentations

Matrix production or contraction: the myofibroblast eternal decision

Raquel BENITEZ[1], Boris HINZ[1,2]

[1]Keenan Research Centre; [2]University of Toronto

Introduction: The irreversible hypertrophic scarring from imperfect wound healing of large area skin wounds 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 (α-SMA).

Hypothesis: We hypothesize that 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.

Material and methods: In vivo MF differentiation was addressed with a Splint Wound model mimicking hypertrophic scarring responses in transgenic dual reporter mice expressing green fluorescent protein (GFP) under control of the collagen type I promoter (Col1α1) and red fluorescent protein (RFP) under α-SMA-promoter control. MF activation kinetics and reporter activities were assessed by analyzing the healing of full-thickness wounds inflicted on the backs of the mice either left to heal normally (relaxed) or splinted with a plastic frame (stressed).
Subcutaneous fibroblasts were isolated from the dual reporter mice previously described and cells were cultured on normal skin-soft or scar-stiff polymer substrates with or without TGF-β1. Ratios of α-SMA and Col1α1 promoter activities were determined using live videomicroscopy and flow cytometry. Cell contraction was assessed using elastomer substrates that exhibit visible 'wrinkling' upon cell force transmission, and expression of ECM and contractile proteins was analyzed by WB, immunofluorescence, qRT-PCR, and bulk RNA-seq.
Activated dual reporter MF on stiff environment were exposed either to soft environment or Knockdown acta2 gene and cell deactivation was analyzed by immunofluorescence, WB and qRT-PCR.

Results: In vivo mechanical stress increased MF participation at the wound edge in correlation with enhanced activity of α-SMA and Col1α1 promoters. Different fibroblast populations were found in the tissue depending on the activity of either α-SMA or Col1α1 promoters at specific areas/time points. Freshly isolated fibroblasts exhibited enhanced α-SMA promoter activity while Col1α1 promoter activity was reduced over time during MF activation. The promoter activity patterns were similar yet accelerated in fibroblasts cultured on stiff versus soft substrates and upon treatment with TGF-β1 in both conditions. Mechanical stress release promoted Col1α1 promoter activity.

Conclusions: MFs adapt ECM production and contraction, in vivo and in vitro, activities to the environmental needs over time based on mechanical and pro-fibrotic stimulation.

Abstract #: 9
Presenting Author: Raquel Benitez
Research Theme: Connective Tissue Repair, Regeneration, Bioengineering
Preferred Presentation: Poster
Presenter Category: Postdoctoral Fellow

Delivery of adipose-derived stromal cells within bioengineered granulation tissue substitutes to enhance skin regeneration

Baasil AFZAL[1]

[1]Western University

Background: Tissue-engineering strategies that enable the localized delivery of pro-regenerative cells, such as adipose-derived stromal cells (ASCs), have attracted interest for wound healing applications. Previously, our team developed novel cell-assembled scaffolds (Moriseette Martin et al., 2021), which have shown efficacy in inducing tissue regeneration. However, questions remain before this technology can be advanced to the clinic. First, comparative testing of scaffolds generated using ASCs from healthy versus type II diabetic (T2D) donors is needed to guide cell source selection (Aim 1). Second, the fabrication methods need to be scaled up to generate scaffolds for testing in a porcine wound healing model (Aim 2).

Methods: Human adipose tissue was decellularized and the decellularized adipose tissue (DAT) was further processed to generate DAT microcarriers, which form the base subunits of the modular scaffolds (Moriseette Martin et al., 2021). Aim 1. Scaffolds generated with mouse ASCs from wild-type C57BL/6 mice versus db/db mice as a model of T2D were compared to enable future testing in a db/db impaired wound healing model. The mouse ASCs were seeded onto the microcarriers in spinner flasks over 24 h, which were then transferred into moulds (3-mm h x 8-mm d) and cultured for 8 days. At the end of this assembly phase, the scaffold physical properties, ASC viability and distribution, cell abundance, and ECM composition were compared between the groups. Samples were also collected for comparative analysis of pro-angiogenic and immunomodulatory factors via ELISA. Aim 2. The fabrication methods were adapted to generate stable scaffolds incorporating porcine ASCs for testing in a porcine wound healing model (5-mm h x 20-mm d). The cell-assembly period was extended to 14 days and in vitro testing was performed to compare scaffolds generated with a low- versus high-seeding density, following methods described in Aim 1.

Results: Following optimization, stable scaffolds meeting the target size criteria were successfully generated using all cell sources, which contained a high density of cells distributed throughout dense ECM. There were no significant differences in cell viability, abundance, or ECM composition in scaffolds generated with wildtype versus diabetic mouse ASCs. Interestingly, the porcine seeding density did not significantly influence scaffold assembly or composition, suggesting there may be differences in cell proliferation between the groups.

Significance: This project represents early steps to validate our patented cell therapy platform for wound healing applications. Next steps include testing in pre-clinical models to advance towards commercialization and clinical translation.

Abstract #: 30
Presenting Author: Baasil Afzal
Research Theme: Connective Tissue Repair, Regeneration, Bioengineering
Preferred Presentation: Either Oral or Poster
Presenter Category: MSc Student

Exploring the long-term effects of a novel media formulation on chondrocyte viability and cartilage quality in stored osteochondral allografts

Sarah ALOI[1,2], Isabel LI[1,2], Margarete AKENS[1], Marc GRYNPAS[1,2], Paul KUZYK[1], Adele CHANGOOR[1,2]

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Background and Hypothesis: Osteochondral allograft transplantation is a surgical technique used to treat large focal cartilage lesions and involves the replacement of degraded cartilage with an osteochondral graft harvested from a cadaveric donor. Success rates of transplantation increase when chondrocyte viability in the donor graft is maintained at a minimum of 70% compared to fresh control. Effective storage of osteochondral allografts is critical for maintaining viability and quality of tissue prior to transplantation to allow sufficient time for mandatory disease testing. The institutional standard for storage used at Mount Sinai Allograft Technologies (Toronto, Canada), consisting of Lactated Ringer's solution, cefazolin, and bacitracin, maintains these levels for up to 14 days. Our group has developed a novel media that includes low glucose Dulbecco's Modified Eagle Medium, high molecular weight hyaluronic acid and doxycycline. It was hypothesized that by storing osteochondral tissues in the novel formulation, chondrocyte viability and histological appearance of the extracellular matrix would be maintained more effectively for up to 56 days of storage compared to the institutional standard.

Methods: Distal femurs from mature rabbits were stored at 4°C in either the novel media formulation or the institutional standard. All underwent weekly media changes. Samples were analyzed at day 0 (fresh control, n = 4), 28 (n = 3), 42 (n = 2), and 56 (n = 2). Chondrocyte viability was analyzed using confocal microscopy to detect cells stained with calcein AM and ethidium homodimer-1 for live/dead quantification. The extracellular matrix was visualized using safranin O/fast green and toluidine blue histological staining.

Results: Absolute cell viability was maintained above the 70% threshold in the novel media for up to 56 days but fell below 60% by 42 days in the institutional standard. Normalized to fresh controls, cell viability was maintained at 93.1% and 75.6% in the novel media at day 42 and 56, respectively, compared to 58.7% and 15.4% in the institutional standard. No qualitative differences in proteoglycan distribution up to 42-day storage were observed in histological sections. Day 56 sections have not yet been analyzed.

Conclusions: The novel media maintained superior chondrocyte viability in rabbit cartilage compared to the institutional standard up to 56 days. Further work is being done to optimize the formulation and characterize the cartilage extracellular matrix structure and function. These results support evaluation of the novel media formulation for the storage of human tissues, potentially enabling its eventual clinical use.

Abstract #: 60
Presenting Author: Sarah Aloi
Research Theme: Cartilage and Intervertebral Disc
Preferred Presentation: Either Oral or Poster
Presenter Category: MSc Student

SESSION 7: Abstracts for Oral Presentations

Regulation of precursor osteoclast proliferation in adolescent idiopathic scoliosis: insights into chondrocyte-osteoclast crosstalk via the TLR-M-CSF axis

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[1]McGill University; [2]Shriners Hospital for Children-Canada; [3]University of Alberta

Facet joint osteoarthritis (OA) is prevalent in young patients with adolescent idiopathic scoliosis (AIS) and might contribute to the disease progression and perceived pain. We previously found a negative correlation between OA severity and subchondral bone quality in facet joints from AIS patients, mirroring early-stage adult OA morphology. Toll-like receptor (TLR) activation, triggered by damage-associated molecular patterns (DAMPs), has been linked to OA pathogenesis in various joints, with its role in inducing pro-inflammatory mediators and proteases that drive cartilage degradation. Many of these pro-inflammatory mediators are believed to affect bone cells either directly or indirectly as they move through subchondral spaces from cartilage. Yet, the specific impact of TLR activation in cartilage on bone-regulating pro-inflammatory factors has not been fully explored in young scoliotic patients' facet joint chondrocytes. In the current study, we employed deep RNA sequencing to compare gene expression in chondrocytes between AIS patients with facet joint OA and age-matched healthy controls. The bioinformatics analysis uncovered 1426 differentially expressed genes (DEGs) that were significantly upregulated in the chondrocytes from AIS patients' facet joints. Notably, these up-DEGs were predominantly enriched in the TLR2 and 4 pathways, as well as chemokine/cytokine-mediated pathways. We also identified TLR2 as a central/hub gene within the top 1 cluster from the protein-protein interaction network, which underscores its pivotal role in cartilage degradation associated with AIS. Moreover, we discovered that M-CSF, vital for the proliferation and survival of osteoclast precursors, is directly regulated by TLR activation. Our experiments showed that media from TLR-activated chondrocytes could enhance the proliferation of osteoclast precursor cells in vitro. Furthermore, we performed EOS reconstructions of the scoliotic spine from patients with Lenke 1 and 2 curves. We also assessed M-CSF gene expression across different spinal levels. This enabled us to link increased M-CSF expression with both heightened cartilage OA severity and intervertebral rotation. In conclusion, our findings suggest that facet joint chondrocytes in AIS patients disrupt bone remodelling by promoting osteoclast formation via the TLR-M-CSF axis. This discovery highlights the potential for TLR inhibitors to treat facet joint osteoarthritis in AIS patients, offering the dual advantages of better cartilage health and bone loss prevention. Such improvements in facet joint health may contribute to spinal stabilization, which could slow down curvature progression and lessen or remove the need for surgical procedures.

Abstract #: 27
Presenting Author: kai sheng
Research Theme: Cartilage and Intervertebral Disc
Preferred Presentation: Oral
Presenter Category: PhD Student

Investigating the role of sex hormones in the intervertebral disc

Jeffrey HUTCHINSON[1], Cheryle SEGUIN[1]

[1]Western University

Introduction: The Global Burden of Disease study identified back pain as the most common cause of years lived with disability with a lifetime prevalence of over 80% in Canada, and socioeconomical impact estimated at $100 billion annually. Though complex, back pain is associated with intervertebral disc (IVD) degeneration in 40% of cases. The current research is based on clinical observations of increased IVD volume in competitive athletes and suspected anabolic steroid users. As increased IVD volume is not normally seen it may provide a therapeutic target for IVD degeneration. Moreover, sex hormone injections are more commonly being administered for the treatment of low back pain, despite limited knowledge on their effects in IVDs. Given this, we hypothesize that exposure to sex steroids will alter cell signaling within the IVD, resulting in matrix synthesis, and attenuation of matrix degradation associated with IVD degeneration.

Methods: Nucleus pulposus (NP) and annulus fibrosus (AF) cells were isolated separately from bovine caudal IVDs and treated with increasing doses of sex steroids (to 125 nM) for 72 h alone or following pre-treatment with TNFα (25 ng/mL) or IL-1β (10 ng/mL) (N=5) to model early IVD degeneration. Cells were harvested for RT-PCR analysis of extracellular matrix genes and markers of degeneration. An in-vivo murine model of age- and caudal injury- associated IVD degeneration was used to assess the effect of supraphysiological testosterone enanthate exposure on IVD health and injury through histopathology and uCT analyses.

Results: Acute exposure to steroid hormones did not alter extracellular matrix gene expression in NP or AF cells. However, both 5α-dihydrotestosterone and 17β-estradiol attenuated the pro-inflammatory response induced by IL-1β or TNFα in a cell type- and cytokine dependent manner. Testosterone and estrogen attenuated inflammatory genes in NP cells when stimulated with IL-1β, while AF cells showed this effect with TNFα stimulation. In vivo, testosterone injections increased body weight and abrogated the loss of disc height following injury. In addition, lower lumbar IVDs (L5/L6, L6/S1) displayed a significant increase in disc height with testosterone exposure as was seen clinically in suspected steroid users.

Discussion: Our data to date suggests that administration of sex hormones to the IVD may not directly regulate extracellular matrix production, but instead attenuate the deleterious effects of inflammation. Subsequent tissue and cell level characterization of our in vivo model will provide key evidence for the therapeutic use of steroids for IVD degeneration.

Abstract #: 45
Presenting Author: Jeffrey Hutchinson
Research Theme: Cartilage and Intervertebral Disc
Preferred Presentation: Oral
Presenter Category: PhD Student

Regenerative approaches to treat disc degeneration and low back pain

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

[1]McGill University; [2]Shriners Hospital for Children-Canada

INTRODUCTION: Low back pain is experienced by ~ 80% of individuals at some time in their lives 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. Despite the prevalence, little is known about the molecular mechanisms leading to IVD degeneration and its associated pain. 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. This study aims to evaluate the therapeutic potential of natural (o-Vanillin) and synthetic (RG7112) senolytics in an in vivo model of low back pain.

METHODS: Treatment of female and male sparc-/- mice began at 3–4 months of age just before the animals started signs of IVD degeneration and low back pain. Animals were treated by oral gavage bi-weekly for 6 months with either RG7112, o-Vanillin, or a combination of the two drugs. Grip strength, acetone-evoked behavior and mechanical sensitivity to von Frey filaments tests were assessed. High-resolution micro-CT scans of the spine were obtained to evaluate IVD volume and vertebral bone quality. IVDs were processed at the termination of the experiment, to evaluate SASP factor release using a Luminex assay.

RESULTS: Senolytic drug treatment of young sparc-/- mice significantly prevented the progression of IVD degeneration and behavioural signs of back pain. The pain scores were significantly lower at the end of the 6 months treatment in all treatment groups. Using Luminex assay we determined reduced levels of SASP factor release in the treated groups. We observed a significantly improved disc volume in the RG7112 and combination groups. In addition, the vertebral bone quality was significantly improved in all treatment groups.

CONCLUSIONS: The senolytic drugs o-Vanillin and RG7112 reduce pain and improve disc structure and bone quality in an in vivo mouse model of IVD degeneration and low back pain. Both drugs reduced pain behaviour, SASP factor release and improved IVD heath and bone quality with a more robust response when the drugs were given as a combination treatment.

Abstract #: 54
Presenting Author: Saber Ghazizadeh Darband
Research Theme: Cartilage and Intervertebral Disc
Preferred Presentation: Either Oral or Poster
Presenter Category: PhD Student

Developing a mineralized collagen-containing hydrogel for repairing bone

Sowmya VISWANATHAN[1,2,3], Eli SONE[1]

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Background: Recent advancements in repairing bone defects include the use of marrow-derived mesenchymal stromal cells (MSC(M)s) as an alternative to bone autografts. MSC(M)s can differentiate into osteoblasts that mineralize the extracellular matrix. However, the effectiveness of injecting cells alone is limited by poor retention at the defect site without an encapsulation material. Thus, there are increased efforts in engineering biomaterials, like injectable hydrogels, to address this challenge.
Objective/Hypothesis
A body of in vitro and in vivo evidence suggests that biomaterials mimetic of bone components (mineralized type I collagen) and substrate stiffness can inherently upregulate MSC(M) differentiation and bone mineral production. Therefore, I hypothesize that a biomimetic and mechanically stiff hydrogel containing mineralized type 1 collagen can accelerate bone repair by enhancing MSC(M) differentiation kinetics.
Methods
Lyophilized mineralized collagen, DMEM, and soluble collagen were combined to create hydrogels that form in situ upon injection into physiological conditions. To develop hydrogels with different stiffnesses, the amount of mineralized collagen, concentration of soluble collagen, and genipin crosslinking were varied. The stiffness was measured as the storage modulus using a rheometer. MSC(M) differentiation in vitro in the hydrogels will be assessed by qPCR and quantitative protein analyses. von Kossa assays and electron microscopy will be used to assess mineral nodule production.
Results
Increasing the concentration of soluble collagen from 3 to 4mg/mL improved the hydrogel stiffness by 3.5-fold. Control hydrogels without mineralized collagen have storage moduli between 150-200Pa. Mineralized collagen-containing hydrogels are significantly stiffer ($p<0.0005$) by 3-fold.
Conclusion
Preliminary rheology results show that the viscosity of soluble collagen and the incorporation of mineralized collagen both improve the stiffness of collagen-based hydrogels. The effect of varying genipin crosslinking on hydrogel stiffness and differentiation kinetics will be explored next.
Significance
My project entails the development of a hydrogel that is mimetic of native bone composition for progenitor cell differentiation and subsequent bone repair. The ability to tune the stiffness of the encapsulation system will broaden the applicability of current MSC(M) therapy.

Abstract #: 21
Presenting Author: Zi Xuan Zhang
Research Theme: Connective Tissue Repair, Regeneration, Bioengineering
Preferred Presentation: Either Oral or Poster
Presenter Category: PhD Student

SESSION 8: Abstracts for Oral Presentations

Cell and transcriptomic diversity of infrapatellar fat pad during knee osteoarthritis

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OBJECTIVE: Knee osteoarthritis (KOA) is the most common form of arthritis. Obesity and female sex are great risk factor for developing this disease. The infrapatellar fat pad (IFP) is the largest FP within the knee however, its role in KOA is not well understood. Furthermore, the cell populations contributing to KOA remain to be fully characterized. This study aims to identify the distinct cell populations within the IFP that may contribute to KOA pathogenesis and how obesity and sex impacts this process using single-nucleus RNA sequencing (snRNA-seq) and spatial transcriptomics.

METHODS: In total, n=21 IFP, n=15 KOA-IFPs (n=15; n=7 normal BMI 18.5-25 kg/m2, n=8 obese BMI 30-40 kg/m2) and n=6 normal IFPs (n=6; obtained <4h post-mortem) were analyzed. Nuclei underwent snRNA-seq on an Illumina NextSeq 550 using the 150bp high output sequencing kit. Data was processed using Cell Ranger while differential gene expression testing determined a gene signature. IFP (n=10) were also spatially sequenced using Visium CytAssist techniques and analyzed using Seurat.

RESULTS: Using snRNA-seq, 73,808 nuclei were analyzed from 21 IFP samples. Cluster analysis revealed eight cell types present, with major populations being fibroblasts, macrophages, adipocytes, and endothelial cells. Independent cluster analysis of each major cell type elucidated multiple subsets, each with a unique transcriptomic profile. Spatial sequencing confirmed fibroblasts, macrophages, adipocytes, and endothelial cells are the major cell types present across the IFP. We also spatially resolved transcriptomic markers of each fibroblast subset. Fibroblasts were the predominant cell type based on proportion of nuclei; thus, downstream analysis focuses on fibroblasts. Within KOA (n=15) compared to healthy IFPs (n=6), 38 transcriptomic markers were differentially expressed: 20 upregulated and 18 downregulated genes. Comparing female KOA-IFP (n=8) vs male (n=7) revealed 105 differentially expressed genes: 35 upregulated and 70 downregulated. Analysis of obese (n=8) vs normal BMI (n=7) KOA-IFP uncovered 21 differentially expressed transcriptomic markers: 10 upregulated and 11 downregulated genes. Of the upregulated genes, 3 were significantly upregulated within spatial analysis. GO and Pathdip analysis identified enriched biological processes and pathways linked to identified transcriptomic markers, suggesting differences in fibroblast function within KOA-IFP based on sex and BMI.

CONCLUSIONS: Using snRNA-seq and spatial sequencing, we have identified distinct cell subsets of fibroblasts, adipocytes, macrophages, and endothelial cells in IFP with KOA, and transcriptomic differences based on OA status, sex, and BMI. Our ongoing efforts will help characterize the role and function of these identified cell subsets in KOA pathogenesis.

Abstract #: 6
Presenting Author: Hayley Peters
Research Theme: Adipose Tissues and Blood Vessels
Preferred Presentation: Either Oral or Poster
Presenter Category: MSc Student

Sequencing identifies microRNAs that distinguish early osteoarthritis and early rheumatoid arthritis

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Background: Osteoarthritis (OA) and rheumatoid arthritis (RA) can present similarly, especially at early stages of single-joint disease when symptoms (e.g., pain) can overlap. OA is commonly assessed by radiographic Kellgren-Lawrence (KL) grading, but these features are less obvious in early stages. While serological markers are primarily used for RA diagnosis, these can overlap with other inflammatory conditions. These limitations can lead to delayed diagnosis and disease management. Accordingly, there is a need for biomarkers that can reliably detect early OA (EOA) and early RA (ERA). Among such promising candidates are microRNAs, small non-coding RNAs, that have emerged as biomarkers and mechanistic regulators of joint diseases. Herein, we aim to use sequencing as an unbiased and comprehensive approach to identify circulating microRNAs that can distinguish EOA, ERA, and non-OA/RA individuals.

Methods: Plasma samples were acquired from the HFH Arthritis Biobank, Nashville Vanderbilt VA Medical Center Biobank, and Osteoarthritis Initiative. EOA (N=12) included OA individuals with symptoms (e.g., pain) and KL grade 0 or 1, ERA (N=6) included treatment-naïve RA individuals with <6 months of symptoms, while non-OA/RA (N=44) individuals had no history of joint disease. Following microRNA-sequencing, analysis through an optimized sequencing pipeline involved alignment to reference databases, filtering, and normalization to total counts. Data clustering was performed using principal component analysis (PCA) and differential expression (DE) analysis was performed using a quasi-likelihood F-test. Finally, proportion analysis with progressing stringency was used to select biologically relevant microRNAs.

Results: PCA showed clustering between EOA versus ERA and non-OA/RA samples, but no clear clustering for ERA versus non-OA/RA nor for confounding variables such as sex, age, or BMI. DE analysis identified 170 and 305 microRNAs significantly dysregulated in EOA versus ERA and non-OA/RA, respectively, but none for ERA versus non-OA/RA. Final filtering of microRNAs based on proportion of expression (higher or lower) across samples within each cohort comparison found 13 upregulated and 151 downregulated microRNAs in ≥83% of EOA samples compared to ERA and non-OA/RA, while 4 microRNAs were upregulated and 4 were downregulated in ≥83% of ERA samples compared to EOA and non-OA/RA.

Conclusion and Future plans: This is the first sequencing study to directly compare circulating microRNAs in EOA, ERA and non-OA/RA individuals. Future experiments are aimed at validating prioritized microRNAs in independent samples, performing pathway analyses to explore their role in each disease, and developing a predictive model to distinguish EOA and ERA.

Abstract #: 25
Presenting Author: Madhu Baghel
Research Theme: Other
Preferred Presentation: Either Oral or Poster
Presenter Category: Postdoctoral Fellow

Spatial transcriptomics of joint space-interfacing tissues using a pre-clinical mouse model of osteoarthritis

Teodora TOCKOVSKA[1], Jason ROCKEL[2], Starlee LIVELY[2], Keemo DELOS SANTOS[2], Himanshi GUPTA[2], Mohit KAPOOR[2]

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Purpose: Osteoarthritis (OA) is a progressive disease that affects multiple joint tissues. Spatial transcriptomics is an emerging technology that allows for the sequencing of transcripts across 5000 co-ordinate spots of 55 μm in diameter in a 2-D histological section. We sought to determine spatially-distributed gene expression changes in synovium, meniscus and articular cartilage, using naïve and surgically-induced OA mice.

Methods: Twelve-week-old C57BL6/J mice were subjected to destabilization of the medial meniscus (DMM) surgery to induce OA (n=3), with surgically-naïve mice as controls (n=3). Limbs were collected 5-weeks post-DMM, or at 17 weeks of age (naïve) and paraffin embedded. Limbs were sectioned (5 μm), and mouse transcriptome probes were hybridized to RNA targets. Libraries of hybridized probes were sequenced at a depth of 25000 read pairs/spot. Spatial transcriptomics analysis was completed using Seurat v5. Synovium, meniscus and articular cartilage were traced on tissue images to identify voxels within tissues of interest. Normalization was completed using SCTransform, followed by PCA. FindMarkers was utilized for the differential expression (DE) analysis (DESeq2 test method). DE analysis compared 5-week DMM against naïve samples. Gene ontology (GO) enrichment analysis was completed using the Goseq R package.

Results: Using spatial voxels only within cartilage, synovium and meniscus, DE analysis yielded 201 significant DEGs (FC ≥ 1.5, q ≤ 0.05). Many of the 181 upregulated DEGs were extracellular matrix genes including collagens (Col2a1, Col1a1) and aggrecan with the top 30 enriched GO terms primarily related to extracellular matrix (ECM). The 20 significantly downregulated DEGs were abundant in histone genes, with the top 30 enriched GO terms related to chromatin structure. The downregulated genes were abundant in histones. The top upregulated DEGs Nfasc and Acan showed spatially-localized changes primarily in the synovium, while Acan was upregulated across all three selected tissues. The top downregulated DEG Hist1h3d was downregulated across all tissues while Hist1h1a was primarily downregulated in meniscus.

Conclusions: Spatial transcriptomics can detect DEGs which can be localized to joint space-interfacing tissues when comparing DMM and naïve mice. Advancements including smaller voxel sizes allowing for higher resolution spatial expression analysis will be key for improved spatial gene expression analysis using small animal models of OA, as tissue size is a limitation for current analyses. We are increasing the sample size of our naive and DMM groups to further refine our dataset.

Abstract #: 13
Presenting Author: Teodora Tockovska
Research Theme: Cell Biology of Connective Tissues
Preferred Presentation: Oral
Presenter Category: Other

SESSION 9: Abstracts for Oral Presentations

Oral delivery of delta-9-tetrahydrocannabinol provides symptom and disease modification in mouse models of knee osteoarthritis

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Objectives: Osteoarthritis (OA) is a joint disease that results in cartilage degeneration and synovitis. Some OA patients use cannabis to alleviate pain. Delta-9-tetrahydrocannabinol (THC), a prominent phytocannabinoid from cannabis, can signal via cannabinoid receptors expressed on joint cells, including chondrocytes and fibroblast-like synoviocytes (FLS). We investigated the effects and signalling mechanisms of THC on pain and disease modification in pre-clinical models of knee OA.

Methods: Destabilization of the medial meniscus (DMM) mice were administered THC (1, 5 or 10 mg/kg) 1 day/week by intra-articular (IA) injection or 5 days/week by oral gavage for 9 weeks. Monosodium iodoacetate (MIA) mice were given THC (5 or 10 mg/kg) 5 days/week by oral gavage for 2 weeks. Von Frey tests were used to evaluate pain at baseline and throughout treatment for DMM and MIA mice. DMM mouse joints were assessed for cartilage degeneration/synovitis (OARSI scoring) and Ki67/αSMA expression (immunohistochemistry [IHC]). Plasma samples collected from DMM mice were subjected to a multiplex immunoassay analysis to detect changes in systemic levels of 23 cytokines, chemokines, and growth factors. RNA sequencing was performed on human OA FLS and chondrocytes treated with 1 uM THC to determine differentially expressed genes (DEGs) and DEG-enriched pathways via PathDIP.

Results: IA administration of THC to DMM mice accelerated cartilage degeneration, increased synovitis, and failed to attenuate pain at all doses (n=5/group). Oral gavage of 10 mg/kg THC provided the most significant pain reduction in DMM and MIA mice (n=15/group). In DMM mice, all THC doses reduced cartilage degeneration, with 10 mg/kg THC reducing synovitis (n=9-10/group) and decreasing αSMA but not Ki67 synovial expression (n=6/group). 10 mg/kg THC did not significantly alter plasma concentrations of the tested cytokines, chemokines, and growth factors (n=9-10/group). RNA sequencing identified 73 DEGs in OA FLS and 21 DEGs in OA chondrocytes after 1 uM THC treatment (n=4). Extracellular matrix (ECM) organization and cholesterol biosynthesis pathways were enriched in upregulated and downregulated genes, respectively, in both cell types.

Conclusions: 10 mg/kg THC reduced pain, cartilage degeneration, synovitis, and synovial αSMA expression in DMM/MIA mouse knee joints. THC treatment of human OA FLS and chondrocytes modified gene expression associated with ECM organization and cholesterol biosynthesis. Ongoing experiments include performing targeted metabolomics analysis on DMM mouse plasma to identify metabolic changes in response to THC and performing single nuclei RNA-sequencing on L3-L5 dorsal root ganglia of MIA-injected mice to identify novel mechanisms by which THC can reduce pain.

Abstract #: 10
Presenting Author: Anca Maglaviceanu
Research Theme: Cell Biology of Connective Tissues
Preferred Presentation: Either Oral or Poster
Presenter Category: PhD Student

An atlas of the synovium in knee osteoarthritis: fibroblast activation defines disease progression

Kabriya THAVARATNAM[1, 2, 3], Eric GRACEY[4], Shabana VOHRA[2, 3], Jason ROCKEL[2, 3], Anusha RATNESWARAN[2, 3], Chiara PASTRELLO[2, 3], Igor JURISICA[2, 3], Paramvir KAUR[2, 3], Starlee LIVELY[2, 3], Himanshi GUPTA[2, 3], Pratibha POTLA[2, 3], Sam DUPONT[4], Raja RAMPERSAUD[2, 3], Nizar N. MAHOMED[2, 3], Rajiv GANDHI[2, 3], Jan VICTOR[4], Nele ARNOUT[4], Dirk ELEWAUT[4], Mohit KAPOOR[1, 2, 3]

[1]University of Toronto; [2]Krembil Research Institute; [3]University Health Network; [4]Ghent University

Purpose: Knee osteoarthritis (KOA) is a progressive joint disease affecting multiple tissues, including the synovium. The synovium is a connective tissue that lines the inner joint cavity ensuring joint lubrication through synovial fluid production. In osteoarthritis (OA), synovium undergoes changes including inflammation, cellular proliferation, and hyperplasia. Studies investigating the contribution of the synovium to OA disease progression, in comparison to cartilage, remain insufficient. To identify the key synovial cellular components in early and late stages of knee OA synovial pathology and characterize their transcriptomic profiles to elucidate their cellular roles we utilized high-throughput single-nuclei RNA sequencing (snRNA), bulk RNA sequencing and flow cytometry on human OA biospecimens and mouse model of knee OA.

Methods: Single nucleus RNA sequencing (snRNAseq) was conducted on synovial tissues from early- (KL1; n=5) and late- (KL3/4; n=4) stage radiographic KOA patients, as well as on synovia from naïve mice (n=3) and mice undergoing destabilization of the medial meniscus surgery at 2 (n=4) or 10 weeks (n=3) post-surgery. Bulk RNA sequencing (KL1; n=6, KL3/4; n=8) and flow cytometry (healthy; n=5, KL1; n=10, KL3/4; n=14) combined with immunohistochemistry were employed on KOA synovia. Bioinformatic analyses identified cell types and subtypes, differentially expressed genes (DEG), pathways and potential transcriptional regulators associated with disease progression.

Results: SnRNAseq discerned nine fibroblast subtypes in KL1 and KL3/4 radiographic KOA synovia. Early-stage KOA synovia exhibited a higher proportion of nuclei in subclusters 1, 2, 4, and 6, while late-stage exhibited a higher proportion in subclusters 0, 3, and 5. Flow cytometry confirmed the expansion of ITGB8+ (cluster 0) and a contraction of DPP4+ (cluster 1) with OA progression. Pathway analyses revealed that human fibroblast clusters 0 and 1 primarily participated in extracellular matrix (ECM)-related pathways. Bulk RNA sequencing confirmed upregulation of late-stage genes enriched for matrisome-related genes. SnRNAseq of mouse synovium unveiled analogous fibroblast subtypes to human synovia. The late-stage human subcluster 0 resembled mouse cluster 1 and the prevalent early-stage human subcluster 1 resembled mouse cluster 4. Catrin analysis identified two putative upstream transcription factors of ECM genes from DEG lists of human clusters 0 and 1, namely ELF1, and BHLHE40, upregulated in late-stage cluster 0.

Conclusions: Our multi-omics approach identified fibroblast subtypes linked to ECM pathways, suggesting their potential role in stage-specific OA pathology.

Abstract #: 22
Presenting Author: Kabriya Thavaratnam
Research Theme: Cell Biology of Connective Tissues
Preferred Presentation: Either Oral or Poster
Presenter Category: PhD Student

The oligomeric structure of microfibril-associated glycoprotein 4 determines its function in elastogenesis

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INTRODUCTION. Microfibril-associated glycoprotein 4 (MFAP4) is a 36-kDa extracellular protein with critical roles in elastic fiber formation in elastic tissues, including the aorta and skin. MFAP4 is associated with microfibrils and elastic fibers, which are essential extracellular fiber systems to confer elasticity. MFAP4 directly interacts with essential elastogenic proteins fibrillin-1 and tropoelastin and with cells via integrins, but its role in elastic fiber formation is unclear.
MATERIALS AND METHODS. We used cryo-electron microscopy and single particle analysis to resolve the structure of purified human MFAP4, atomic force microscopy (AFM), dynamic light scattering (DLS), surface plasmon resonance (SPR) spectroscopy, and fibroblast culture to assess the protein size, conformation, and binding to elastogenic proteins and cells.
RESULTS. Cryo-electron microscopy showed that MFAP4 assembles in the presence of calcium as an octamer, where two sets of homodimers constitute the top and bottom halves of each octamer. This data was further validated by AFM and DLS. Each of the homodimers is linked together by an inter-molecular disulfide bond. An engineered C34S missense mutation in MFAP4 prevented disulfide bonding between monomers, but the mutant still assembles octamers. We build an MFAP4 atomic model at 3.55 Å resolution and describe the intermolecular linkages in the octamer. Salt bridges are important for the interaction of the homodimers, while non-polar interactions are essential for tetramer halves to assemble octamers. In the absence of calcium, MFAP4 dissociates to tetramers, resembling the octamer halves. Replacement of calcium with magnesium does not induce octamer reconstitution from tetramers. SPR binding studies with elastogenic proteins, including fibrillin-1, tropoelastin, LTBP4, and small fibulins, showed that MFAP4 has multiple surfaces for protein-protein interactions, which depend upon the higher-order assembly of MFAP4. While the disulfide bond mediated by C34S contributes little to those protein interactions, it modulates cell interaction. When MFAP4 forms assemblies with fibrillin-1, it abrogates MFAP4 interactions with cells.
CONCLUSIONS. MFAP4 forms octamers in the presence of calcium and tetramers in the absence of calcium or the presence of magnesium. This study provides detailed molecular structure-function relationships of MFAP4 interactions with elastogenic proteins and cells.

Abstract #: 24
Presenting Author: Dieter Reinhardt
Research Theme: Skin and Matrix Biology
Preferred Presentation: Oral
Presenter Category: Principal Investigator

Investigating the use of intra-articular injections of GSK3787 for osteoarthritis

Ermina HADZIC[1], Xueli MEI[1], Holly DUPUIS[1], Garth BLACKLER[1], C. Thomas APPLETON[1], Elizabeth GILLIES[1], Frank BEIER[1]

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Background: Osteoarthritis (OA) is a whole-joint disorder with no disease modifying drug (DMOAD) to stop, slow, or prevent the disease, so there is a research emphasis on therapeutic targets. Nuclear receptors are transcription factors that are of particular interest, as 23 nuclear receptors are dysregulated in osteoarthritic cartilage. The peroxisome proliferator activated receptor (PPAR) is one of the altered nuclear receptors in OA. Chondrocytes treated with a PPARδ agonist upregulate ADAMTS and MMP genes, which may lead to increased cartilage breakdown. A previous study noted that surgically-induced OA was attenuated in cartilage-specific Ppard deficient mice. A follow-up pilot study was conducted and found that pharmacological inhibition of PPARδ (GSK3787) may decrease pain-related OA behaviours, although the daily systemic injections presented logistical issues. In the present study, we utilized hydrogels to encapsulate GSK3787 for intra-articular (IA) injections in rats, which is more feasible than the previous systemic injections.

Methods: OA was surgically-induced in 9.5 week old male Sprague Dawley rats via anterior cruciate ligament transection with destabilization of the medial meniscus (ACLT-DMM). The control group underwent a sham surgery at the same timepoint. Animals were then randomized to receive either PBS, hydrogel only, or hydrogel+GSK3787 IA injections at 1-week post surgery and 8-weeks post-surgery. Rats were taken to an 8-week endpoint (N = 48) or 16-week endpoint (N = 48) for a total of N = 8 per group. Behavioural data and knee joints were collected for analysis.

Results: Mechanical allodynia was increased at weeks 4 – 12 in the PBS treated OA rats compared to baseline but did not significantly change in the hydrogel+GSK3787 group. Pressure hyperalgesia was increased at week 4 in the hydrogel+GSK3787 treated OA rats, but this was significantly improved at week 8 and 16 compared to baseline. Cartilage damage is significantly decreased in the OA hydrogel and OA hydrogel+GSK3787 rats compared to the OA PBS group, and subchondral bone damage in the hydrogel+GSK3787 trends towards less damage than the PBS group at the 8-week endpoint. Tissue damage in the lateral compartment was not significantly different between the OA and sham groups, regardless of treatment.

Conclusions: Treatment of hydrogel+GSK3787 appears to modulate pain behaviours and there may be structural benefits to hydrogel+GSK3787. Future analysis is in progress to comprehensively understand the feasibility of this treatment.

Abstract #: 36
Presenting Author: Ermina Hadzic
Research Theme: Cartilage and Intervertebral Disc
Preferred Presentation: Oral
Presenter Category: PhD Student

Characterizing PANX mutants and identifying their implications in erosive osteoarthritis

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Erosive osteoarthritis (EOA) is a degenerative joint disease characterized by severe inflammation in the interphalangeal joints. Due to the multifactorial nature of EOA and an unclear understanding of the biological mechanism that contribute to disease progression, there are no cures for EOA. Recently, we identified two families at high-risk of EOA with dominant mutations in PANX1 and PANX3. These genes encode for two mechanosensitive channel-forming glycoproteins, Pannexin 1 (PANX1) and Pannexin 3 (PANX3) respectively. Channels formed by PANX1 and PANX3 are important in the development and maintenance of joint tissues by facilitating ion and metabolite passage during autocrine and paracrine signalling. We aim to characterize the effect of the PANX1 intracellular loop mutation (PANX1 IL-MT) and the PANX3 N-terminal mutation (PANX3 NT-MT) on protein biochemistry, localization, stability, and cell viability. Both mutants were observed to have an affected glycosylation status, with an increase in the high-mannose (Gly1) species and a decrease in the complex glycosylation (Gly2) species in comparison to wild-type (WT) PANX1 and PANX3 when overexpressed in Hs578T and U2OS cell models (lacking endogenous PANX1 and PANX3 respectively). Furthermore, PANX1 IL-MTs have the presence of an Endoglycosidase H-resistant intermediate glycosylation species reflecting the impediment of the glycosylation modification mechanism from Gly1 into Gly2. However, this PANX1 IL-MT was not observed to have a change in localization when compared to PANX1 WT. In contrast, the PANX3 NT-MT was observed to have increased intracellular colocalization with calnexin at the endoplasmic reticulum (ER) and in lysosomes, thereby reflecting potential ER-Phagy. Furthermore, energy minimization modelling has identified altered pore-channel structures for both PANX1 IL-MT and PANX3 NT-MT. More specifically, the PANX1 IL-MT channel has a conical frustrum shaped-pore on the intracellular mouth with increasing diameter, while the PANX3 NT-MT has both an increased pore-diameter on the intracellular mouth and a conical frustrum shaped-pore on the extracellular mouth with increasing diameter, in comparison to their respective wild-types. Furthermore, we discovered a destabilization of the PANX3 NT-MT subunit and altered pore-lining electrostatic densities for both mutants. Our findings suggest that the PANX1 IL-MT and PANX3 NT-MT may contribute to the development of EOA by changing the biochemistry and localization of the protein. These mutations are amongst the first genetic factors identified for EOA, and the first-ever reported germline mutation for PANX3. Understanding these mutations will elucidate the role that PANX1 and PANX3 plays in EOA development and their potential as novel targets of EOA treatments.

Abstract #: 41
Presenting Author: Justin Tang
Research Theme: Cell Biology of Connective Tissues
Preferred Presentation: Poster
Presenter Category: PhD Student

Non-invasive electroarthrography to monitor cartilage in an equine model of early osteoarthritis

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INTRODUCTION: Osteoarthritis (OA) is a degenerative joint disease characterized by progressive and permanent cartilage degradation. Low-grade, asymptomatic cartilage deterioration occurs early in the progression of OA, however, clinical assessments, including radiography and physical examination, are unable to detect these changes. Monitoring of early OA may identify opportunities for interventions to slow disease progression. Electroarthrography (EAG) is a non-invasive method for measuring the electrical signals produced by cartilage during load bearing that correlate to cartilage biomechanical properties. This study aimed to determine if EAG could track cartilage changes in an equine model of early OA.

METHODS: Six standardbred horses underwent surgical creation of an osteochondral fragment on the medial aspect of the proximal phalanx and daily post-operative treadmill running to induce changes similar to naturally occurring OA. Six weeks after surgery, horses received intra-articular treatment with a biologic (n = 3) or saline (n = 3). EAG measurements involved placing six adhesive electrodes on skin around the joint line and compressing the joint by lifting the contralateral leg to shift weight to the instrumented forelimb. EAG was collected 2 weeks before surgery as well as 2, 4, 8, and 10 weeks after surgery. Ground-reaction force (GRF) was captured using a custom-built force mat. EAG amplitudes from two electrodes in closest physical proximity to the osteochondral fragment were normalized to GRF and combined (EAGcomb). Lameness was quantified subjectively (graded 0-5) and objectively using a kinematic tool (Equinosis). MRI was performed before treatment (week 4) and after treatment (week 10) and graded using the Whole-Organ Magnetic Resonance Imaging Score (WORMS). One-way ANOVA was used for statistical comparisons.

RESULTS: Five weeks after surgery all horses had significantly lower EAGcomb compared to pre-operative measurements ($p < 0.001$). Saline-treated horses displayed no statistical difference in EAGcomb when comparing values after surgery (weeks 2 and 4) to values after treatment (weeks 8 and 10, $0.096 < p < 0.146$). Biologic-treated horses displayed significantly higher EAGcomb after treatment (weeks 8 and 10), compared to values before treatment (weeks 2 and 4, $p < 0.001$). EAGcomb was significantly correlated with subjective ($r=-0.494$, $p=0.014$) and objective ($r=-0.521$, $p=0.009$) lameness. A weak correlation was observed with WORMS scoring ($r=-0.467$, $p=0.126$).

CONCLUSION: EAG, a non-invasive measure of cartilage quality, successfully tracked cartilage degradation after surgery and subsequent changes due to treatment. These data support development of EAG into a clinical methodology for monitoring and diagnosis of OA and other degenerative joint diseases.

Abstract #: 64
Presenting Author: Peter Suderman
Research Theme: Cartilage and Intervertebral Disc
Preferred Presentation: Either Oral or Poster
Presenter Category: PhD Student

Lysophosphatidylcholine and microvascular dysfunction in knee osteoarthritis

Hanyu JIANG[1], Tom APPLETON[1]

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Synovial microvessels provide the joint organ with oxygen and nutrients. Angiogenesis, or the growth of new microvessels, and its normal function are crucial in the wound healing process in knee osteoarthritis (OA). OA causes failure of the joint organ system, resulting in pain and damage to the joint, but the mechanisms are unclear. We found high levels of lysophosphatidylcholines (LPC) in synovial fluid (SF) and synovial microvessel dysfunction (MVD) are both associated with worse pain and tissue damage in patients with knee OA. However, little is known about the role of LPCs in OA. LPC can be metabolized to lysophosphatidic acid (LPA) via the enzyme autotaxin (ATX). From literature, LPA is abundant in the synovium and may act on LPA receptors (LPAR1-6). Since LPA increases pain in animal models and endothelial cell dysfunction in other diseases, LPA may be a mediator of MVD in OA.

We will characterize LPAR expression profile in synoviocytes using single-cell RNA sequencing and immunofluorescence. The effects of knee OA patient SF and LPAR inhibitors on human microvascular endothelial cells (HMEC-1) will be explored in vitro using wound closure, permeability, and proliferation assays, as well as staining/gene analyses (such as Rho/ROCK and actin polymerization/depolymerization pathways). Additionally, the effects of LPA and LPAR inhibitors in male and female rat synovial microvasculature in vivo will be examined using histology and micro-CT.

Our scRNAseq dataset of knee OA patient synovium revealed endothelial cells as well as lining and perivascular fibroblasts expressed high levels of ATX, LPAR1, and LPAR6. Immunofluorescence confirmed the LPAR results. Immunocytochemistry showed human microvascular endothelial cells expressed high levels of LPAR1 and LPAR6. Calcium assay using HMEC1 exposed to LPC or LPA with LPAR1 inhibitor showed decreased signalling intensity, suggesting that HMEC1 may be converting LPC to LPA endogenously. Preliminary results also showed HMECs exposed to oppositely sexed OA patient SF show faster scratch wound closure, whereas LPAR1 and 6 inhibitors reduced most of the closure effects induced by SF.

We have found higher abundance of LPC-LPA-LPAR signalling intermediaries in OA synovium as well in HMEC-1 cells. HMEC1 assays suggest that LPC-LPA in SF may have the potential to increase angiogenesis mediated by LPAR1/6. Future work should investigate the effects of LPC and LPA on microvascular dysfunction, including long-term effects of chronic LPA exposure. The LPC-LPA-LPAR axis may hold important implications for microvascular health in the context of knee OA.

Abstract #: 33
Presenting Author: Hanyu Jiang
Research Theme: Adipose Tissues and Blood Vessels
Preferred Presentation: Either Oral or Poster
Presenter Category: MSc Student

Chitosan/platelet-rich plasma implants in an ovine arthroscopic model of meniscus repair

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Introduction: Meniscal tears stand as the most common knee condition. Despite the availability of various repair techniques, most tears are considered irreparable often leading to meniscectomy, which increases the risk of developing osteoarthritis. A freeze-dried chitosan-based formulation reconstituted in autologous platelet-rich plasma (CS-PRP) has shown great potential in pre-clinical studies for enhancing tissue repair in orthopaedic indications including suture-based meniscus repair.

Objective: The objectives of this study were to develop a fully arthroscopic ovine model of meniscus repair and to test the efficacy of CS-PRP as an augmentation strategy for suture-based meniscus repair.

Design: Unilateral radial lesions were created in the mid-body of the medial menisci of 22 skeletally mature ewes. Tears were sutured inside-out with two 2-0 Fiberwire meniscus repair needles. Two groups received a CS-PRP injection into the sutured lesions (0.5 or 1 mL doses, n=6/group). One group was injected with 1 mL autologous PRP. The control group was only sutured (n=5/group). One sheep from each CS-PRP group, were euthanized at 1day. The 20 other sheep were euthanized at 6 months post-operative. The Biomomentum multiaxial mechanical MACH-1 tester was used for mechanical testing (instantaneous modulus and thickness) and the Biomomentum Artho-BST probe was used for electromechanical testing (quantitative parameter). Mappings of the repaired and contralateral cartilaginous surfaces (menisci as well as femoral and tibial articular surfaces) were generated. Their histological and biochemical properties were also acquired.

Results: CS-PRP was resident in the meniscal tears at 1 day and triggered the recruitment of polymorphonuclear cells. Histological scores revealed that the menisci and articular surfaces from the knees treated with CS-PRP had a significantly better structural appearance than those of controls. Tissue biopsies from the treated knees had higher average glycosaminoglycan content than tissue biopsies from the control knees. Mechanical properties from treated groups were better than those of other groups (for medial menisci in the « Horns » region, groups 0,5 mL and 1mL CS-PRP had respectively a median instantaneous modulus of 2.6 and 2.3 MPa against 0.3 MPa for group 1mL PRP, and 0.4 MPa for group Sutures only. Contralateral group had a median value of 4.8 MPa)

Conclusion: To our knowledge, this is the first time that an arthroscopic meniscal repair was attempted in sheep. CS-PRP implants seem to limit cartilaginous degradation in joints with radially incised medial menisci and show promise as a novel treatment for meniscal tears.

Abstract #: 48
Presenting Author: Margaux Delvaux
Research Theme: Connective Tissue Repair, Regeneration, Bioengineering
Preferred Presentation: Oral
Presenter Category: MSc Student

Abstracts for Poster Presentations

CCN1 and CCN3 protein expression in Duchenne muscular dystrophy

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Introduction: Duchenne muscular dystrophy (DMD) is a genetic disorder characterized by progressive muscle degeneration due to a mutation in the dystrophin gene. This disease affects 1 in 5,000 boys, making it the most common childhood-onset dystrophy. In response to ongoing muscle degeneration, the body initiates a reparative process leading to abnormal collagen deposition in the muscle tissue, ultimately forming fibrotic scars.
The CCN protein family consists of six proteins (CCN1-6). CCNs are involved in many fibrotic diseases, such as idiopathic lung fibrosis, systemic scleroderma, etc. In the context of DMD, anti-CCN2 antibody is currently being tested in clinical trials as a potential treatment option. However, the role of CCN2 natural antagonists – CCN1 and CCN3 – is yet to be determined in DMD. In this study, we aim to characterize CCN1 and CCN3 protein expression in skeletal muscle at different stages of DMD progression.

Methods: Gastrocnemii muscle of wild-type (ScottySnell, ScSn), mdx (DMD model lacking dystrophin) and mdx utrn+/- (DMD model with a more severe phenotype) mice of 4, 8 and 15 weeks of age (n=6 for each genotype and age group) were collected. CCN1 and CCN3 expression was estimated using ELISA and normalized based on total protein concentration measured by Pierce BCA Assay. A three-way ANOVA was performed to compare the groups. The impact of age, genotype, and sex were analyzed. If significant differences were detected, Tukey's post-hoc test was performed.

Results: No significant differences in CCN1 expression were observed between the groups (Genotype: $p=0.22$, Age: $p=0.19$, Sex: $p=0.33$). CCN3 expression was significantly higher in DMD models compared to controls (ScSn vs mdx: $p<0.01$, ScSn vs mdx utrn +/-: $p<0.01$); however, there were no differences between the two DMD models ($p=0.99$). A significant interaction between sex and genotype ($p<0.01$) but not genotype and age ($p=0.08$) on CCN3 expression was observed. CCN3 expression was significantly lower at 15 weeks compared to 4 weeks ($p<0.01$), but no differences at 15 and 8 weeks ($p=0.09$) or 8 and 4 weeks ($p=0.24$) were observed.

Discussion: This study is the first to analyze the expression of CCN1 and CCN3 in DMD. CCN3, but not CCN1, protein expression was altered in DMD mouse models compared to controls, which suggests that CCN3 might be involved in DMD pathology at the beginning stages of the disease. Further exploration of molecular pathways involving CCN3 is needed to identify the role of this protein in DMD pathogenesis.

Abstract #: 3
Presenting Author: Margarita Egiian
Research Theme: Cell Biology of Connective Tissues
Preferred Presentation: Poster
Presenter Category: PhD Student

Short link N peptide modulates inflammasome activity via CD14 interaction: Potential therapeutic for IVDD

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Introduction: Intervertebral disc degeneration disease (IVDD) is a cause of chronic back pain. This condition involves complex interactions between neural and inflammatory pathways, leading to hyperinnervation and nociception of dorsal root ganglia neurons. The NLRP3 inflammasome is a multiprotein complex that plays a critical role in the immune response and is implicated in the processing of interleukin-1 beta (IL-1β), a cytokine that contributes to inflammation and pain. Activation of the inflammasome is intricately linked to toll-like receptor (TLR) stimulation by various ligands including pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). TLRs require the cofactor receptor CD14 for full activation. Despite the prevalence and impact of IVDD, current treatments remain inadequate, underscoring the urgent need for innovative therapeutic approaches. Our previous work has shown that sLN, an 8-residue peptide derived from link protein, can mitigate IVD degeneration in various model systems. In this study, we investigate a mechanism of sLN in modulating inflammation and alleviating pain in IVDD.

Methods: Western blotting and RT-PCR was used to assess sLN on the activation of caspase-1 and the production of interleukin-1 beta (IL-1β), as well as on the expression of inflammasome markers in human nucleus pulposus cells (hNP) treated with lipopolysaccharide (LPS). Co-culture assays were conducted on hNP and RAW macrophages to evaluate the influence of sLN on macrophage polarization following LPS stimulus. Peptide docking studies and immunoprecipitation experiments were employed to investigate the interaction between sLN and CD14.

Results: Our findings reveal that sLN markedly inhibits the activation of NF-κB and caspase-1, leading to a significant reduction in the maturation and secretion of IL-1β in nucleus pulposus cells, as demonstrated through Western blot analysis. RT-PCR results further confirm the downregulation of key inflammasome markers, including NLRP3 and caspase-1 following sLN treatment. Co-culture experiments showed a decrease in pro-inflammatory M1 macrophage markers, indicative of an anti-inflammatory shift. In silico modelling and immunoprecipitation studies suggest a direct interaction between sLN and CD14, indicating the ability of sLN in blocking LPS from triggering an inflammatory cascade.

Conclusion: We have demonstrated that sLN significantly reduces inflammasome activity and IL-1β production by interacting with the CD14 receptor. This interaction interrupts the pro-inflammatory cascade, offering a novel mechanism for mitigating discogenic pain. Given the pressing need for effective treatments for IVDD, sLN represents a promising candidate for future clinical research and application in chronic back pain.

Abstract #: 4
Presenting Author: Muskan Alad
Research Theme: Cartilage and Intervertebral Disc
Preferred Presentation: Poster
Presenter Category: PhD Student

Role and expression of 15-prostaglandin dehydrogenase in cartilage tissue

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Background. Osteoarthritis (OA) is the most typical type of joint disease. OA is distinguished by several characteristics, including synovitis (synovial membrane inflammation), progressive cartilage degradation, subchondral bone remodeling, and discomfort. Prostaglandin E2, which has been implicated in the regulation of inflammation and cartilage biology, is catabolized by 15-hydroxyprostaglandin dehydrogenase (15-PGDH). This study is intended to evaluate the expression and function of 15-PGDH in cartilage.

Methods. The expression of 15-PGDH mRNA and protein in cartilage was investigated using real-time reverse transcriptase-polymerase chain reaction (RT-PCR), immunoblotting, and immunohistochemistry. IL-1 was applied to stimulate chondrocytes. Western blotting and real-time RT-PCR were utilized to measure 15-PGDH expression, and 18-Glycyrrhetinic acid was used to examine how 15-PGDH activity affected the expression of significant inflammatory and anabolic genes.

Results. 15-PGDH expression was lower in OA tissues compared to its expression in both healthy human and murine cartilage, suggesting that the reduced levels of 15-PGDH may contribute to OA development. Treatment with IL-1β downregulated 15-PGDH expression in human OA chondrocytes. We also showed that treatment with 18 βGA, an inhibitor of 15-PGDH activity, up-regulated the expression of key inflammatory genes, including iNOS, COX-2, and mPGES-1, and down-regulated the expression of the main anabolic genes, type II collagen and aggrecan, in cartilage.

Conclusion. Together, these data suggest that 15-PGDH is involved in the pathogenesis of OA. They also suggest that targeting 15-PGDH expression and activity could be a new anti-OA therapy that specifically modulates PGE2 levels.
Keywords: Cartilage, Osteoarthritis, 15 prostaglandin dehydrogenase, Prostaglandin E2, Chondrocytes, Inflammation.

Abstract #: 7
Presenting Author: SAMI ALSABRI
Research Theme: Cartilage and Intervertebral Disc
Preferred Presentation: Poster
Presenter Category: PhD Student

Mechanically activated myofibroblasts drives macrophages into profibrotic phenotype through direct cell contact

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Background: Aberrant crosstalk of fibroblasts and macrophages (Mϕ) 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 and contact 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 communication restricted to the exchange of soluble factors. Cells obtained from all experimental combinations were separately 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 cultured alone on stiff substrates exhibit MF protein and RNA profiles absent from soft-cultured fibroblasts. Substrate stiffness in the chosen range does not affect RNA profiles of Mϕ in monoculture. 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 the activation of IL-17, TNF, NF-kB, and C-type lectin signaling. Immunofluorescence and flow cytometry analysis validate RNA sequencing data.

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

Abstract #: 8
Presenting Author: Li Diao
Research Theme: Skin and Matrix Biology
Preferred Presentation: Poster
Presenter Category: Postdoctoral Fellow
CCTS Travel Award Competition: No
Invited Speaker Abstract: No

Mechanical environment of mesenchymal stromal cells controls production of exosomes

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

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Mesenchymal stromal cells (MSC) are used in cell therapies to repair severely burned skin that is subject to 'hypertrophic' scarring. MSC are chosen due to their availability from human donor tissue biopsies and their regenerative capabilities of damaged tissues when re-transplanted. To obtain the MSC required for therapies small biopsy material is expanded in cell culture and bioreactors. However, the cell culturing process involves growth of MSC on stiff plastic surfaces (dishes or small beads), which mechanically activates MSC into scar-promoting cells that lose their regenerative potential.
My host lab developed culture substrates with the softness of skin that preserve MSC regenerative potential and prevents the scar-inducing properties. MSC harvested for such 'soft cultures' and transplanted to animal models of wound healing promoted scarless healing while MSC from conventionally stiff surfaces increased scarring. Because the transplanted MSC disappear much earlier from the recipient tissue (1-4 days) than scarring takes place (9-12 days), we hypothesize that soft-cultured MSC produce soluble factors that instruct the host wound cells to make 'better' wound tissue. It is the aim of my project to isolate and characterize the so-called extracellular vesicles, specifically exosomes, that are secreted by MSC cultured on soft substrates compared to those produced by stiff-cultured MSC. We will use these exosomes from differently stiff environments to treat fibroblasts and quantify the myofibroblast activation, which promotes scarring. These exosomes will also be used to determine their effect on macrophage polarization from a pro-inflammatory to a pro-repair phenotype. Lastly, we aim to elucidate the mechanism of how the mechanical environment affects exosome production. We will examine potential mechanical mediators that are responsible for the production and packaging of these exosomes.
By optimizing the growing conditions of the MSCs, we propose that these secreted factors can be isolated and used in controlled applications to treat wounds that are at risk to turn into severe scars.

Abstract #: 16
Presenting Author: Kyle Lam
Research Theme: Connective Tissue Repair, Regeneration, Bioengineering
Preferred Presentation: Poster
Presenter Category: MSc Student

Fibronectin isoforms regulate postnatal skeletal development

Neha DINESH[1], Justine ROSSEAU[2], Ling LI[1], Ronit MOHAPATRA[1], Philippe CAMPEAU[2], Dieter REINHARDT[1]

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Aim: Fibronectin (FN) is a crucial matrix glycoprotein essential for the development of vertebrate organ systems, yet its specific contribution to skeletal development remains poorly understood. Therefore, our study seeks to comprehensively investigate the physiological role of FN isoforms during postnatal skeletal development using a range of advanced methodologies and conditional knockout mouse models.

Methods and Results: We employed the Cre-lox system to generate conditional knockout mouse models targeting cellular FN in cartilage (cFNKO), plasma FN in liver and circulation (pFNKO), and both FN isoforms (FNdKO). Bone samples from these mice at various developmental stages (from embryonic day 16.5 to 2 months postnatal) were extracted and analyzed using histological, immunohistochemical, and computed tomography-based techniques. Our findings revealed a distinct topological pattern of FN in the mouse limb, with prominent FN levels observed in the resting and hypertrophic chondrocyte zones and in trabecular bone. Deletion of FN isoforms in the cartilage of FNKO knockout mice was confirmed at embryonic day 16.5. Immunostaining analysis demonstrated that circulating pFN was restricted to the primary ossification center, while cartilage-specific cFN was predominant in the epiphyseal cartilage. Single knockout of either cFNKO or pFNKO showed subtle changes, whereas complete ablation of both cFN and pFN in FNdKO mice resulted in significantly reduced postnatal body weight, body length, and bone length. Micro-CT analysis of FNdKO adult bone microarchitecture revealed reduced trabecular bone volume, trabecular network, and bone mineral density. Additionally, the FNdKO was characterized by increased bone marrow adiposity. Chondrogenesis analysis in FNdKO mice exhibited alterations in the length of proliferating and hypertrophic growth plate zones, accompanied by dysregulation of the chondrogenic markers collagen type II and type X. Further, hypertrophic chondrocytes in FNdKO showed reduced levels of apoptosis, and these mice also exhibited reduced levels of key bone formation markers. Mechanistic analysis further revealed reduced levels of transforming growth factor-β1 (TGFβ1) and downstream phospho-AKT levels in FNdKO mice, indicating disrupted chondrogenic differentiation and regulation of bone formation.

Conclusion: Our study provides evidence for the essential role of FN in chondrogenesis and postnatal bone development. Simultaneous deletion of both FN isoforms in developing cartilage leads to critical TGFβ-mediated alterations in chondrogenic differentiation, highlighting FN's significance in maintaining skeletal integrity and function.

Abstract #: 20
Presenting Author: Neha Dinesh
Research Theme: Bone and Developmental Biology
Preferred Presentation: Poster
Presenter Category: Postdoctoral Fellow

Developing a cartilage-on-a-chip and vascularized synovium-on-a-chip model for joint diseases

Lauren BANH[1,2], Kevin PERERA[1], KaKit CHEUNG[1], Kebin LI[3], Byeong-Ui MOON[3], Edmond YOUNG[1], Sowmya VISWANATHAN[1,2]

[1]University of Toronto; [2]University Health Network; [3]National Research Council Canada

Osteoarthritis (OA) is a degenerative joint disease with no effective treatments. This is partly because multifactorial parameters are not adequately represented in conventional laboratory models. Thus, we are using novel organ-on-a-chip engineering techniques to develop “cartilage-on-a-chip” (CoC) and “synovium-on-a-chip” (SoC) models for capturing OA hallmarks. For the CoC model, we fabricated a device that compresses OA patient-derived articular cartilage biopsies, obtained from both male and female patients. Hyperphysiological compression (1Hz, 35% depth) was applied for several cycles over 14 days. The degree of cartilage degradation was measured by sulphated glycosaminoglycans (sGAG) in the conditioned medium and Safranin-O staining for proteoglycan content. For the SoC model, human umbilical vein endothelial cells (HUVECs) with OA fibroblast-like synoviocytes (FLS) were co-cultured in a 3D fibrin hydrogel for 7 days. Changes in cell organization and lumen formation were monitored through confocal microscopy. In the CoC model, hyperphysiological compression increased the sGAG concentration in the conditioned medium and decreased proteoglycan content in the tissue relative to static unloaded controls, demonstrating that OA-like traits were induced by mechanical degradation. Biological readouts such as cartilage gene expression and degraded extracellular matrix protein concentrations are currently being added to the panel. In the SoC model, HUVECs spontaneously self-assembled into vascular-like networks with the support of periphery FLS by day 3 of culture and collapsed by day 7. Ongoing experiments are being done to move to the complete use of synovium-specific cells, including FLS and human synovial microvascular endothelial cells (HSyMVECs). The vascular network stability will be optimized by adjusting FLS and HySMVEC cell concentration ratios. Overall, CoC and SoC are more physiologically relevant joint models of cartilage degradation and synovial inflammation, respectively. These modules will be valuable tools for studying OA pathophysiology, and potential drug screening purposes when combined to reflect a “joint-on-a-chip” model.

Abstract #: 23
Presenting Author: Lauren Banh
Research Theme: Connective Tissue Repair, Regeneration, Bioengineering
Preferred Presentation: Poster
Presenter Category: PhD Student

Chitosan and platelet-rich plasma biomaterial improves healing of skin wounds in diabetic rats

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[1]Polytechnique Montreal; [2]Université Laval (LOEX)

Introduction: Diabetic foot ulcers (DFUs) represent a significant challenge in clinical management due to their propensity for chronicity and associated complications. Current treatments often yield suboptimal outcomes, necessitating the exploration of novel therapeutic approaches to improve wound healing and reduce the risk of amputation. The Biomaterial and Cartilage Laboratory (BCL) has developed a biomaterial composed of freeze-dried chitosan (CS) and platelet-rich plasma (PRP, a fraction of the blood). It is an injectable hybrid material that solidifies in situ once mixed and releases growth factors.

Objectives: The study aimed to assess the potential of CS-PRP to improve wound healing in diabetic rats.

Methods: The study included 20 diabetic male Sprague Dawley rats injected with streptozotocin, with two full-thickness 8-mm splinted excisional wounds on their back. Nine rats were treated with CS-PRP and inactive jelly was applied in 11 rats (as controls). Wound closure rate was followed with images taken every 3 to 5 days until terminal analyses. For each rat, both wounds were retrieved and frozen 7 or 28 days after surgery and treatment. The presence of the biomaterial in wounds was assessed by fluorescence microscopy to detect rhodamine-labeled chitosan. Histology was conducted with Hematoxylin and Eosin, Masson's Trichrome and CD31 immunostaining (for blood vessels detection). A 7-criteria semi-quantitative histological scoring system was used to assess the state of tissue healing 7- and 28-days post-treatment. Mechanical indentation tests were performed to determine skin thickness and elasticity.

Results: The biomaterial remained in all treated wounds, below the epidermis at 7 days and in the lower dermis after 28 days. Global wound closure was significantly faster with CS-PRP ($p<0.01$). After 7 days, CS-PRP treated wounds (n=18 wounds) were half closed (52% of initial area) compared with only 37% for controls (n=22). After 12 days, 82% closure was achieved for the CS-PRP group (n=8) compared to 69% for controls (n=11)
After 7 days, neo-epidermis covered more surface on the treated wounds (n=10 each group, $p<0.01$). After 28 days, neodermis of the treated wounds (n=7) displayed enhanced and more homogenous extracellular matrix deposition than controls (n=11). Overall, the treated wounds featured histological scores significantly more similar to those associated with undamaged skin at both timepoints ($p<0.01$). Skin was significantly thinner and more rigid in wounded areas than undamaged skin.

Conclusion: CS-PRP increases the closure rate of skin wounds in diabetic rats and improves within a month the histological features of the healed tissue.

Abstract #: 26
Presenting Author: Laura Ahunon
Research Theme: Skin and Matrix Biology
Preferred Presentation: Oral
Presenter Category: PhD Student
CCTS Travel Award Competition: Yes
Invited Speaker Abstract: No

Development of an engineered synovium model for studying synovial cell-cell interactions

Paul COLLIE[1], Dr. Lauren FLYNN[1], Dr. Tom APPLETON[1], Garth BLACKLER[1]

[1]Western University

Background. The synovium (joint lining) plays a critical role in joint health and homeostasis, and deranged synovial physiology leads to the development of osteoarthritis (OA). Changes in the synovium may precede cartilage damage in OA, with evidence that synovial inflammation contributes to disease progression, joint pain, and joint failure. Progress in understanding how synovial cell dysfunction contributes to OA has been impeded by the lack of physiologically relevant culture models.

Objective and Approach. Recognizing this need, this project focuses on designing 3D-engineered synovium models that mimic native synovium. Our lab previously developed “cell-assembly” methods that harnessed the capacity of human adipose-derived stromal cells (hASCs) to remodel decellularized tissue bioscaffolds to generate engineered connective tissues with cells dispersed throughout a 3D extracellular matrix (ECM) network [1], similar to native synovium. The current project is adapting these methods to generate synovium models using human synovial fibroblasts as the primary cell source.

Methods. Applying a modular approach, the fibroblasts are seeded onto custom-fabricated microcarriers composed of decellularized adipose tissue (DAT), selected based on its compositional similarity to synovial ECM and abundant availability from human surgical waste. The cell-seeded microcarriers are transferred into moulds and cultured under conditions that promote cell expansion and ECM production. Over an in vitro culture period, the cells remodel the ECM, fusing the microcarriers into cohesive tissues with a well-defined 3D geometry. Cell number, distribution, and phenotype are being assessed through immunofluorescence staining with cell-type specific markers, complemented by quantitative assessment of cell abundance via the Picogreen assay and cell viability by flow cytometry. Upcoming studies will characterize ECM composition via biochemical assays, and perform mechanical testing to compare the properties to native synovial tissues.

Results to Date. The cell-assembly methods have been successfully adapted to generate stable engineered synovial tissues (d~6 mm, h~2 mm) over an 8-day culture period. Preliminary flow cytometry and PicoGreen data support that the synovial fibroblasts remain viable and expand within the scaffolds. Staining confirmed the cell distribution was similar to native tissues, and that the synovial fibroblasts expressed lubricin, a key component of synovial fluid essential for joint lubrication.

Significance. These studies will provide valuable insight into the role of the 3D cellular microenvironment in directing synovial cell phenotype and function. In the future, the model will be extended to include other synovial cell populations, including macrophages, to study their roles in the development and progression of OA. [1] Morissette Martin, Biomaterials, 2021.

Abstract #: 28
Presenting Author: Paul Collie
Research Theme: Cell Biology of Connective Tissues
Preferred Presentation: Poster
Presenter Category: MSc Student

Continuous expansion of human cruciate ligament cells for ligament tissue engineering

Tarek KLAYLAT[1,2], Paul MARTINEAU[1,2], Chan GAO[1,2], Julie FRADETTE[3], Showan NAZHAT[1], Derek RO SENZWEIG[1,2], Rahul GAWRI[1,2]

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INTRODUCTION: Anterior cruciate ligament (ACL) ruptures are prevalent musculoskeletal injuries. Due to biomechanics and poor blood supply, ligament tissues fail to heal properly after injury. The standard of care, ACL reconstruction surgery (ACL-R), is associated with high failure, complications, and revision rates, thus, creating an unmet clinical need for alternative solutions. Tissue engineering regenerative medicine (TERM) for ligament tissues is an emerging field. Nevertheless, uncovering the optimal cell source for such applications remains a challenge. Stem cells from diversified sources have been examined with limited success. Using primary ligament cells for TERM applications has dual limitations of limited cell numbers and loss of phenotype (dedifferentiation) on cell passaging. In the current study, we investigate cell number expansion using a biaxial strain device (cellarator) that provides a continuously expanding cell culture surface, thereby expanding primary ligament cell populations without the need for passaging and the resulting dedifferentiation.

METHODS: Primary human ACL cells were isolated from resected surgical samples (per IRB-approved protocols and consent) and seeded on the collagen-coated cellarator dish with a 10-day continuous expansion protocol. Collagen-coated static culture dishes with cells serially passaged to match the increasing cell growth surface served as a control. At the experimental endpoint, the continuously expanded cells were in passage P1, whereas the cells on the surface area-matched Petri dish were in P3. The relative gene expression of collagen type-I, III, and VI, tenascin C, decorin, α-SMA, and scleraxis, cell morphology and arrangement, growth rate, and metabolic activity were assessed.

RESULTS: Continuously expanded (CE) ACL cells showed a more spindle-like morphology and parallel arrangement than their serially passaged counterparts. Similarly, CE ACL cells maintained their growth rate, while serially passaged ACL fibroblasts showed an altered growth rate. In addition, CE primary ACL cells displayed a higher metabolic activity compared to their passaged counterparts. Our gene expression analysis results show a declining trend in the transcriptional expression of ligament-specific markers collagen type-I, III, VI, scleraxis, decorin and tenascin C from P0 to P3. However, CE ACL fibroblasts had a significantly higher gene expression (3 to 5-fold changes) of scleraxis, and tenascin C compared to their passaged counterparts (at P3).

CONCLUSIONS: This demonstrates that CE enables expanding primary ACL cell populations with the preservation of phenotype for TERM applications while impeding unwanted dedifferentiation. These cells can be seeded on bioengineered ligament scaffolds to be used in ACL-R, thus enhancing surgical outcomes and patients' quality of life.

Abstract #: 34
Presenting Author: Tarek Klaylat
Research Theme: Connective Tissue Repair, Regeneration, Bioengineering
Preferred Presentation: Poster
Presenter Category: PhD Student

Development of an explant model of tendon injury to study heterotrophic ossification

Tarek KLAYLAT[1,2], Cameliya CAMELIYASINHA ROY[1,2], Paul MARTINEAU[1,2], Mohan RADHAKRISHNA[1,2], Chan GAO[1,2], Rahul GAWRI[1,2]

[1]McGill University; [2]McGill University Health Center

INTRODUCTION: Neurogenic heterotopic ossification (NHO) refers to abnormal calcification/ossification in soft tissues like tendons at periarticular sites following trauma to the central nervous system, such as in spinal cord injuries (SCI). The etiology remains largely unknown, thus limiting available therapeuties. Our collaborative group developed a novel and clinically relevant murine model of concomitant transectional SCI and musculotendinous injury (MTI). Nevertheless, animal models remain cumbersome and costly to develop, utilize, and sustain. This study aims at developing and characterizing a tendon crush injury explant model mimicking polytrauma to musculotendinous sites and using it to develop a high throughput co-culture system with neurons isolated from healthy and injured spinal cords to elucidate the neuronal mechanism(s) involved in SCI-induced periarticular NHO.

METHODS: All procedures received IACUC approval. The mice underwent non-invasive crush injury with mosquito forceps with crushing pressure applied for 20 seconds at the patellar and Achilles tendon sites. Immediately after the injury, the mice were euthanized and mouse Achilles and patellar tendons were harvested to isolate primary tendon cells (TDCs) and establish tendon explant cultures. TDCs and explants were cultured in osteogenic media for 7,14, and 21 days. The mineralization potentials of tendon-derived cells and explants were determined using appropriate staining and compared to those of bone marrow-derived stem cells. Expression of stemness markers (CD73, CD90, and CD105) in TDCs was quantified by fluorescence-assisted cell sorting (FACS).

RESULTS: Our findings show that tendon-derived cells have a higher potential for mineralization compared to bone marrow-derived stem cells. The Achilles and patellar tendon explants, both injured and uninjured, show sufficient mineralization in osteogenic media. Our data also shows that the injured tendons have more mineral deposition compared to uninjured tendons. The tendon-derived stem cell (TDSC) niches are thought to cause this mineralization. We observed tendon-derived stem cell populations that are highly variable in their CD marker expression profile for stemness. We identified a substantial stem cell population that is triple positive (CD73, CD90 & CD105), providing the osteogenic potential to the tendons in states of disease and pathology.

CONCLUSION: This explant culture method will allow us to study ex vivo interactions of tendons with injured and uninjured neurons in a co-culture system to elucidate the mechanisms behind NHO and potential therapeutics. The novel explant model developed in this study will help advance the research applications aimed at promoting the 3Rs of animal research to reduce the number of animals used in health research.

Abstract #: 37
Presenting Author: Tarek Klaylat
Research Theme: Bone and Developmental Biology
Preferred Presentation: Poster
Presenter Category: PhD Student

Analyzing the effects of PGC1α overexpression in a post-traumatic mice model of osteoarthritis

Sepideh TAGHIZADEH[1], Matthew GROL[1], Frank BEIER[1]

[1]Western University

Background: Osteoarthritis (OA) is a multifactorial disease characterized by extracellular matrix (ECM) breakdown within articular cartilage, proinflammatory cytokine activation, and dysregulation of cellular processes and structures such as the mitochondria. Recent findings suggest that the disruption of mitochondrial balance plays a significant role in the development of OA. The downregulation of peroxisome proliferator-activated receptor-gamma co-activator 1-alpha (PGC1α), a key regulator of mitochondrial function, has been observed in both aging and post-traumatic mouse models of OA. Preliminary work from Dr. Beier's lab indicates that loss of PGC1α leads to early OA pathology during aging; however, it's unclear if overexpression of PGC1α would have the opposite outcome and slow disease progression in different forms of OA.

Hypothesis: We hypothesize that local overexpression of PGC1α achieved using helper-dependent adenovirus will slow OA progression in a mouse model of post-traumatic OA.

Methods: To generate our overexpression vectors, we used PCR amplification and Gibson assembly to insert either β-galactosidase (LacZ) or the cDNA encoding PGC1α (Ppargc1a) downstream of the constitutive elongation factor-1 promoter (EF-1) within the pLPBL1 shuttle vector (EF-1-LacZ-bGH PolyA or Ef1-Ppargc1a-bGH PolyA, respectively). We confirmed that our constructs are functional by transfecting them into the C3H10T1/2 murine embryo fibroblast cell line using lipofectamine reagent and confirming PGC1α gene expression by qPCR with future experiments planned to quantify protein levels using Western blot. In parallel with our in vitro studies, our constructs will be moved into the helper-dependent genome (pΔ28E4) and we will subsequently generate helper-dependent adenoviruses for use in vivo. Following virus production, we will evaluate therapeutic efficacy in vivo using the destabilization of the medial meniscotibial ligament (DMM) model of post-traumatic OA. Mice will be evaluated at 8-, 14-, and 20-weeks post-DMM using behavioral assays, histological staining, phase-contrast μCT imaging, and qRT-PCR and immunohistochemistry for molecular profiling.

Results: We have successfully generated our overexpression constructs and confirmed expression of PGC1a by qPCR in transfected C3H10T1/2 cell cultures. Compared to cells transfected with the control plasmid (lacking PGC1α) or untransfected cells, we observed a significant overexpression of PGC1α at 24 and 48 h post-transfection. We are currently evaluating the level of PGC1α protein produced from our overexpression constructs, after which we will proceed with production of our virus.

Future Direction: This study will enhance our understanding of PGC1α's role and therapeutic potential in osteoarthritis. Further analysis of molecular pathways will be conducted to expand our knowledge of this complex condition.

Abstract #: 39
Presenting Author: Sepideh Taghizadeh
Research Theme: Cartilage and Intervertebral Disc
Preferred Presentation: Poster
Presenter Category: PhD Student

Growth factor cocktail: In vitro phenotypic modulation of human periodontal ligament and gingival fibroblasts

Chengyu GUO[1], Amin RIZKALLA[1], Douglas W HAMILTON[1]

[1]Western University

Introduction: Periodontal diseases are prevalent chronic inflammatory conditions affecting the tooth-supporting structures, ultimately leading to tooth loss if left untreated. While significant efforts have focused on bone tissue regeneration in the context of periodontal diseases, the understanding of soft tissue regeneration remains relatively limited. Soft tissues in the periodontium, gingiva and periodontal ligament (PDL), play a crucial role in supporting and protecting teeth and underlying bone structures; thus, essential for successful periodontal regeneration. Growth factors, including transforming growth factor-beta 1 (TGF-β1), TGF-β3, fibroblast growth factor-2 (FGF-2), and FGF-9, are known to influence cellular behavior and augment tissue regeneration. However, their specific effects on human gingival fibroblasts (hGF) and human PDL cells (hPDL), are yet to be fully characterized.

Methods: Primary hGF and hPDL cells were isolated from gingival and periodontal tissues obtained from five patients. Expression levels of major extracellular matrix (ECM) components, including fibronectin, periostin, collagen I, and versican, were evaluated using RT-qPCR, immunofluorescent imaging, and Western blotting. Chemokinesis and chemotaxis properties were measured via wound healing and trans-well assays, respectively. α-smooth muscle actin (α-SMA) quantity and fiber formation were used to evaluate myofibroblast activation. Proliferation was assessed using the CyQUANT proliferation assay. Results were compared to untreated controls. Upon examining the individual growth factors, we found two candidate growth factors, each with distinct functions. These factors were combined and simultaneously introduced in vitro. Western blotting of p-SMAD3 and p-ERK were used to assess the signalling pathways of the combined treatment.

Results: In both hGF and hPDL, TGF-β1 and TGF-β3 significantly enhanced ECM production while FGF-2 promoted chemokinesis. While hGF did not display chemotactic responses to any growth factors, hPDL cells exhibited chemotactic migration towards FGF-2 and attenuation in the presence of TGF-β1. Meanwhile, TGF-β1 induced the greatest amount of myofibroblast differentiation followed by TGF-β3. Proliferation rates remained unchanged following treatment. Combination treatment with TGF-β3 and FGF-2 retained their individual effects on matrix production and migration, with minimal myofibroblast activation observed. Both TGF-β3 and FGF-2 signalling pathways are activated simultaneously.

Conclusion: This study advanced our understanding of soft tissue periodontal regeneration by exploring the individual and combined effects of growth factors on cellular behavior in hGF and hPDL. Our study is the first to investigate the combined TGF-β3 and FGF-2 signalling; these findings offer insights into the potential applications of combined growth factors not only for periodontal regeneration but also for other tissues within the body.

Abstract #: 40
Presenting Author: Chengyu Guo
Research Theme: Connective Tissue Repair, Regeneration, Bioengineering
Preferred Presentation: Oral
Presenter Category: PhD Student
CCTS Travel Award Competition: No
Invited Speaker Abstract: No

Inflammation-inducible strategies for growth factor gene therapy to promote joint tissue repair: Applications for osteoarthritis therapy

Anisha THOMAS[1], Matthew W. GROL[1]

[1]Western University

Rationale: Osteoarthritis (OA) is a multifactorial synovial joint disease that leads to chronic pain. Despite its economic and health impacts, there are no disease-modifying therapies for OA progression. Many growth factors essential for joint development and homeostasis, including transforming growth factor beta (TGF-β), are dysregulated in OA; however, constitutive overexpression of any growth factor as a therapeutic can cause aberrant growth of other joint tissues besides the intended target – diseased articular cartilage. To address this, our objective was to develop and validate an inflammation-inducible strategy to deliver TGF-β to OA joints using cell culture models of disease.

Hypothesis: We hypothesize that inflammation-inducible expression of TGF-β will be elicited upon exposure to synovial fluid from human OA patients and that it will better protect from OA synovial fluid-induced loss of anabolic gene expression in chondrocyte cultures.

Experimental Approach: To generate constitutive and inflammation inducible Tgfb1 expression vectors, we cloned the murine Tgfb1 open reading frame (ORF), an internal ribosome entry site (IRES), and the β-galactosidase gene (LacZ) into the pLPBL plasmid downstream of either the inflammation-inducible endothelial leucocyte adhesion molecule-1 (ELAM-1) promoter (pLPBL-ELAM1-Tgfb1) or the constitutive elongation factor 1 (EF1) promoter (pLPBL-EF1-Tgfb1) using Gibson assembly. Plasmids without Tgfb1 were also generated as controls (pLPLB1-EF1-LacZ, pLPLB1-ELAM1-LacZ). To verify the functional expression of our transgene, pLPLB1-EF1-Tgfb1 was transfected into C3H10T1/2 cells, and Tgfb1 expression was quantified by qPCR. Compared to control plasmid, we observed a significant increase in Tgfb1 gene expression of (25956 ± 3455 vs 0.6812 ± 0.3173) after 48 hours. To test for functional expression in our inflammation-inducible construct, C3H10T1/2 will be transfected with pLPBL-ELAM1-Tgfb1 or control plasmid, treated with LPS at various concentrations, and Tgfb1 expression analyzed by qPCR. Finally, to demonstrate that inflammation-inducible Tgfb1 can protect chondrocytes from OA synovial fluid-driven changes in cartilage-specific gene expression, ATDC5 chondrocyte-like cells will be transfected with pLPBL-ELAM1-Tgfb1, pLPLB1-EF1-Tgfb1, or the control plasmids, plated in micromass culture to promote differentiation, and then treated with control of OA patient human synovial fluid. RNA will be isolated at different time points, and qPCR performed to examine changes in the expression of chondrocyte-related genes.

Significance: The findings of this work will demonstrate that an inflammation-inducible strategy for Tgfb1 expression can protect chondrocytes exposed to OA synovial fluid. Next steps include the incorporation of these novel constructs into helper-dependent adenoviral vectors and their application to a preclinical mouse model of post-traumatic OA.

Abstract #: 42
Presenting Author: Anisha Thomas
Research Theme: Cartilage and Intervertebral Disc
Preferred Presentation: Poster
Presenter Category: MSc Student

Localization and quantification of stem cells in salivary glands

Mariia MIELKOZOROVA[1], Lander MANRIQUE[1], Ola M MARIA[1], Simon D TRAN[1]

[1]McGill University

Salivary glands are vulnerable to a range of diseases that can severely impact the quality of life, affecting functions such as swallowing, chewing, and speech. From these conditions arise discomfort, pain, and potential complications. Stem cells, present in salivary glands like in other tissues, play pivotal roles in maintaining tissue repair and regeneration, offering promising therapeutic avenues. While some stem cell therapies have shown efficacy, ongoing efforts aim to identify and characterize optimal stem cells for specific therapeutic applications. The ongoing endeavor to identify and characterize salivary stem cells underscores the importance of advancing stem cell therapies for both scientific and medical progress and enhancing accessibility for patients.

This study aimed to refine current methodologies and detect/quantify stem cells in salivary glands across different demographic and disease groups. Immunofluorescence staining was performed on samples from 14 patients, utilizing specific stem cell markers (CD34, CD90, Vimentin) and salivary gland markers (AQP5, CK7, aSMA). Custom MATLAB-based code facilitated image processing for correction, merging, and cell counting. Lastly, statistical tests were performed on the acquired data. Results indicated the presence of stem cells in submandibular salivary glands across 392 processed images. Semi-quantification of stem cells relative to salivary cells was achieved through an innovative image processing application, reducing processing time with a minimal error rate of 2% and allowing manual cell count adjustments. Moreover, quantification of red and green channel overlaps revealed the nature of detected stem cells (acinar, ductal, or myoepithelial).

Interestingly, correlations emerged between patient demographics (age, sex) and certain medical conditions impacting the abundance of CD34+ or CD90+ cells. These insights are crucial for understanding the slow regeneration observed in radiotherapy-treated salivary tissues.

In conclusion, this study contributes to refining image processing and cell counting methodologies while shedding light on salivary stem cell dynamics across diverse demographic and disease groups. Future research aims to compare healthy and irradiated salivary tissues, advancing the field of stem cell therapies.

Abstract #: 43
Presenting Author: Mariia Mielkozorova
Research Theme: Stem Cells and Therapy
Preferred Presentation: Poster
Presenter Category: MSc Student

Effects of obesity/metabolic syndrome on synovial microvasculature during knee osteoarthritis

Qinli GUO[1], Dan KLAPAK[1], Garth BLACKLER[1], Benoit FISET[2], Holly PHILPOTT[1], Geoffrey PICKERING[1], Tom APPLETON[1]

[1]Western University; [2]McGill University

Osteoarthritis (OA), obesity/metabolic syndrome (MetS) are prevalent diseases worldwide and are common causes for disability, while MetS are also strong risk factor for OA. Patients with MetS in addition to OA have faster OA progression and worse OA outcomes including joint damage and pain. While the mechanism underlying the pathophysiology remains unclear, our previous clinical observation suggests that synovium, a key tissue in maintaining joint homeostasis, is affected by MetS. Specifically, we found reduced vascularization and signs of vascular pathology in OA synovium from patients with MetS, which was also associated with more OA pain. Reduced synovial vascularization suggests not only a compromised blood supply to OA joint, but also a dysfunctional synovial microvasculature in the presence of MetS.

To understand the mechanism underlying MetS-OA pathophysiology, we employed an experimental rat post-traumatic knee OA (PTOA) model coupled with diet-induced obesity (DIO) to recapitulate the clinical manifestations of MetS-OA. We focused on the effects of MetS on pain, joint structural damage and synovial microvasculature expansion in OA joints. We also applied single-cell RNA sequencing to assess synovial transcriptome, which helped us to understand the vascular cells in response to MetS-OA. We found our PTOA model demonstrated neovascularization in synovial lining region, however, the DIO-PTOA model demonstrated worse pain, cartilage damage and failure in synovial lining neovascularization. Our single-cell transcriptome analysis identified a unique group of endothelial cells underwent transitioning towards mesenchymal phenotype (Endothelial-to-mesenchymal transition, EndoMT) in OA pathophysiology. Expansion of EndoMT cells at 4-week after PTOA induction suggests normal wound-healing response of endothelial cells during OA, however, these cells diminished after OA induction when MetS was pre-established. In addition, DIO-PTOA model demonstrates an imbalanced mural-to-endothelial ratio at 4-week after PTOA induction, which indicates a potential dysregulation within the microvascular niche.

In summary, our finding suggests a synovial endothelial response that likely represents the wound healing response to OA which is likely the pivotal target for MetS in compromising joint homeostasis and thus contributing to worse OA progression.

Abstract #: 44
Presenting Author: Qinli Guo
Research Theme: Adipose Tissues and Blood Vessels
Preferred Presentation: Poster
Presenter Category: PhD Student

Ultrasound-measured synovitis is associated with movement-evoked pain in knee osteoarthritis: A cross-sectional and within participant design.

Brent WAKEFIELD[1,2], Trevor B. BIRMINGHAM[1,2], Hayden ATKINSON[1,2], Holly PHILLPOTT[1,2], Robert DIMA[1,2], Tamara TOMPKINS[1,2], Songlin ZHU[1,2], C. Thomas APPLETON[1,2]

[1]Western University; [2]Lawson Health Research Institute

Objective: To examine the association between synovitis and movement-evoked pain (MEP) in patients with knee osteoarthritis (OA).

Methods: 342 participants with radiographically (Kellgren-Lawrence [KL] arthritis grading scale) mild, moderate, and severe OA and frequent symptoms underwent ultrasound measures of inflammation using the Outcome Measures in Rheumatology (OMERACT) knee ultrasound scoring system. Measures of MEP, BMI, age, and sex were collected. MEP was assessed using the 6-minute walk test and a numerical rating scale for knee-specific pain before and after walking. A series of logistical regression analyses adjusted for age, sex, and BMI were used to determine the association between the presence of MEP and synovitis across KL grades. A secondary analysis using a conditional logistic regression in a subset of participants with knees discordant for MEP was performed. Synovial biopsies were taken during high tibial osteotomy or total knee replacement surgery in a subset of patients with late-stage OA. Synovium was scored for histopathological features of inflammation and damage and was assessed for associations with MEP.

Results: Moderate-to-severe synovitis was associated with increased odds of MEP [OR = 1.47 95%CI 0.92 – 2.35] in the entire cohort. Synovitis was associated with increased odds of experiencing MEP in patients with mild-moderate OA [OR = 2.32 95%CI 1.40 – 3.84], and decreased odds of MEP in knees with severe radiographic damage [OR = 0.19 95%CI 0.03 – 1.0]. Our conditional logistical regression model strengthened the association between synovitis and MEP in patients with mild-moderate OA [OR = 4.75 95%CI 1.62 – 13.97]. There were no significant histological associations of inflammation and damage with MEP.

Conclusions: In knee OA with KL grade ≤ 3, moderate-to-severe synovitis is associated with MEP. Targeting synovial inflammation may be beneficial in early OA. In severe OA, additional sources, such as bone deformity, may contribute to MEP. Synovial samples from early OA knees may elucidate histological features of synovitis associated with MEP in OA.

Abstract #: 47
Presenting Author: Brent Wakefield
Research Theme: Other
Preferred Presentation: Poster
Presenter Category: Postdoctoral Fellow

Retinoid signaling in the modulation of ectopic mineralization in a mouse model of DISH

Alexander RICO[1], Mayu NAGAO[1], Geoffrey KERR[1], Cheryle SEGUIN[1]

[1]Western University

Introduction: Diffuse Idiopathic Skeletal Hyperostosis (DISH) is a disease marked by dystrophic calcification of the soft tissues surrounding the anterolateral spine. Affecting 1 in 10 individuals in North America over the age of 60, it is a common disease whose etiology remains unknown. Symptoms vary from pain and stiffness to dysphagia and nerve compression in severe cases. Risk factors associated with DISH include metabolic disorders, coronary artery disease, and prolonged use of isotretinoin. Interestingly, individuals with DISH exhibit elevated endogenous levels of 13-cis retinoic acid (isotretinoin) and all-trans retinoic acid (ATRA), the primary ligands of the retinoid signaling pathway. ENT1 knockout (ENT1-/-) mice show mineralization of spinal tissues including the intervertebral discs (IVDs) resembling DISH. Analysis of changes associated with ectopic mineralization in these mice suggests the involvement of Retinoic Acid Receptor Gamma (RARγ) as a candidate regulator. We hypothesize that modulation of retinoid signaling in ENT1-/- cells would regulate ectopic mineralization.

Methods: Annulus fibrosus (AF) cells were isolated from the IVD's of ENT1-/- and Wild-type (WT) mice and cultured in micromass. Cells were treated in with an RARγ agonist (CD1530) or DMSO and assessed for mineral deposition. RNA was also isolated for bulk RNA sequencing, and subsequent bioinformatics analysis.

Results: Treatment with the RARγ agonist decreased mineralization in both the WT and the ENT1-/- cells, relative to controls. Out of 32884 genes with non-zero read counts, differential gene expression (DEG) analysis identified 3470 DEG's following RARγ agonist treatment (1827 increased, 1643 decreased), including genes involved in extracellular matrix remodeling, cell differentiation, and cartilage formation. Pathway enrichment of gene ontology (GO) biological process (BP) identified pathways involved in extracellular matrix organization, ossification and connective tissue development. Similarly, GO cellular component (CC) analysis identified enrichment in collagen-containing extracellular matrix, while GO molecular function (MF) showed enrichment in actin and cell adhesion molecule binding. Interestingly, KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analysis further identified changes in the PI3K-Akt and MAPK pathways, including the Ras and Rap1 upstream signaling pathways. Further, Hallmark gene set enrichment analysis (GSEA) identified enriched KRAS signaling as well as several pathways associated with inflammation, cytokine signaling, estrogen response and apoptosis.

Discussion: These findings suggest that the PI3K-Akt and MAPK signaling pathways may be influenced by RARγ agonism. Further exploration into the role of retinoid signaling in ectopic AF mineralization may prove useful in our understanding of DISH pathology and inform the development of novel therapeutics.

Abstract #: 49
Presenting Author: Alexander Rico
Research Theme: Cartilage and Intervertebral Disc
Preferred Presentation: Poster
Presenter Category: MSc Student

The relationship between TGFα and mitochondrial function in chondrocytes

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Introduction: Mitochondrial dysfunction has been implicated in many pathological processes, including osteoarthritis (OA), a widespread degenerative disease that affects the entire joint. Several hallmark pathologies of OA may be influenced by mitochondrial dysfunction, such as chondrocyte apoptosis and increased catabolic gene expression. Mitochondrial function can be extrapolated from assessment of the mitochondrial membrane potential (ΔΨm), which is required for oxidative phosphorylation and efficient ATP production. A sustained disruption of ΔΨm will lead to decreased cell viability and increased pathology. A decrease in ΔΨm has previously been demonstrated in human OA chondrocytes, accompanied by an increase in mitochondrial mass. Transforming growth factor alpha (TGFα) has been recognized as a key player in the pathogenesis of OA. The role of TGFα is context-dependent, as it can exhibit either protective or destructive effects on the progression of OA depending on age, stage of disease and subtype. In chondrocytes, TGFα binds to and activates the epidermal growth factor receptor (EGFR), triggering a downstream signalling cascade. Activation and inhibition of EGFR signalling in intestinal stem cells and non-small cell lung cancers has been shown to alter ΔΨm and mitochondrial function, however the effect of TGFα /EGFR signalling on mitochondrial function in chondrocytes has not yet been studied. The objective of this project is to characterize the relationship between TGFα and mitochondrial function in chondrocytes, which could provide a novel mechanism by which TGFα/EGFR signalling contributes to the pathogenesis of OA. We hypothesize that primary chondrocytes treated with TGFα in vitro will undergo alterations to their ΔΨm and mitochondrial mass.

Methods: Primary cultures of immature murine articular chondrocytes (iMACs) will be obtained from 5-day old C57Bl/6 mice as previously described, and will undergo treatment with increasing concentrations of recombinant human TGFα. ΔΨm and mitochondrial mass will be analyzed by administration of JC-1 and Mitotracker Green staining, respectively, and imaged with fluorescence microscopy.

Results and Discussion: We anticipate that chondrocytes treated with TGFα in vitro will undergo a significant change in both ΔΨm and mitochondrial mass. Significant results would lead to further studies on mitochondrial respiration in response to TGFα treatment in chondrocytes.

Abstract #: 50
Presenting Author: Emily White
Research Theme: Cartilage and Intervertebral Disc
Preferred Presentation: Poster
Presenter Category: MSc Student

Fibulin-4 and LTBP-4 regulate skin elastogenesis via syndecan interaction

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AIM: Elastogenesis is a crucial process in skin physiology, orchestrated by interactions among various extracellular matrix (ECM) proteins. Among these, fibulin-4 (FBLN4) and latent TGF-β binding protein-4 (LTBP4) play pivotal roles. Despite their recognized importance, the precise cell receptors and molecular pathways governing their participation in elastogenesis remain less understood. This study aims to elucidate these mechanisms using elastogenic dermal skin fibroblasts (NSF) and vascular smooth muscle cells (SMC).

METHODS AND RESULTS: Our investigations reveal robust and specific interactions of NSF and SMC with FBLN4 and LTBP4, with FBLN4 notably engaging as multimers. Through detailed mapping using deletion mutant constructs, we identified novel cell interaction epitopes on FBLN4 within cbEGF2-3 and the C-terminal domain, with multimerization sites mapped to cbEGF4-5 and the C-terminal domain. Similarly, a previously unknown cell interaction site was identified within the N-terminal half of LTBP4. Notably, cell binding to FBLN4 and LTBP4 was disrupted by heparin and attenuated by heparan sulfate or heparinase treatment, implicating heparan sulfate proteoglycans as essential cell surface receptors mediating these interactions. Moreover, the global knockdown of syndecans (SDC1-4) led to the complete abrogation of NSF interaction with FBLN4 and LTBP4. Specific knockdown experiments further delineated the distinct roles of SDC-2 and SDC-3 in mediating FBLN4 and LTBP4 interactions, respectively. Direct binding assays between recombinant proteins validated SDC-2 and SDC-3 as critical cell receptors for FBLN4 and LTBP4, respectively. Functionally, the interaction of FBLN4 and LTBP4 with syndecans stimulated tropoelastin assembly and elastic fiber formation through activation of focal adhesion kinase (FAK), RhoA, and ERK signaling pathways. Pharmacological inhibition of FAK, ERK1/2, or RhoA markedly attenuated tropoelastin assembly and elastic fiber formation, underscoring the significance of these pathways in elastogenesis.

CONCLUSION: In summary, our findings unravel the syndecan-mediated mechanisms through which FBLN4 and LTBP4 drive elastogenesis by enhancing focal adhesion formation and cell contractility via FAK, ERK1/2, and RhoA activation. These data contribute significantly to our understanding of the molecular interactions governing skin elastogenesis and offer potential therapeutic targets for skin-related pathologies characterized by altered elastin synthesis and assembly.

Abstract #: 51
Presenting Author: Neha Dinesh
Research Theme: Skin and Matrix Biology
Preferred Presentation: Poster
Presenter Category: Postdoctoral Fellow

Pannexin 3 deletion in male and female mice results in severe osteoarthritis in aging.

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Background: Osteoarthritis (OA) is a multi-factorial disease that is strongly associated with aging. As the molecular mechanisms underpinning the pathogenesis of this disease are unclear, there are no disease-modifying drugs to combat OA progression. The mechanosensitive channel Pannexin 3 (PANX3) has been shown to promote cartilage loss during posttraumatic OA. In contrast, the ablation of Panx3 in male mice results in superficial cartilage lesions with forced treadmill running, and spontaneous full-thickness cartilage lesions at 24 months of age. Additionally, while protected from traumatic intervertebral disc (IVD) degeneration, Panx3 knockout (KO) mice show signs of IVD disease with altered disc mechanics. The objective of this study was to determine the effects of deleting Panx3 results in OA during aging in male and female mice, and if forced treadmill running influences OA progression. Methods: Male and female wildtype (WT) and global Panx3 KO C57Bl6 mice were aged to 18 months of age. Mice were then randomized to sedentary (SED) or forced treadmill running (FEX) for 6 weeks (N = 5-14). Knee joint tissues including patellar tendon, quadriceps and distal patellar enthesis, and synovium were analyzed histologically, along with lumbar spine IVDs. Additionally, subchondral bone and tibial cartilage thickness were measured using micro-computed tomography.

Results: Approximately half of male and female Panx3 KO mice developed full-thickness cartilage lesions and synovial damage, including ectopic fibrocartilage and calcification deposition of the knee joints. Additionally, Panx3 KO mice with severe OA show signs of quadriceps and patellar enthesitis, characterized by bone and marrow formation. Forced treadmill running did not seem to exacerbate these phenotypes in male or female Panx3 KO mice; however, it may have contributed to the development of lateral compartment OA. Micro-CT analysis revealed that Panx3 KO mice have reduced cartilage thickness, thinner trabecular bone, and reduced bone volume fraction compared to wildtype counterparts. The IVDs of aged Panx3 KO mice displayed no apparent differences to control mice, and forced treadmill running had no overt effects in either genotype.

Conclusion: Aged Panx3 KO mice show features of late-stage primary OA including full-thickness cartilage erosion, subchondral bone thickening, and severe synovitis. This data suggests the deletion of Panx3 is deleterious to joint health in aging.

Abstract #: 52
Presenting Author: Brent Wakefield
Research Theme: Cartilage and Intervertebral Disc
Preferred Presentation: Poster
Presenter Category: Postdoctoral Fellow

Optimizing a novel media formulation for osteochondral allograft preservation

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Marc D. GRYNPAS[1,2], Adele CHANGOOR[1,2]

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Introduction: Osteochondral allograft transplantation (OCAT) is a surgical approach for treating large focal cartilage lesions that involves replacing damaged cartilage with osteochondral grafts from a cadaveric donor. A minimum of 70% chondrocyte viability, compared to the day of procurement, is commonly considered the threshold for transplantation.

However, donor tissues must be stored prior to transplantation to allow sufficient time to undergo required microbiologic and serologic screening and scheduling of the surgical procedure, which reduces chondrocyte viability. Storage protocols currently employed can maintain viability up to 14-56 days but are difficult to replicate. Our lab has designed a novel media formulation that includes Dulbecco's Modified Eagle Medium (DMEM), hyaluronic acid (HA) and doxycycline (DOX) that can maintain viability in rabbit cartilage for up to 56 days of storage. DOX is an antibiotic and it has been shown to inhibit cartilage degrading enzymes. This study aimed to determine the optimal concentration of doxycycline (DOX) to maximize chondrocyte viability at lengthy storage times.

Methods: The humeral heads of 10 male New Zealand white rabbits were isolated and stored in the novel media formulation, consisting of DMEM supplemented with 0.1% HA and either 1 µg/mL (DOX1), 5 µg/mL (DOX5) or 10 µg/mL (DOX10) of DOX. Tissues were stored for 0 days (n=4), 42 days (n=8) or 56 days (n=8) at 4°C, protected from light, and subjected to weekly media changes. Live and dead chondrocytes in the full thickness of the cartilage were stained using Calcein AM and Ethidium Homodimer, respectively. The percent chondrocyte viability of samples stored for 42 days and 56 days were normalized to fresh controls. Statistical analyses were performed using Welch's unequal variance t-test to compare the percent viable chondrocytes per storage conditions and storage time (GraphPad Prism 10.0.2).

Results: After 56 days of storage, DOX1 and DOX5 samples had significantly greater ($p = 0.0364$ and $p = 0.003$, respectively) normalized percent chondrocyte viability compared to DOX10 samples. Viability in DOX1 samples stored for 42 days were not statistically significant compared to DOX10 samples. Adverse effects of DOX10 on chondrocyte viability could be due to the higher dosage producing cytotoxic effects on chondrocytes.

Conclusion: These data support the selection of either 1 µg/mL or 5 µg/mL of DOX for maximizing chondrocyte viability over 56 days of storage in rabbit cartilage. Future work will investigate performance of the optimized novel media for storage of human osteochondral tissues.

Abstract #: 57
Presenting Author: Isabel Li
Research Theme: Cartilage and Intervertebral Disc
Preferred Presentation: Poster
Presenter Category: Undergraduate Student

Effects of Ras-associated binding protein Rab7b and its nucleotide mutants on vacuolar type H+-ATPase (V-ATPase) a3 subunit localisation in HeLa cells

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Rab7, a small GTPase and a member of the Rab family, is involved in lysosomal trafficking. There are two paralogues of Rab7, Rab7a and Rab7b. Rab7a has been found to interact with the a3 subunit of the vacuolar-type ATPase (V-ATPase) to facilitate its localisation to osteoclast plasma membranes for bone resorption. Rab7a regulates maturation from early to late endosomes and facilitates the transportation and fusion of late endosomes and lysosomes at the perinuclear region. The latter isoform, Rab7b, was found to function similarly to Rab7a. Besides localising at the late endosomes, there has been evidence demonstrating Rab7b localising at the Trans Golgi Network (TGN). We are not aware of any experimental evidence demonstrating the interaction between a3 subunit and the Rab7b paralogue. According to published literature, Rab7b should interact similarly to Rab7a. While cloning Rab7b, we identified two splice isoforms, Rab7b2 and Rab7bx8. Rab7b2 lacks exon 8 with a deletion of the nucleotide binding G3 conserved motif. Rab7bx8 lacks exon 7 with a deletion of the PM3 motif and the nucleotide binding G2 conserved motif. We hypothesize that the Rab7b splice isoforms and nucleotide mutants will affect localisation of the a3 subunit. To test this hypothesis, we aim to look at the differences in colocalization and vesicular transport to characterize the potential biological functions of Rab7b splice isoforms and its nucleotide mutants, including Rab7bT22N (Rab7bT), Rab7bQ67L (Rab7bQ), and Rab7bN125I (Rab7bN).

Abstract #: 58
Presenting Author: Wing Hei Wong
Research Theme: Bone and Developmental Biology
Preferred Presentation: Poster
Presenter Category: Undergraduate Student

Exploring the long-term effects of a novel media formulation on chondrocyte viability and cartilage quality in stored osteochondral allografts

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Background and Hypothesis: Osteochondral allograft transplantation is a surgical technique used to treat large focal cartilage lesions and involves the replacement of degraded cartilage with an osteochondral graft harvested from a cadaveric donor. Success rates of transplantation increase when chondrocyte viability in the donor graft is maintained at a minimum of 70% compared to fresh control. Effective storage of osteochondral allografts is critical for maintaining viability and quality of tissue prior to transplantation to allow sufficient time for mandatory disease testing. The institutional standard for storage used at Mount Sinai Allograft Technologies (Toronto, Canada), consisting of Lactated Ringer's solution, cefazolin, and bacitracin, maintains these levels for up to 14 days. Our group has developed a novel media that includes low glucose Dulbecco's Modified Eagle Medium, high molecular weight hyaluronic acid and doxycycline. It was hypothesized that by storing osteochondral tissues in the novel formulation, chondrocyte viability and histological appearance of the extracellular matrix would be maintained more effectively for up to 56 days of storage compared to the institutional standard.

Methods: Distal femurs from mature rabbits were stored at 4°C in either the novel media formulation or the institutional standard. All underwent weekly media changes. Samples were analyzed at day 0 (fresh control, n = 4), 28 (n = 3), 42 (n = 2), and 56 (n = 2). Chondrocyte viability was analyzed using confocal microscopy to detect cells stained with calcein AM and ethidium homodimer-1 for live/dead quantification. The extracellular matrix was visualized using safranin O/fast green and toluidine blue histological staining.
Results: Absolute cell viability was maintained above the 70% threshold in the novel media for up to 56 days but fell below 60% by 42 days in the institutional standard. Normalized to fresh controls, cell viability was maintained at 93.1% and 75.6% in the novel media at day 42 and 56, respectively, compared to 58.7% and 15.4% in the institutional standard. No qualitative differences in proteoglycan distribution up to 42-day storage were observed in histological sections. Day 56 sections have not yet been analyzed.

Conclusions: The novel media maintained superior chondrocyte viability in rabbit cartilage compared to the institutional standard up to 56 days. Further work is being done to optimize the formulation and characterize the cartilage extracellular matrix structure and function. These results support evaluation of the novel media formulation for the storage of human tissues, potentially enabling its eventual clinical use.

Abstract #: 60
Presenting Author: Sarah Aloi
Research Theme: Cartilage and Intervertebral Disc
Preferred Presentation: Poster
Presenter Category: MSc Student

Cell-autonomous ECM regulation of MuSC function

Yuguo LIU[1]

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Loss of Fibronectin (FN) from the skeletal muscle stem cell (MuSC) niche represents a root cause of regenerative failure in aging. While FN is abundant and has pleiotropic functions during healthy skeletal muscle regeneration, it remains unclear how its spatiotemporal specificity for MuSCs is established. Here, we demonstrate that activated MuSCs secrete an autoregulatory FN splice variant containing the EDB extra domain (EDB(+) FN), which is not expressed by accessory cells in the niche. The biogenesis of EDB(+) FN in MuSCs depends on serine/arginine-rich splicing factor 1 (Srsf1) whose promoter is controlled by Smad3. EDB(+) FN knockdown in adult MuSCs or downregulation in aging impairs skeletal muscle regeneration. A single timed injection of transforming growth factor-beta (TGFβ) restores the myogenic capacity of aged MuSCs by stimulating EDB(+) FN secretion through the Smad3-Srsf1 axis. Our work identifies autoregulatory FN in the MuSC niche as a key target for skeletal muscle rejuvenation.

Abstract #: 61
Presenting Author: Yuguo Liu
Research Theme: Stem Cells and Therapy
Preferred Presentation: Poster
Presenter Category: Postdoctoral Fellow

The relationship between centre-of-pressure and electroarthrography, a non-invasive method for cartilage assessment

Josephine LUI[1], R. Peter SUDERMAN[1,2], Rodrigo MUNEVAR LUQUE[3], Marc GRYNPAS[1,2], Hani NAGUIB[2], Judith KOENIG[3], Adele CHANGOOR[1,2]

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Background: Osteoarthritis (OA) is characterized by progressive deterioration of cartilage. Detecting early cartilage degeneration, before OA becomes symptomatic, may identify opportunities for slowing disease progression. Electroarthrography (EAG) is a method by which electrical signals produced by cartilage during compression are measured non-invasively through electrodes placed on the skin around an articular joint. As EAG is acquired during joint loading, biomechanical factors may offer critical insights into EAG signal properties. Centre of pressure (COP), which denotes the point on the joint surface at which ground reaction force (GRF) is concentrated, is expected to influence EAG signal magnitude and propagation. This study aimed to investigate the effect of COP on EAG and to explore the influence of a cartilage lesion on the relationship between EAG and COP.

Methods: An equine forelimb explant, consisting of the metacarpus to the hoof, was subjected to simulated physiological loading on a mechanical testing system to approximate the forces experienced during walking. A series of standardized wedges were placed beneath the hoof to shift COP between 0° and 2° medially. The explant was tested before and after the creation of a focal cartilage lesion on the medial-dorsal aspect of the distal metacarpus. EAG was measured by placing electrodes on the skin around the metacarpophalangeal joint. GRF was measured simultaneously using a custom-built force mat and used to calculate COP. EAG amplitudes from the electrode in the closest physical proximity to the lesion were normalized to GRF. Paired t-tests were performed to compare measurements taken during the following conditions: pre-lesion, post-lesion, 0°, and 2° incline. To determine whether there were correlations with significant differences from zero, 95% confidence intervals ($p < 0.05$) were used.

Results: EAG signal amplitude and distribution varied based on magnitude and direction of COP movement. When comparing 0° to a 2° medial incline, EAG was significantly lower at no incline both before the lesion was created ($p < 1 \times 10\text{-}6$) and after ($p < 1 \times 10\text{-}4$). When comparing EAG pre- and post-lesion, the averages were significantly higher before the lesion was created at both 0° ($p < 1 \times 10\text{-}5$) and 2° ($p < 1 \times 10\text{-}6$). Conclusion: COP modified the amplitude and distribution of EAG indicating the significance of COP in the interpretation of EAG in both the presence and absence of cartilage lesions. These data contribute to improving EAG accuracy and may eventually aid in identifying cartilage lesion locations.

Abstract #: 63
Presenting Author: Josephine Lui
Research Theme: Cartilage and Intervertebral Disc
Preferred Presentation: Poster
Presenter Category: Undergraduate Student

Role of fibulin-5 citrullination in elastogenesis

Ramshaa MAHALINGAM[1], Valentin NELEA[1], I-Cheng HO[2], Dieter Peter REINHARDT[1]

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INTRODUCTION: The elastic fiber system confers elasticity to various vital organ systems and connective tissues, including blood vessels, skin, and lungs. Elastogenesis involves multiple extracellular matrix proteins such as tropoelastin (TE), fibulin-4 and -5 (FBLN4/5), fibrillin-1 and -2, and latent transforming growth factor beta binding protein-4 (LTBP4). Previous findings from the Ho and Reinhardt labs showed that FBLN5 citrullination by peptidylarginine deiminases-2 promotes elastogenesis. Other data from the Reinhardt lab demonstrated that knockdown of the cell surface proteoglycan receptors syndecan-2, -3, -4 reduces elastic fiber formation.

HYPOTHESIS: The elastogenesis-promoting effects of FBLN5 citrullination are mediated through interactions with syndecans.

METHODOLOGY AND RESULTS: We performed immunofluorescence staining to delineate the distribution and timeline of essential elastogenic proteins, including TE, FBLN4, FBLN5, LTBP4, fibronectin (FN), and syndecan-4 using dermal skin fibroblast. Fibers containing FBLN4, FBLN5, LTBP4, and FN were typically discernible after 3 days of culture. Mature TE fibers developed later, starting around day 10. Staining of syndecan-4 resulted in a homogenous punctate-like staining of the cell bodies from about day 3 onwards, consistent with a cell surface-located plasma membrane proteoglycan. We explored the interaction between citrullinated FBLN5 and syndecans by surface plasmon resonance using recombinantly produced human FBLN5 immobilized on the gold chip and recombinantly generated syndecan ectodomains (lacking the transmembrane region) as soluble analytes. Syndecan-3 and -4 showed a fast association followed by a slow dissociation from FBLN5, consistent with a strong interaction of these syndecan ectodomains to FBLN5. On the contrary, the syndecan-2 ectodomain did not interact with FBLN5. To analyze the specific role of FBLN5 citrullination in this interaction and the larger context of elastogenesis, we set out to inactivate the previously identified citrullination sites in FBLN5 (R75K, R107K, R349K). We designed synthetic DNA constructs coding for full-length wild-type FBLN5 and FBLN5 containing modifications of the three arginine to lysine mutations. The commercially synthesized DNA was introduced into an episomal expression plasmid pCEP4 using Gibson assembly. Sanger sequencing resulted in a series of additional mutations, all of which were corrected. The wild-type and citrullination mutants were transfected into HEK 293 EBNA cells. Analysis of the FBLN5 expression levels in the selected recombinant cell clones is currently pending.

CONCLUSIONS: These preliminary results partially support the hypothesis that citrullination of FBLN5 mediates the interaction with syndecan-3 and syndecan-4. After the recombinant constructs are available, further validation will be performed.

Abstract #: 65
Presenting Author: Ramshaa Mahalingam
Research Theme: Cell Biology of Connective Tissues
Preferred Presentation: Poster
Presenter Category: MSc Student

A metabolomic approach to understanding the pathogenesis of ectopic spine mineralization

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INTRODUCTION: In North America, 15%-25% of people over 50 suffer from diffuse idiopathic skeletal hyperostosis (DISH) – 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 mineralization 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.

METHODS: AIM 1- Candidate pathways and proteins were assessed using in situ localization and quantitative analyses of IVD tissues from wild-type (WT) and ENT1-/- mice at 2 and 6 months of age. Targets of interest include apoptosis pathways, PI3K/Akt, and S100A9.
AIM 2- Bioinformatics analyses of untargeted metabolomic data generated from AF tissues and plasma from WT and ENT1-/- mice at 2 and 6 months of age was conducted to identify altered pathways and metabolites.

RESULTS & DISCUSSION: Immunolocalization of cleaved caspase-3 suggested an enrichment of caspase 3 activation at both timepoints in the AF of ENT1-/- mice relative to WT. Immunolocalization of S100A9 showed no significant difference in ENT1-/- mice at either timepoint. No difference in Akt activation was detected in either male or female ENT1-/- mice by western blot at either timepoint; however, Akt levels were significantly decreased in both male and female ENT1-/- mice compared to WT at 6 months. Metabolomic analyses found 1 metabolite at 2 months and 6 metabolites at 6 months were significantly altered in the AF of ENT1-/- mice compared to WT, and 22 metabolites were significantly altered in the plasma at 6 months. Numerous metabolites were also found to be altered when comparing ENT1-/- mice at 2 and 6 months in both tissues.

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 #: 66
Presenting Author: Fang Chi Wang
Research Theme: Cartilage and Intervertebral Disc
Preferred Presentation: Poster
Presenter Category: MSc Student

The impact of bone morphogenic protein-2 on SATB2 mediated gingival heterotopic ossification

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Introduction: Peripheral ossifying fibromas (POFs) are non-neoplastic oral lesions that present with nidi of bone and are proposed to originate from the periodontal ligament (PDL). They are localized to the gingiva, which is embryologically derived from neural crest cells. POFs show consistent nuclear expression of SATB2, an osteoinducive transcription factor that has been shown to be upregulated by bone morphogenic protein-2 (BMP-2) in mesenchymal cells. This current study attempts to determine the effect of BMP-2 on SATB2 expression and ossification rate in human gingival fibroblasts (hGFs) and human periodontal ligament fibroblasts (hPDLFs). It also aims to clarify the origin of POFs by elucidating which oral fibroblast populations are capable of ossifying. We hypothesize that BMP-2 will upregulate SATB2 expression and ossification rate in hPDL fibroblasts, but not hGFs, supporting the theory that POFs originate from the PDL. Through delineating how BMP-2 impacts SATB2 expression and ossification in POFs, we hope to provide insight into heterotopic ossification pathways in other regions derived from the neural crest.

Methods: Healthy hPDLFs and HGFs were cultured and analyzed for SATB2 expression via immunofluorescence staining. Furthermore, healthy hPDLFs were cultured in osteogenic cell culture media and are being treated with BMP-2 concentrations of either 0, 2, 4, 8, 10, 20, 40 or 80 ng/mL every two days over 6 weeks. Control cell cohorts received Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum and 2% antibiotic/antimycotic. These cell cultures were stained with Alizarin Red S to assess for mineralization after the 6 weeks are completed. QuPath was utilized to quantify the amount of mineralization.

Results: Both healthy hGFs and hPDL fibroblasts showed nuclear translocation of SATB2. Furthermore, after 7 days of BMP treatment, BMP-2 concentration and hPDLF mineralization appeared to exhibit a positive association until concentration surpassed 10 ng/mL. At higher concentrations, there appeared to be reduced mineralization and increased rates of cell death. The DMEM control group exhibited no mineralization.

Discussion: The results weaken the theory that POFs originate from the PDL, as both healthy hGFs and hPDLFs express SATB2. They also suggest that the effect of BMP on hPDLF ossification is dose dependent. Future experiments will assess the influence of BMP on hGFs osteogenic capacity, as well as assessing BMP expression in human POF tissues.

Abstract #: 67
Presenting Author: Esther Chang
Research Theme: Cell Biology of Connective Tissues
Preferred Presentation: Poster
Presenter Category: MSc Student

The role of adjuvant in the severity of collagen induced arthritis

Gabrielle BUCKLEY[1], Jaspreet KAUR[1], Ewa CAIRNS[1], Lillian BARRA[1]

[1]Western University

Background: Rheumatoid arthritis (RA) is a chronic, inflammatory autoimmune disease that primarily affects synovial joints. Citrullination and homocitrullination, post-translational modifications of arginine and lysine, respectively, are observed in endogenous synovial proteins, including type II collagen (CII). Tolerance breakdown against these modifications result in the typical RA histological characterizations: bone erosion, cartilage damage, synovitis, and pannus formation. The resulting inflammatory environment further increases citrullinating and homocitrullinating enzymes, thus supporting disease progression. Presently, no RA-specific therapies exist, and pre-clinical testing of novel therapies frequently employ the collagen induced arthritis (CIA) mouse model due to similar disease manifestations. CIA induction occurs through immunization with CII in Complete Freund's Adjuvant (CFA). However, CFA concentration varies amongst studies, thus requiring standardization.

Hypothesis: Histological damage, and citrullinated and homocitrullinated joint proteins in the CIA model will increase as the primary immunization's CFA concentration increases.

Methods: Synovial joints from CIA mice immunized with bovine CII (bCII) emulsified in 0.5, 1.0, or 2.0 mg/mL CFA (N = 6) were sectioned and stained with hematoxylin and eosin to examine joint structures and infiltration or toluidine blue to assess cartilage health. Furthermore, citrullinated and homocitrullinated protein levels were determined through immunofluorescence microscopy.

Results: 1.0 mg/mL CFA induced CIA most closely mimicking human RA histology. However, 2.0 mg/mL CFA exhibited minimal histological damage but had overall increased citrullinated and homocitrullinated protein levels, although primarily not significant. Significant differences were observed in the ankle homocitrullinated protein levels of the 2.0 mg/mL CFA group compared to the 0.5 and 1.0 mg/mL CFA groups ($p = 0.0019$, $p = 0.002$). Similarly, the ankle homocitrullinated protein levels of the 2.0 mg/mL CFA group in the bone marrow were significantly greater than the 1.0 mg/mL CFA group ($p = 0.0402$).

Significance: Histopathology suggests 1.0 mg/mL CFA is optimal for CIA induction; however, further immunofluorescence analysis is required to support usage in CIA model future applications.

Abstract #: 68
Presenting Author: Gabrielle Buckley
Research Theme: Cell Biology of Connective Tissues
Preferred Presentation: Poster
Presenter Category: Undergraduate Student

Topographical modulation of human dermal keratinocyte behaviour

Justin CARMICHAEL[1]

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Chronic wounds present a serious and growing clinical condition where wounds fail to heal within three months and lead to a lack of mobility, re-current infections, and amputations. Keratinocytes on the edge of chronic wounds are still proliferative and capable of migration; however, their migration is misguided which can lead to epibole or undermining of the epithelium into the dermis (Tinney, unpublished data). Micrometer topographical cues, specifically groove ridge topographies, are able to stimulate directed migration of many cell types and offer an opportunity to correct the misguided migration of the epithelium. Topographies of various periodicities, groove and ridge widths and depths were investigated. Polycaprolactone (PCL) is a versatile FDA and Health Canada approved polymer that was selected to create a thin membrane containing micrometer topographical cues. Polycaprolactone membranes were fabricated by placing beads of PCL into a negative impression mold of the desired topography, heated to 150°C with 25 PSI exerted on the PCL to facilitate topography recapitulation. Primary normal human dermal keratinocytes purchased from Lonza were used as a model system. Cytokeratin (CK) phenotyping of the cells revealed that they closely resemble normal human skin cytokeratin expression. Single keratinocytes seeded directly on topographies with a depth of 10 µm resulted in significant orientation to the long axis of the grooves compared to the flat control surface. Shallow grooves with a depth of 1 µm resulted in significant orientation to the long axis of the grooves compared to the flat control surface only when the keratinocyte was in contact with a sufficient number of topographical cues. Basal keratinocytes are identified by positive CK 14 expression, whereas suprabasal keratinocytes are identified by positive CK 10 expression (Patel et al, 2006). Basal (CK 14+) and suprabasal (CK 10+) keratinocytes were seeded as individual cells on the various topographies. On all topographies, the number of CK 10+ cells decreased as well as total cell count indicating that the topographies did not stimulate differentiation or dedifferentiation of the keratinocytes. Cytokeratin 6 is proposed to be anti-migratory (Wang et al, 2018), however single cells expressing CK 6 are capable of migration on all topographies. These results demonstrate that groove depths of 10 µm result in significant orientation to the direction of the grooves, induce migration regardless of CK 6 expression and do not cause differentiation or differentiation of the keratinocytes, culminating to a surface that is capable inducing directed migration of single keratinocytes.

Abstract #: 69
Presenting Author: Justin Carmichael
Research Theme: Skin and Matrix Biology
Preferred Presentation: Poster
Presenter Category: MSc Student

Phase-contrast microcomputed tomography for the evaluation of cartilage loss in a genetic mouse model of mild, early-onset osteoarthritis

Kevin FAN[1], Brendan H. LEE[2], Matthew W. GROL[1]

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BACKGROUND: Osteoarthritis (OA) is a multifactorial synovial joint disease characterized by articular cartilage degradation, subchondral bone sclerosis, and synovial inflammation that cause chronic pain. Preclinical models have provided great insights into OA pathogenesis; however, these are driven largely by histological evaluations of disease, which are prone to sampling bias and technical variability. To address these limitations, high-resolution phase-contrast microcomputed tomography (μCT) has been established to quantify changes in intact murine with surgically-induced moderate-to-severe OA. In these contexts, phase-contrast μCT provides 3-D assessments of articular cartilage and subchondral bone changes that are free of sampling bias with increased statistical power. However, this imaging modality has not been applied to OA disease models where the pathological changes are more subtle. Thus, the goal of this study was to determine if our phase-contrast μCT protocol could be adapted to characterize milder forms of early-onset disease.

HYPOTHESIS: Phase-contrast μCT will agree with the histopathological changes in articular cartilage observed for the cho/+ mouse model of mild, early-onset OA.

METHODS: Mice heterozygous for the cho mutation, which is a spontaneous single nucleotide deletion in the ColXIa1 gene, develop mild OA beginning at 12-15 months. For our study, male cho/+ and wildtype C57BL/6J mice were aged to 18-months, euthanized, and knee joints collected for histopathological scoring (i.e., OARSI) or phase-contrast μCT. For the phase-contrast μCT analysis, samples were fixed in glutaraldehyde and stained with ruthenium (III) hexamine and osmium tetroxide to enhance soft tissue contrast. Samples were then embedded in paraffin wax and scanned at 4-μm resolution using 40 kV and 200 μA (no filter). Quantitative analysis of articular cartilage volume and surface were conducted using Dragonfly commercial software by Object Research Systems (ORS).

RESULTS: Using scans from wildtype mice, a protocol was successfully developed that allowed for quantification of articular cartilage volume and surface. When comparing preliminary results (n = 3 mice per group), we observed little-to-no difference in articular cartilage volume and surface in cho/+ mice at 18-months compared to wildtype. On the other hand, cho/+ mice had a modestly elevated OARSI score compared with wildtype mice indicative of mild early-onset OA.

CONCLUSIONS: Based on our preliminary findings, the current phase-contrast μCT protocol is not sensitive enough to detect subtle changes in articular cartilage in a genetic mouse model of early-onset OA. Future studies will explore alternate scan settings as well as additional contrast agents to further enhance the features of joint tissues

Abstract #: 72
Presenting Author: Kevin Fan
Research Theme: Cartilage and Intervertebral Disc
Preferred Presentation: Poster
Presenter Category: Undergraduate Student

Cold Plasma-based Redox Therapy for Breast-to-Bone Metastasis Tumor Growth Control

Laura BOURET[1], Jean-Baptiste BILLEAU[1], Michael WEBER[2], Stephan REUTER[1], Derek ROSENZWEIG[2]

Polytechnique Montreal[1]; McGill University[2]

Background: Bone, particularly the spine, is a frequent site of metastasis for breast, lung, and prostate cancers, presenting a considerable challenge. Current treatment options such as chemotherapy and invasive surgery often require extensive tissue removal, risking infection and necessitating reconstruction. Cold plasma therapy, operating below 40°C, offers a non-invasive alternative by locally delivering reactive oxygen and nitrogen species (RONS). Though promising, understanding the interaction between plasma and tissues, as well as determining optimal treatment dosage and RONS composition, remains an ongoing area of research.

Hypothesis and objective: This project aims to develop and characterize a cold plasma source and explore its potential in mitigating bone cancer metastasis, hypothesizing its anti-tumor effects. The overall objective is to create a tissue-plasma platform for cold plasma therapy, aiming to control the metastatic spread of breast cancer cells to bone tissue.

Methods: We have developed a platform combining tailored plasma reactivity through a kHz coaxial dielectric barrier discharge source and a highly reproducible bioprinted circular bone tissue model. The bone tissue model was bioprinted (Cellink BioX) using cell-laden hydrogel. A multi-well plate was generated with identical “breast-to-bone” metastasis as a coculture model of MDA-MB-231 and human bone marrow mesenchymal stem cells (hbmMSCs). Liquid RONS were quantified using UV-VIS colorimetry. Metabolic activity was assessed using Alamar blue assays on days 1, 2, and 3 post-plasma treatment, and live/dead measurements were used to evaluate the biological response of tumor cells.

Results: Results have shown that A1G7 cell-laden hydrogel was bioprinted with reproducible results in a model of cocultured MDA-MB-231 breast cancer cells and hbmMSCs. Dose responses of plasma on cancer cells and healthy cells were assessed in 2D and 3D cultures, including 3D coculture models. Plasma exhibited a selective antitumoral effect on MDA-MB-231 cancer cells compared to hbmMSCs healthy cells in both 2D and 3D settings. Additionally, colorimetric assays confirmed the ability to customize long-lived species (H_2O_2 and NO_2^-) by adjusting plasma parameters such as energy, distance, treatment duration, and atmospheric composition.

Conclusion: Our platform enables the creation of unique biological chemistry for plasma redox-based cancer treatment. Utilizing a bioprinted model ensures reproducibility and precise control, facilitating detailed studies of tumor migration. With a tailored plasma jet, our platform explores novel therapeutic approaches using exogenous reactive species. This aims to develop a personalized, non-invasive treatment to reduce bone cancer metastasis, potentially integrating the technology into clinical applications.

Abstract #: 73
Presenting Author: Laura Bouret
Research Theme: Connective Tissue Repair, Regeneration, Bioengineering
Preferred Presentation: Poster
Presenter Category: PhD Student

Poster Judging Schedule

Abstract #	Title	Presenting Author	Poster Judging
3	CCN1 and CCN3 protein expression in Duchenne muscular dystrophy	Margarita Egiian	Tuesday
4	Short link N peptide modulates inflammasome activity via CD14 interaction: Potential therapeutic for IVDD	Muskan Alad	Monday
7	Role and expression of 15-prostaglandin dehydrogenase in cartilage tissue	Sami Alsabri	Monday
8	Mechanically activated myofibroblasts drives macrophages into profibrotic phenotype through direct cell contact	Li Diao	Tuesday
16	Mechanical environment of mesenchymal stromal cells controls production of exosomes	Kyle Lam	Tuesday
20	Fibronectin isoforms regulate postnatal skeletal development	Neha Dinesh	Monday
23	Developing a cartilage-on-a-chip and vascularized synovium-on-a-chip model for joint diseases	Lauren Banh	Tuesday
26	Chitosan and platelet-rich plasma biomaterial improves healing of skin wounds in diabetic rats	Laura Ahunon	Tuesday
28	Development of an engineered synovium model for studying synovial cell-cell interactions	Paul Collie	Tuesday
34	Continuous expansion of human cruciate ligament cells for ligament tissue engineering	Tarek Klaylat	Tuesday

37	Development of an explant model of tendon injury to study heterotrophic ossification	Tarek Klaylat	Monday
39	Analyzing the effects of PGC1α overexpression in a post-traumatic mice model of osteoarthritis	Sepideh Taghizadeh	Monday
40	Growth factor cocktail: In vitro phenotypic modulation of human periodontal ligament and gingival fibroblasts	Chengyu Guo	Tuesday
42	Inflammation-inducible strategies for growth factor gene therapy to promote joint tissue repair: Applications for osteoarthritis therapy	Anisha Thomas	Monday
43	Localization and quantification of stem cells in salivary glands	Mariia Mielkozorova	Tuesday
44	Effects of obesity/metabolic syndrome on synovial microvasculature during knee osteoarthritis	Qinli Guo	Monday
47	Ultrasound-measured synovitis is associated with movement-evoked pain in knee osteoarthritis: A cross-sectional and within participant design	Brent Wakefield	Monday
49	Retinoid signaling in the modulation of ectopic mineralization in a mouse model of DISH	Alexander Rico	Monday
50	The relationship between TGFα and mitochondrial function in chondrocytes	Emily White	Monday
51	Fibulin-4 and LTBP-4 regulate skin elastogenesis via syndecan interaction	Neha Dinesh	Tuesday
	Pannexin 3 deletion in male and female mice results in	Brent Wakefield	Tuesday

	severe osteoarthritis in aging52		
57	Optimizing a novel media formulation for osteochondral allograft preservation	Isabel Li	Monday
58	Effects of Ras-associated binding protein Rab7b and its nucleotide mutants on vacuolar type H+-ATPase (V-ATPase) a3 subunit localisation in HeLa cells	Wing Hei Wong	Monday
61	Cell-autonomous ECM regulation of MuSC function	Yuguo Liu	Tuesday
63	The relationship between centre-of-pressure and electroarthrography, a non-invasive method for cartilage assessment	Josephine Lui	Monday
65	Role of fibulin-5 citrullination in elastogenesis	Ramshaa Mahalingam	Tuesday
66	A metabolomic approach to understanding the pathogenesis of ectopic spine mineralization	Fang Chi Wang	Monday
67	The impact of bone morphogenic protein-2 on SATB2 mediated gingival heterotopic ossification	Esther Chang	Tuesday
68	The role of adjuvant in the severity of collagen induced arthritis	Gabrielle Buckley	Tuesday
69	Topographical modulation of human dermal keratinocyte behaviour	Justin Carmichael	Tuesday
72	Phase-contrast microcomputed tomography for the evaluation of cartilage loss in a genetic mouse model of mild, early-onset osteoarthritis	Kevin Fan	Monday

73	Cold Plasma-based Redox Therapy for Breast-to-Bone Metastasis Tumor Growth Control	Laura Bouret	Tuesday

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