Canadian Connective Tissue Society

30th Canadian Connective Tissue Conference 2026
#CCTC2026

30th Canadian Connective Tissue Conference (CCTC)

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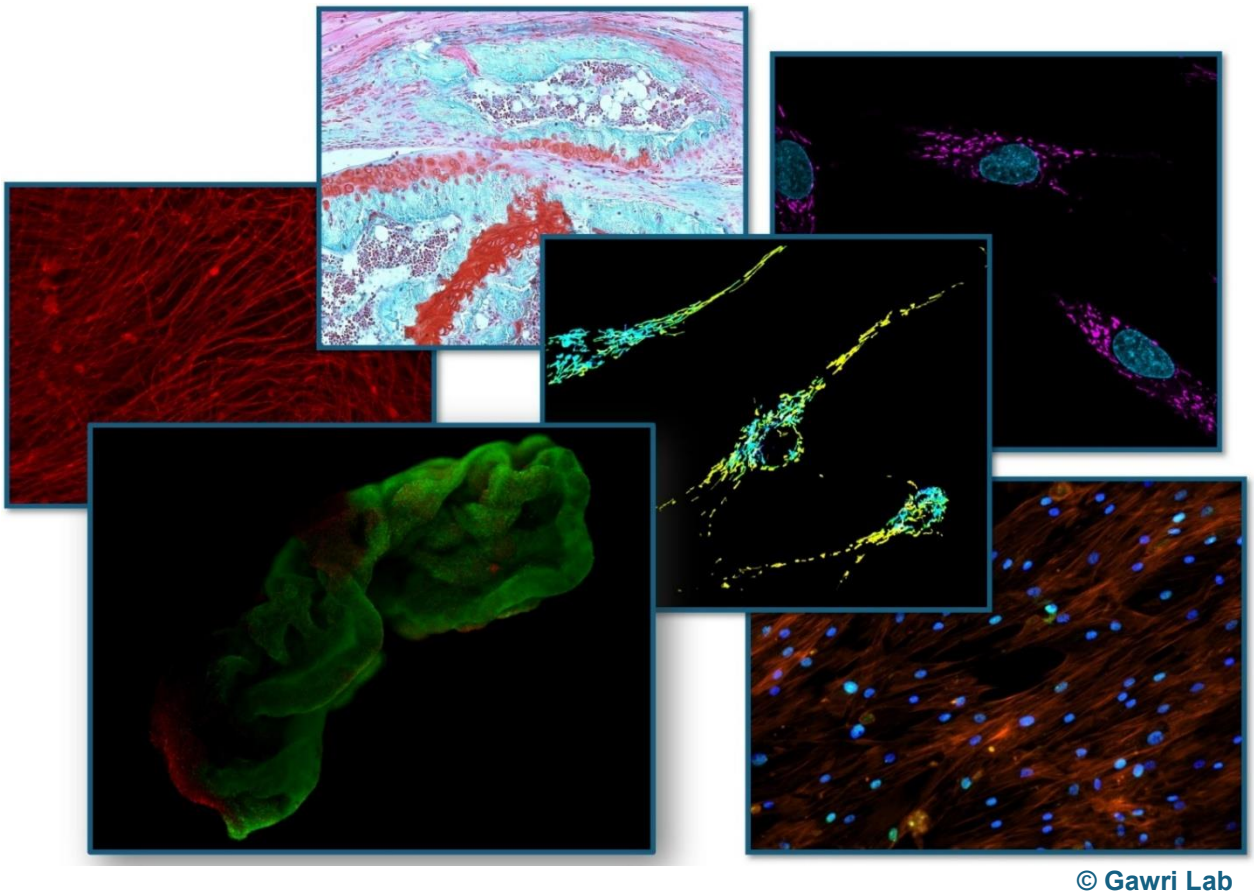
#CCTC2026

May 25-27th, 2026

Polytechnique Montreal, Montréal, Québec



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Message from the CCTS President

Chers amis/Dear Friends,

Bienvenue à la 30e conférence canadienne sur le tissu conjonctif!

Welcome to the 30th Canadian Connective Tissue Conference!

I am delighted to welcome you not only to this year's meeting but also to the Canadian Connective Tissue Society community. Whether you are attending for the first time or are a seasoned participant, I encourage you to fully immerse yourself in this supportive environment, which is a place where young scientists thrive and collaborations flourish.

The CCTC was founded by Dr. Robin Poole in 1994 as he realized there was a need for Canadian researchers, ranging from trainees to established scientists, to connect with one another to share their work and exchange ideas. He organized the very first CCTC meeting at McGill with virtually no budget and low expectations for attendance. As it turned out, many others saw value in building these connections and the CCTC has proceeded annually (with the exception of the COVID years) ever since. In 2012, the Canadian Connective Tissue Society was established and the CCTC became the annual meeting of the Society. It has been my great privilege to serve as President of the CCTS and help continue building this vibrant and collaborative society.

Our unwavering commitment to trainees is what makes the CCTS special. Every aspect of the conference is thoughtfully designed to nurture the development of our young Canadian scientists by offering opportunities to present your research and build professional networks that will serve you throughout your careers. I attended this conference as a student myself. I remember being intimidated by how little I knew of the vast field of connective tissue research, but I was reassured to discover that there were fellow trainees that shared these feelings. I was also very nervous about presenting my work, but I persevered, received thoughtful questions from the audience that challenged my thinking, and felt more confident the next time I gave a talk. These experiences were instrumental in my development as a scientist, and I hope this year's conference provides you with the same encouragement, growth, and sense of belonging.

I am very happy that this year's conference is being held at Polytechnique Montréal, where I was once a student. Poly is renowned for innovation making it a fitting place to host the outstanding program assembled by our conference co-chairs. Over the next three days, I encourage you to take full advantage of opportunities to share knowledge, step outside your comfort zone, and engage in thoughtful discussions. This is how great science and strong networks are built.

I wish you a wonderful conference ! Je vous souhaite une bonne conférence !

Adele Changoor
CCTS President

Message from CCTC 2026 Co-Chairs

Florian Bentzinger
Co-Chair CCTC 2026

Houman Savoji
Co-Chair CCTC 2026

Dear Friends, Colleagues, and Trainees,

On behalf of the **Canadian Connective Tissue Society (CCTS)**, it is our great pleasure to welcome you to the **30th Annual Canadian Connective Tissue Conference (CCTC 2026)**, taking place in Montréal, Québec, from May 25 to 27, 2026. Reaching the 30th edition of this conference is a wonderful milestone for our community. Over the years, the CCTC has become much more than an annual meeting. For many of us, it is where collaborations begin and where scientific ideas take shape through discussion, often leading to lasting connections and friendships. The impact of this community on Canadian connective tissue research has been remarkable, but its influence on the people within it may be even greater. This spirit continues to guide the vision of the CCTS. Scientific excellence remains central, while the culture of openness and mentorship has helped generations of trainees and investigators grow and find their place in the field.

CCTC 2026 brings together a vibrant program that reflects both the breadth and energy of connective tissue research today. Alongside keynote lectures and selected presentations, we are also introducing a new preconference workshop entitled "***From Research and Innovation to Commercialization and Impact***" This initiative aims to open discussions beyond the laboratory and to give trainees a broader perspective on how scientific discoveries can ultimately shape society. This year's meeting also offers the opportunity to recognize two exceptional members of our community. We are honoured to celebrate **Marc D. McKee**, recipient of the **CCTS Lifetime Achievement Award**, whose pioneering work on skeletal mineralization and extracellular matrix biology has had a lasting influence on the field and has inspired outstanding mentorship. We also extend our sincere congratulations to **Julie Fradette**, recipient of the **Robin Poole Investigator Award**, for her innovative contributions to tissue engineering and regenerative medicine and for the impact her work continues to have across disciplines.

A defining feature of the CCTC is the strong involvement of trainees at all levels of the meeting. Seeing younger scientists take ownership of sessions and discussions remains one of its most rewarding aspects. In many ways, the continuity between generations gives the CCTS its unique identity. Recent meetings have brought together multiple generations of scientists connected through the mentorship lineage of Dr. Robin Poole, a powerful reminder of how knowledge and curiosity continue to be passed forward. We would like to sincerely thank everyone who contributed to CCTC 2026, including participants, reviewers, volunteers, the organizing committee, and our sponsors. Their efforts made this meeting possible. We are delighted to welcome you to Montréal and hope you will find the meeting both engaging and rewarding.

We look forward to celebrating this special anniversary together.

With our warmest regards

Florian Bentzinger and Houman Savoji
Co-chairs, CCTC 2026

CCTC 2026 Organizing Committee:

Dieter Rienhardt
Professor
McGill University

Rahul Gawri
Associate Professor
McGill University

Tarek Klaylat
PhD Student
McGill University

Neha Dinesh
Post-doctoral Fellow
McGill University

CCTC 2026 Volunteers

We would like to extend our sincere thanks to our CCTC 2026 volunteers from the Savoji Lab at Polytechnique Montreal, Research Center of Sainte Justine Hospital, and University of Montreal.

Shervin Foroughi
Post-doctoral Fellow

Arman Jafari
Post-doctoral Fellow

Arezoo Shamloo
PhD Student

Alireza Nemati
PhD Student

Amir Aidun
PhD Student

CCTC 2026 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 2026.

Matthew Grol, Western University

Boris Hinz, University of Toronto

Dieter Reinhardt, McGill University

Rahul Gawri, University Health Network

Derek Rosenzweig, McGill University

Adele Changoor, University of Toronto

Lidan You, University of Toronto

Marc Grynpas, University of Toronto

Morris Manolson, University of Toronto

Carl Richards, McMaster University

Roshni Rainbow, Queen's University

CCTC 2026 Student Session Co-Chairs

We would like to extend our sincere thanks to the Student Session Co-Chairs for their valuable contributions and support in moderating the scientific sessions.

• Session 1 — Bone Biology and Tissue Mineralization
Vincenzo Marchese (Queens University) & **Laura Ahunon** (Polytechnique Montreal)

• Session 2 — Bioprinting and Additive Manufacturing in Connective Tissue Biology
Shervin Foroughi (Polytechnique Montreal) & **Hosni Cherif** (McGill University)

• Session 3 — Cell Biology of Connective Tissues
Iram Fatima Siddiqui (McGill University) & **Hayley Murray** (University of Western Ontario)

• Session 4 — Functional Biomaterials for Connective Tissue Repair
Arezoo Shamloo (University of Montreal) & **Sami Alsabri** (McGill University)

• Session 5 — Matrix Biology in Connective Tissue Research
Camille Ingrand (Université de Sherbrooke) & **Amir Aidun** (University of Montreal)

• Session 6 — Repair and Regeneration of Biomechanically Loaded Tissues
Dantha Sitompul (McGill University) & **Arianne Graveline** (Université de Sherbrooke)

• Session 7 — Clinical Perspectives in Connective Tissue Pathobiology
Liyang Zhong (University of Toronto) & **Juan Duque** (Université de Sherbrooke)

• Session 8 — Cartilage Biology and Pathobiology
Julia Velez (McGill University) & **Alireza Nemati** (University of Montreal)

CCTC 2026 Sponsors

BIOMOMENTUM

MDPI

Cell

Réseau de thérapie cellulaire, tissulaire et génique du Québec

Rheolution
TEST SMARTER

UNIVERSITÉ DE SHERBROOKE
Faculté de médecine et des sciences de la santé

POLYTECHNIQUE MONTRÉAL

CCTC 2026 Travel Award Winners

Lauren Banh, University of Toronto

Nabangshu Das, University Health Network

Neha Dinesh, University of Toronto

Xinying Guo, University of Toronto

Fahad Ehsan, University of Toronto

Simon Dumontier, University of Sherbrooke

Moatter Syed, University of Ottawa

Qinli Guo, Western University

Brandon Vaz, University Health Network

Quan Zhou, McMaster University

CCTS international travel awardees

Jeffrey Hutchison, Western University

Tarek Klaylat, McGill University

CCTC 2026 Conference Overview

Scientific Sessions:

All oral presentations in Sessions 1–8 of the Scientific Program, as well as the Pre-Conference Workshop, will take place in the **Amphitheatre (6th Floor, Principal Building)** at **Polytechnique Montréal**, Montréal, Québec.

All oral presenters are requested to bring their presentation on a USB key, ready to be uploaded during the break proceeding their respective session.

Registration and Poster Sessions:

Registration and Poster Sessions will take place in **Galerie Rolande (Room B-600.16)** on the **6th Floor of the Principal Building** at **Polytechnique Montréal**.

Poster judging will be held on Tuesday, May 26th, (Session 1) and Wednesday, May 27th (Session 2). Each poster will be judged by two experts. Poster presentations consist of a 5-minute presentation at the poster, followed by 2 minutes Q&A with each of the assigned experts.

Gala Dinner:

The **Gala Dinner** will take place on **May 26, 2026, from 6:00 PM to 10:30 PM**, in **Galerie Rolande (Room B-600.16)** on the **6th Floor of the Principal Building** at **Polytechnique Montréal**. **Ticket is mandatory for attendance.**

This fun-filled evening will include an award presentation ceremony for the CCTS Lifetime Achievement Award, Robin Poole Investigator Award, and their respective acceptance lectures.

Venue & Access Information:

ACCESS TO POLYTECHNIQUE MONTRÉAL BY CAR

Adress: Lassonde Buildings – Polytechnique Montréal, 2700 Chemin de la Tour, Montréal, QC H3T 1J4.

Take Ramp Road toward the Main Building. At the second stop sign, make a U-turn. The parking entrance will be on your right (Lassonde Building).

Parking: Take a ticket at the entrance. Park in sections **4A** or **4B** on **Level 4**. Please keep your ticket, as it is required to exit the parking facility.

ACCESS TO THE MAIN BUILDING FROM THE LASSONDE VISITOR PARKING

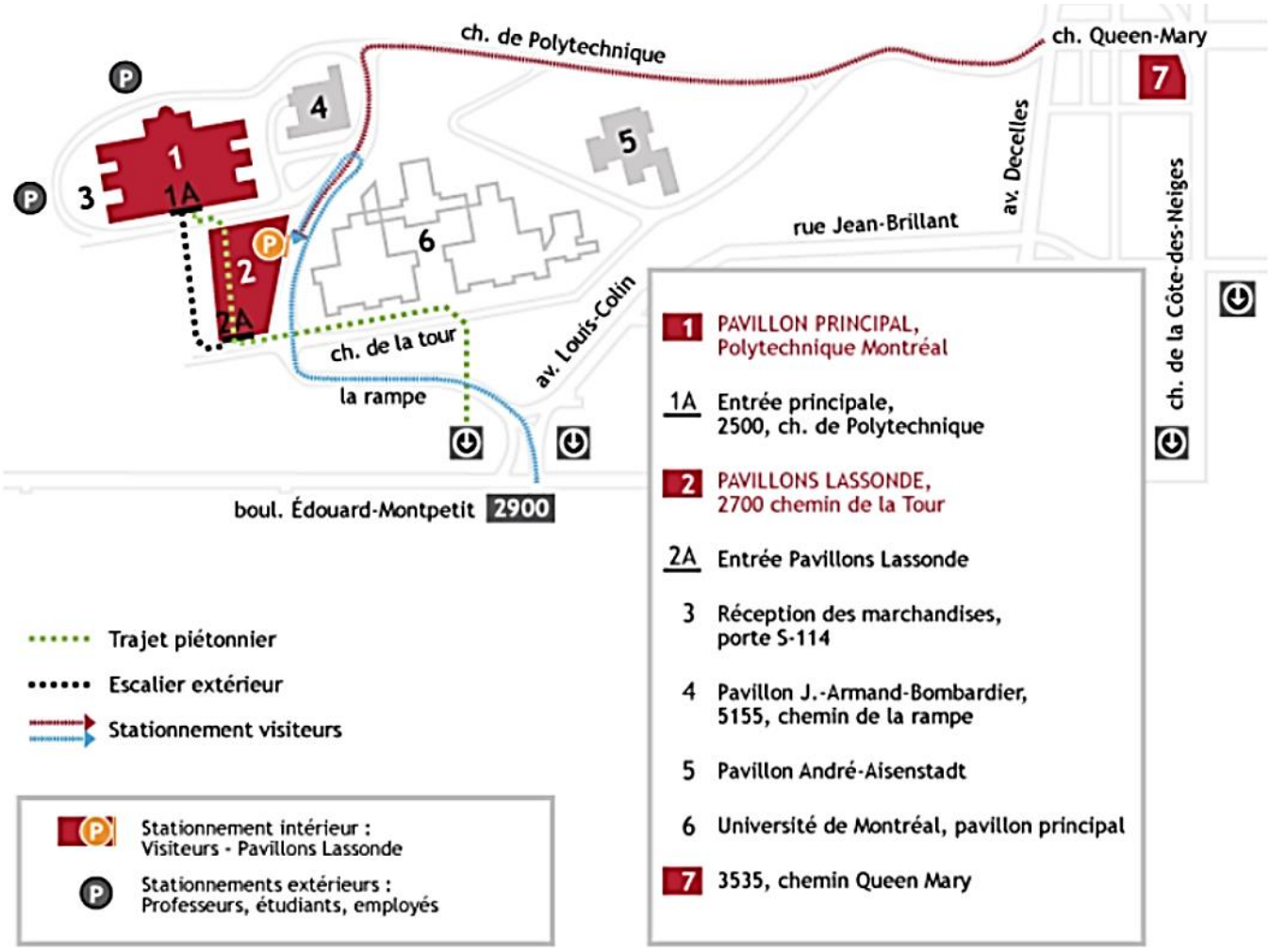
From the Lassonde visitor parking, exit via **Level 4** and take the escalators to **Level 6**. Proceed straight through the indoor tunnel connecting the Lassonde and Principal Buildings. Upon exiting the tunnel, turn

left and follow the cafeteria corridor. At the intersection, turn left and take the escalators to access the **6th Floor of the Principal Building**, where the conference activities will take place.

ACCESS BY SUSTAINABLE TRANSPORT

Access from Université de Montréal Metro Station (Blue Line): After exiting the turnstiles, turn left and proceed past the convenience store. Take the stairs to the station exit, then use the escalators on your right. At the top of the escalators, turn left and follow the corridor. Continue up the stairs leading outside, then turn right onto **Chemin de la Tour** and follow the signs to the **Lassonde Buildings**, where conference signage will direct you to the venue.

Access to the Principal Building from the Lassonde Buildings: Enter through the Lassonde Buildings main entrance (**2A**) and take the escalators to **Level 6**. Proceed through the indoor tunnel linking the Lassonde and Principal Buildings. Upon exiting the tunnel, turn left and follow the cafeteria corridor. At the intersection, turn left and take the stairs to the **Principal Building entrance (1A)**. From there, take the escalators to the **6th Floor**, where registration, poster sessions, oral presentations, and conference events will be held.

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1A
2
2A
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Stationnement intérieur :
Visiteurs - Pavillons Lassonde
Stationnements extérieurs :
Professeurs, étudiants, employés
1 PAVILLON PRINCIPAL,
Polytechnique Montréal
1A Entrée principale,
2500, ch. de Polytechnique
2 PAVILLONS LASSONDE,
2700 chemin de la Tour
2A Entrée Pavillons Lassonde
3 Réception des marchandises,
porte S-114
4 Pavillon J.-Armand-Bombardier

CCTC 2026 Scientific Program

Polytechnique Montréal, Montréal, Québec, Canada
May 25–27, 2026
(Please see footnote for talk format information)

PRE-CONFERENCE WORKSHOP

From Research and Innovation to Commercialization and Impact

Monday, May 25, 2026

09:00 – 09:15 | WELCOME & OPENING

Morning - Expert Talks

09:15 – 09:45 | **Jérôme Feige** (Nestlé Research / EPFL, Switzerland) *"Publishing and Careers at the Academia Industry Interface"*

09:45 – 10:15 | **Patrice Leclerc** (TransferTech Sherbrooke) *"Why Intellectual Property Matters: From Research to Value Creation"*

10:15 – 10:30 | REFRESHMENT BREAK

10:30 – 11:00 | **Amir Hooshiar** (SuPER Centre, McGill University, Vitruvius Venture Studio) *"Translational Research from Bench to Bedside – with a "Start-up" in the middle"*

11:00 – 11:30 | **Marie Lyne Nault** (CHU Sainte-Justine) *"Clinical Innovation and Career Paths: Industry vs Academia"*

11:30 – 12:00 | **Panel-style Open Discussion**

12:00 – 13:00 | LUNCH & NETWORKING

Afternoon - Interactive Sessions

13:00 – 13:15 | **Instructions & Group Setup**

13:15 – 14:15 | **Group rotations (4 x 15 min)**

14:15 – 15:00 | FINAL WRAP UP

CONFERENCE DAY 1 - Tuesday, May 26, 2026

07:30 – 08:30 | REGISTRATION ● BREAKFAST ● POSTER SETUP

08:30 – 08:45 | WELCOME REMARKS - Adele Changoor (President, CCTS; University of Toronto), Maud Cohen (President, Polytechnique Montréal) & Conference Chairs

SESSION 1 - Bone Biology and Tissue Mineralization

08:45 - 09:15 | KEYNOTE LECTURE - Maryam Tabrizian (McGill University) *"Subchondral Bone Matters: A Disease-Modifying Approach for Osteoarthritis Using Mesenchymal Stem Cell-Derived Nanoghosts"*

09:15 - 09:30 | Kimberly Seaman (University of Toronto) *"Examining mechanical regulation of disseminated prostate cancer cells using an in vitro model of the bone marrow perivascular niche"*

09:30 - 09:45 | Laura Bouret (Polytechnique Montréal) *"Localized Cold Atmospheric Plasma Treatment Following Tumor Resection Improves Outcomes in a Rat Model of Breast Cancer Bone Metastasis"*

09:45 - 10:00 | Derek Rosenzweig (McGill University) *"A Novel Rat Caudal Vertebra Metastasis Model Demonstrates Suppression of Tumor Recurrence and Preservation of Bone by Doxorubicin-Releasing PMMA Cement"*

10:00 - 10:15 | Shazah Waqar (University of Ottawa) *"Evaluation of eggshell-based constructs for bone regeneration in rat calvaria critical-size defect model"*

10:15 – 10:30 | REFRESHMENT BREAK

SESSION 2 - Bioprinting and Additive Manufacturing in Connective Tissue Biology

10:30 – 11:00 | KEYNOTE LECTURE - Matt Kinsella (McGill University) *"Biomaterials from Decellularized Extracellular Matrix Molecules"*

11:00 - 11:15 | Arman Jafari (University of Montréal) *"3D Printed Tissue-Engineered Heart Valves Using Light-based Printing Technology"*

11:15 - 11:30 | Seong Yeon Kim (McGill University) *"Bioengineering a 3D Intestinal Co-Culture Model for In Vitro Therapeutic Screening in Inflammatory Bowel Disease"*

11:30 - 11:45 | Moatter Syed (University of Ottawa) *"Characterization of 3D bioprinted eggshell membrane-loaded alginate biomaterials for wound healing applications"*

11:45 - 12:00 | Aidee Arizpe-Tafoya (McMaster University) *"Multiplexed Multi-Material 3D Bioprinted Platform to Model Mechanical Heterogeneity for Disease Microenvironments"*

12:00 – 13:30 | LUNCH ● POSTER SESSION 1 ● CCTS Board Meeting (Board members only)

SESSION 3 - Cell Biology of Connective Tissues

13:30 - 14:00 | KEYNOTE LECTURE - **Jean-François Beaulieu** (Université de Sherbrooke) *"Extracellular matrix molecules and their receptors in the human intestinal epithelium. From the bench to the clinical lab."*

14:00 - 14:15 | **Li Diao** (St. Michael's Hospital, Toronto) *"Contact with myofibroblasts activates macrophages into profibrotic states that depend on the proto-cadherins Dchs1 and FAT1/4"*

14:15 - 14:30 | **Simon Dumontier** (Université de Sherbrooke) *"Laminin receptor Integrin β1 and Dystroglycan signaling regulates Muscle Stem Cell Fate and Enhances Repair"*

14:30 - 14:45 | **Elham Karimizadeh** (St. Michael's Hospital, Toronto) *"Endothelial Cells Drive Local Pericyte Activation in Response to Injury"*

14:45 - 15:00 | **Avesta Gheysari** (McGill University) *"Thrombin-Induced Dose-dependent Effects of Aspirin on Endothelial Cell Function"*

15:00 - 15:15 | REFRESHMENT BREAK

SESSION 4 - Functional Biomaterials for Connective Tissue Repair

15:15 – 15:45 | KEYNOTE LECTURE - **May Griffith** (Université de Montréal) *" Nature Knows Best: Biomimetic Materials from Bench to Bedside"*

15:45 - 16:00 | **Fanny Brunard** (Université Laval) *"A Histological assessment of irradiated murine skin: effects of bioactive dressings engineered from adipose-derived mesenchymal cells"*

16:00 - 16:15 | **Fahad Ehsan** (University of Toronto) *"Injury-Induced LTBP2 Promotes Adverse Remote Myocardial Remodeling After Myocardial Infarction"*

16:15 - 16:30 | **Jannik Herold** (University of Reutlingen) *"B-type natriuretic peptide acts as an anti-fibrotic agent in the dermal environment"*

16:30 - 16:45 | **Tarek Klaylat** (McGill University) *"Physioxic Preconditioning Improves Functional Maturation of hBM-MSC-Seeded Dense Aligned Collagen Gel Ligament Constructs"*

16:45 - 18:00 | FREE TIME / BREAK

18:00 – 22:30 | GALA DINNER & AWARD LECTURES

Marc D. McKee (McGill University) *"Some reflections on biomineralization science that quite naturally I am very familiar with"*

Julie Fradette (Université Laval) *"Beyond Skin Deep: Growing a Career in Tissue Engineering"*

CONFERENCE DAY 2 - Wednesday, May 27, 2026

07:30 – 08:30 | **BREAKFAST● POSTER SETUP**

08:30 – 08:45 | **OPENING REMARKS**

SESSION 5 - Matrix Biology in Connective Tissue Research

08:45 – 09:15 | KEYNOTE LECTURE - **Darcy Wagner** (McGill University) *"The role of the extracellular niche in regulating lung epithelium"*

09:15 - 09:30 | **Adel Batal** (McGill University) *"CD109 Restrains Fibroblast–Immune Crosstalk by Limiting NF-κB–Driven Skin Inflammation"*

09:30 - 09:45 | **Elias Cuartas-Gómez** (Université Laval) *"Differential effects of extracellular matrix-derived fragments on adipocyte differentiation using a human biomimetic adipose tissue model"*

09:45 - 10:00 | **Neha Dinesh** (St. Michael's Hospital, Toronto) *"Uncoupling Matrix Production from Contraction During Fibroblast Activation"*

10:00 - 10:15 | **Iram Fatima S. Siddiqui** (McGill University) *"A novel dual role of fibrillin-1 in adipose tissue early development and late maintenance"*

10:15 - 10:30 | **REFRESHMENT BREAK**

SESSION 6 - Repair and Regeneration of Biomechanically Loaded Tissues

10:30 – 11:00| KEYNOTE LECTURE - **François Bordeleau** (Université Laval) **"***Understanding cell adaptation to the evolving tumor stroma: from mechanism to engineered models"*

11:00 - 11:15 | **Nabangshu Das** (University Health Network) *"Uncovering the role of progesterone receptor in synovial pathology during osteoarthritis"*

11:15 - 11:30 | **Xinying Guo** (Unity Health Toronto) *"The 'Soft Skills' of Sall1-Engineered Mesenchymal Stromal Cells Prevent Hypertrophic Scarring"*

11:30 - 11:45 | **Arsal Khan** (Western University) *"Transcriptomic Profiling and Histopathological Assessment of Testosterone-Mediated Changes in Appendicular and Axial Tendons"*

11:45 - 12:00 | **Quan Zhou** (McMaster University) *"Lung stiffness disrupts gremlin mediated fibroblast-AT2 interactions in pulmonary fibrosis"*

12:00 – 13:30 | **LUNCH ● POSTER SESSION 2**

SESSION 7 - Clinical Perspectives in Connective Tissue Pathobiology

13:30 – 14:00| KEYNOTE LECTURE - **Michelle Bendeck** (University of Toronto) *"The role of discoidin domain collagen receptors in fibrotic vascular disease"*

14:00 - 14:15 | **Lucie Germain** (Université Laval) **"***First-in-Human Clinical Trial of Bilamellar Gene-Corrected Skin Substitutes for the Treatment of Recessive Dystrophic Epidermolysis Bullosa"*

14:15 - 14:30 | **Qinli Guo** (Western University) *"Effects of Diet-induced Metabolic Disease on Synovial Microvasculature in Knee Osteoarthritis"*

14:30 - 14:45 | **Hosni Cherif** (McGill University) *"Chondrocyte senescence in facet joint cartilage of adolescent idiopathic scoliosis and the therapeutic potential of senolytic drugs"*

14:45 - 15:00 | **Brandon Vaz** (University Health Network) *"Generating a high-resolution spatial transcriptomic atlas of the synovium during osteoarthritis"*

15:00 – 15:15 | REFRESHMENT BREAK

SESSION 8 - Cartilage Biology and Pathobiology

15:15 – 15:45 | KEYNOTE LECTURE - **Rahul Gawri** (University Health Network) *"The Role of Physioxia in Bioengineering of Musculoskeletal Tissues"*

15:45 - 16:00 | **Lauren Banh** (University of Toronto) **"***Knee osteoarthritis patient-derived cartilage-on-a-chip model can evaluate mechanical and inflammatory clinical phenotypes"*

16:00 - 16:15 | **Emily White** (Western University) *"TGFα drives metabolic reprogramming and mitochondrial membrane depolarization in primary articular chondrocytes"*

16:15 - 16:30 | **Fajer Yousef** (McGill University) *"Nanodiamond-Conjugated Link N Peptide for Sustained Intra-Articular Delivery in Osteoarthritis"*

16:30 – 17:30 | CCTS GENERAL ASSEMBLY ● AWARDS CEREMONY ● CLOSING REMARKS AND CONFERENCE CONCLUSION

Presentation format:

Keynote and award lectures: *25 minutes + 5 minutes for Q&A*

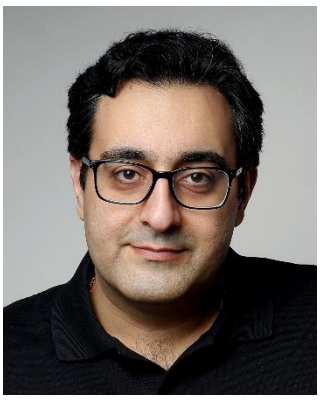
Short talks: *12 minutes + 3 minutes for Q&A*

Poster presentations: *5-minute presentation at the poster, followed by 2 minutes Q&A with assigned experts*

CCTC 2026 Workshop Guest Speakers

Jerome N Feige, PhD | Dr. Jerome N. Feige is a recognized expert in muscle and aging biology currently appointed as senior expert leading the Adult Health science strategy at Nestlé Research and adjunct principal investigator at the Ecole Polytechnique Fédérale de Lausanne in Lausanne, Switzerland. He holds a degree in Bioengineering and a PhD in Biology from the University of Lausanne. Dr Feige performed post-doctoral research at the Institute of Genetics, and Molecular and Cellular Biology in Strasbourg, France on the molecular regulation of energy metabolism. He subsequently worked as laboratory head at the Novartis Institute of Biomedical Research in Basel, Switzerland, where he performed drug discovery for muscle diseases and contributed to the development of new therapies. Since 2012, Dr Feige has held increasing responsibilities in the Nestlé Institute of Health Sciences where he established a research program studying muscle biology and lead a translational department developing nutritional therapies to support the Musculo-Skeletal system. His research programs have led to the commercialization of several products for infant and medical nutrition, and to the creation of a start-up. His research focuses on mechanisms and clinical translation of skeletal muscle aging with focus on metabolic fluxes, mitochondria, and stem cell / regeneration for which he developed innovative bioactive nutrients (Migliavacca et al, Nature Comms 2019; Membrez et al Nature Metabolism 2024; Ancel et al, JCI 2024; Gherardi et al, Cell Metab 2025). In parallel to his research activities, Dr Feige teaches nutrition and entrepreneurship, and trains PhD students in biomedical science at EPFL.

Patrice Leclerc, PhD | Dr. Patrice Leclerc has more than 20 years of experience in biotechnology and life sciences. Holder of a doctorate from the Université de Sherbrooke, he has worked for more than six years at Tranzyme Pharma Inc., he was responsible for the development of screening and preclinical validation programs for the compounds of Tranzyme's R&D portfolio. In 2012, he became Coordinator at the Pharmacology Institute of Sherbrooke (IPS), where he is in charge of scientific animation, scientific support to members and developing networking activities at Ud for multidisciplinary research projects and partnerships with the industry. Since is recruitment at TransferTech Sherbrooke (TTS) in 20218, Patrice has been in charge of maturation and licensing of life sciences technologies from Université de Sherbrooke. Since 2025, he acts as CEO of TTS.



Amir Hooshiar, PhD | Dr. Amir Hooshiar is an Assistant Professor of Surgery at McGill University and Founding Director of the Surgical Performance Enhancement and Robotics Centre. With a PhD in Mechanical Engineering and more than 15 years of experience across academic research, medical device innovation, and regulatory strategy, his work focuses on translating advanced technologies into clinically impactful solutions. Dr. Hooshiar leads research on intelligent surgical systems that combine robotics, artificial intelligence, and image guided technologies to improve precision, safety, and efficiency in care delivery. His program emphasizes the full innovation pathway from foundational research and system development to clinical translation, intellectual property creation, and commercialization, supported by major competitive funding and multiple patents. His work demonstrates how interdisciplinary research can move from discovery to real world impact, enabling technologies that enhance surgical performance and expand access to advanced care.

Marie Lyne Nault, MD, PhD

Dr. Marie-Lyne Nault is a Canadian pediatric orthopedic surgeon, clinician-scientist, and academic leader recognized for her expertise in pediatric spine deformities, scoliosis, and complex musculoskeletal disorders in children and adolescents. She is affiliated with CHU Sainte-Justine and the Université de Montréal, where she contributes to both clinical care and translational research in pediatric orthopedics. She has published numerous peer-reviewed papers in leading orthopedic and biomedical engineering journals and collaborates extensively with multidisciplinary teams in orthopedics, biomechanics, and medical imaging. In addition to her research activities, Dr. Nault is actively involved in medical education, resident training, mentorship of graduate students and clinical fellows, and entrepreneurship. Through her combined contributions to clinical excellence, scientific innovation, entrepreneurship, and academic leadership, Dr. Nault has become a recognized figure in pediatric orthopedic research and care in Canada.

CCTC 2026 Awards

✳ CCTS LIFETIME ACHIEVEMENT AWARD ✳

Marc D. McKee, PhD

Professor Marc McKee holds the Canada Research Chair in Biomineralization at McGill University's Faculty of Dental Medicine and Oral Health Sciences, and the Faculty of Medicine and Health Sciences. He received his Ph.D. degree from McGill University in cell biology, followed by postdoctoral training at Harvard and the Children's Hospital Boston, and then appointments at the Forsyth Institute in Boston and University of Montreal. McKee's research focuses on biomineralization in bones, teeth, otoconia and eggshells, and in pathologic ectopic calcification. With 260 scientific papers, an h-index of 94, and over 38,000 citations of his research, he has received two Distinguished Scientist Awards from the International Association for Dental Research, the Adele Boskey Esteemed Scientist Award from the American Society for Bone and Mineral Research, and the C.P. Leblond Award From the FRQS Quebec Network for Bone and Oral Health Research.

✳ ROBIN POOLE INVESTIGATOR AWARD ✳

Julie Fradette, PhD

Dr. Julie Fradette is full Professor at Université Laval, department of Surgery, faculty of Medicine. She is a researcher at the Centre de recherche en organogénèse expérimentale (LOEX), at the CHU de Québec-Université Laval Research Center since 2005. She is a stem cell (epithelial and mesenchymal) and tissue-engineering expert focusing on human adipose-derived stem/stromal cells (ASCs) and their use in regenerative medicine. Her most significant contributions are related to the development of tissue-engineered substitutes mimicking specific microenvironments for various biological systems: namely adipose tissue, skin, and bone-like tissues. Her research program covers various aspects of basic science (adipogenesis/angiogenesis) and translational research. Her team currently perform preclinical studies using xenogen-free culture systems for ASCs to further future applications for difficult to heal skin wounds. She contributed to the development of a wide range of engineered tissues through collaborations with clinicians (urologic tissues), engineers (heart valves) and other biomedical scientists (orthopedic applications)..

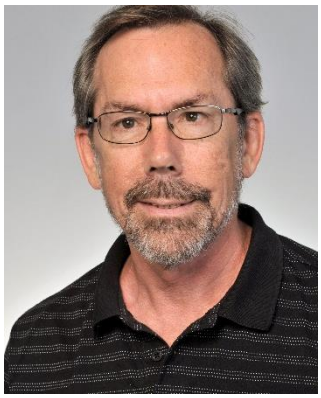
CCTC Keynote Speakers

Maryam Tabrizian, PhD

Maryam Tabrizian is a James McGill Professor in the Departments of Biomedical Engineering and Dental Medicine and Oral Health Sciences at McGill University. She holds a Tier I Canada Research Chair in Regenerative Medicine and Nanomedicine. She focuses her research on designing biomimetic materials, nanomedicine, and microfluidic platforms to promote tissue regeneration. These platforms are used as nanocarriers for therapeutic and imaging applications, for real-time monitoring of molecular and cellular events at biointerfaces, and for biosensing and disease modeling. Professor Tabrizian has received numerous prestigious awards and fellowships, including the FRQS Chercheur National Fellowship, the Guggenheim Fellowship, and election to the Royal Society of Canada, the Canadian Academy of Health Sciences, and the Canadian Academy of Engineering. She is also a Fellow of the American Institute for Medical and Biological Engineering. She has published over 300 peer-reviewed articles with nearly 22,000 citations. Professor Tabrizian serves as Editor-in-Chief of Materials and is the founding Editor-in-Chief of Exploration of Biomat-X.

Matt Kinsella, PhD

J. Matt Kinsella is an Associate Professor of Bioengineering at McGill University. His research focus is on the development of protein-based biomaterials, tissue engineering, nanomedicine, and biofabrication. These efforts are focused on developing patient-derived preclinical disease models to evaluate modern therapeutics (cell, biologic, or nonmaterial-based). The team has recently developed decellularized extracellular matrix (dECM) materials with tunable viscoelastic properties to understand how the mechanobiology of the ECM affects drug efficacy. Prior to joining McGill Matt was an American Cancer Society Postdoctoral fellow at UCSD and the Sanford Burnham Prebys Medical Discovery Institute after receiving his PhD in Biomedical Engineering from Purdue University.



Jean François Beaulieu, PhD

Dr. Jean François Beaulieu is a Professor of Cell Biology at the Université de Sherbrooke and a member of both the Centre de recherche du CHUS and the Sherbrooke Cancer Research Institute. He completed his doctoral training at the Université de Sherbrooke, followed by postdoctoral work in molecular genetics at Université Laval and in intestinal physiology at Cornell University. His research focuses on the fundamental biology of the intestinal epithelium, with particular emphasis on how interactions between cells and the extracellular matrix regulate tissue development, renewal, and repair. His work has advanced understanding of the mechanisms controlling cell proliferation, differentiation, and stem cell function in the intestine, both in normal physiology and in diseases such as inflammatory bowel disease and colorectal cancer. More recently, his research has contributed to the identification of diagnostic and predictive biomarkers for intestinal disorders, including necrotizing enterocolitis and other inflammatory and neoplastic conditions.

May Griffith, PhD

May Griffith is a Professor at the Department of Ophthalmology, University of Montreal, Canada, working at the Maisonneuve-Rosemont Hospital Research Centre. She holds the Canada Research Chair in Biomaterials and Stem Cells in Ophthalmology and the Caroline Durand Foundation Research Chair in Cellular Therapy of the Eye. MG is known for pioneering work in translational regenerative medicine, successfully achieving the first-in-human regeneration of the human cornea in a clinical trial using a cell-free biosynthetic implant to stimulate in situ tissue regeneration. This was followed by incorporating an inflammation-suppressing polymer network into their implants, allowing stable regeneration in patients at high risk of rejecting conventional allografts. Most recently, MG and team developed a synthetic, biocompatible, and adhesive liquid hydrogel (LiQD Cornea) to fill wounds, perforations, and replace partial thickness anterior grafts, to allow for prevention and a revolutionary treatment of patients in the clinic and not the operating room, thereby enhancing accessibility to patients.

Darcy Wagner, PhD

Professor Darcy Wagner joined McGill in January 2024 as the Canadian Excellence Research Chair in Lung Regenerative Medicine with a co-appointment in Medicine and Biomedical Engineering. Darcy started her independent laboratory at Lund University in Sweden in 2017 as a Wallenberg Molecular Medicine Fellow and European Research Council Starting Grant recipient. The broad interest of her laboratory is the development of bioengineering strategies to generate lung tissue ex vivo as well as to develop new therapies to regenerate tissue in vivo. Her laboratory combines novel biomaterials, including deriving and using tissue-specific extracellular matrix, with advanced manufacturing approaches to generate this tissue. Her laboratory was the first to 3D bioprint human airways containing regionally specified primary cells using tissue-specific bioinks. The long-term goal of the lab is to develop transplantable tissue to meet the unmet clinical need for solid organ transplantation but also to use these bioengineering advances to develop new ex vivo humanized models of disease.

François Bordeleau, PhD

Dr Bordeleau is an Associate Professor and the titular of the Canada Research in Tumor Mechanobiology and Cellular Mechanoregulation at Université Laval. His lab is interested in the mechanism influencing the interplay between cell mechanoregulation, matrix mechanical properties and local invasion. This has led the lab to expand its expertise in the design of novel tissue engineered tumor microenvironment and the development of enabling imaging systems to tackle the underlaying biological questions. The efforts of his lab are interdisciplinary and combine engineering, material science, physics and cellular and molecular biology approaches. His expertise encompasses the use and development of stiffness tunable/biomimetic in vitro engineered tumor models to address biological questions, the development of state-of-the-art imaging methods for mechanobiology-based readouts, and computational modeling that are then applied to interrogates the underpinning molecular mechanisms.

Michelle Bendeck, PhD

in the Translational Biology and Engineering Program (TBEP) at the Ted Rogers Centre for Heart Research. The TBEP program was initiated to promote multi-disciplinary work between medicine and engineering and is the first of its kind. Her current research is focused on understanding the molecular mechanisms involved in atherosclerosis and heart failure. She uses experimental animal models and cell culture assays to study cell adhesion and cell-matrix remodeling. One major project is focused on mechanosensing and cell differentiation during vascular fibrosis and calcification. In another, translational research project, she is developing peptide conjugated nanoparticles to treat vascular disease.

Rahul Gawri, PhD

Rahul Gawri is a Scientist with the Schroeder's Arthritis Institute, University Health Network, and an Assistant Professor at the University of Toronto. Rahul is a recognized expert in the field of musculoskeletal (MSK) biology, pathobiology, and regeneration. His research program intersects with clinical, translational, and bioengineering fields, developing applications in musculoskeletal physiology and pathophysiology. He has expertise in biomarker discovery, charting the natural course and endophenotyping of degenerative MSK diseases, therapeutic target discovery, and working on rare and orphan lysosomal storage disorders with profound MSK involvement. He also has expertise in the bioengineering of hypoxic (physioxic) musculoskeletal tissues, such as ligaments, tendons, cartilage, and intervertebral discs, for applications in arthritis and intervertebral disc degeneration. At the Schroeder's Arthritis Institute, Dr. Gawri is expanding his research program to study the epigenetics and endotyping of multijoint osteoarthritis (including osteoarthritis of the spinal column), discover predictive biomarkers for pain phenotypes, and develop novel therapeutic strategies with a central aim to improve post-op outcomes and recovery in patients suffering from degenerative MSK disorders.

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ABSTRACT #1

Can the identifiable biomarkers of therapeutic response in adult rheumatoid arthritis be used to assemble a tool aiming at implementing clinical practice in the future? A systematic review and meta-analysis

May Al-Sakkal[1,2]

[1]Royal college of physician of Edinburgh ; [2]Royal college of physician and surgeon of Glasgow

Background: Rheumatoid arthritis(RA)is a progressive inflammatory autoimmune condition characterised by an ongoing inflammatory process that results in damage to the bony and cartilaginous components of the joint and extra-articular organs. Achieving remission in RA is paramount to controlling inflammation and preventing joint destruction, as well as subsequent permanent articular dysfunction. Therefore, ensuring the administration of effective treatment as early as possible is essential. Besides the availability of different therapeutic options for RA, only 10% achieve ACR70, and a fifth of the RA population requires DMARD escalation to the third level due to lack of a satisfactory response. Hence, exploring the presence of drug resistance-associated biomarkers is beneficial and cost-effective.

Objectives: This systematic review aimed to gather evidence on circulating biomarkers in adult Rheumatoid arthritis patients to assist in populating a drug resistance predictive tool for future clinical application.

Methods: This systematic review and meta-analysis, composed of 19 studies, the majority of which employed a prospective cohort study design. Multiple software programmes were used to conduct the meta-analysis and to calculate missing data. Each article was critically appraised using an appropriate tool suitable for the study design. Statistical analysis was employed to identify the biomarkers with statistical significance that can be used to assemble a tool to predict drug resistance in adult RA patients.

Results: Nineteen studies were included in this SR; the quality of each was assessed manually using validated tools. Visual inspection of the Forest and funnel diagrams suggested the presence of heterogeneity and possible publication bias, respectively. Eighteen biomarkers were filtered out to build a prediction tool.

Conclusion: This systematic review and meta-analysis have established the strength of evidence for the association between lack of response to the disease–modifying anti-rheumatic drugs and specific biomarkers. Most of the studies focused on a single biomarker, whilst this study provided an overview of the published markers and identified the precision of each marker to predict therapeutic response in RA. Populating a predictive tool to predict DMARD resistance may be practical; however, a further large Cohort study is necessary to validate this predictive tool before applying it in routine clinical practice. Addressing the issue of drug resistance in the management of RA could improve the overall outcome of

patients with RA by switching the DMARDs earlier in the management to avoid disease progression, permanent articular damage, and it may save our patients the burden of continuing to consume ineffective treatment for them.

ABSTRACT #2

B-type natriuretic peptide acts as an anti-fibrotic agent in the dermal environment

Jannik Herold[1], Xinying Guo[1], Boris Hinz[1,2]

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

Background: Every year, over six million people seek medical attention for burn injuries. Current therapies allow healing without major loss of tissue function after most injuries. However, if tissue damage is too substantial or repetitive, healing occurs with excessive collagen accumulation, a process known as hypertrophic scarring in the skin and fibrosis in other organs. One therapeutic approach to support endogenous healing is to obtain autologous or allogenic mesenchymal stromal cells (MSCs) from donor biopsies, expand them ex vivo, and reimplant them at the patient's injury site. This approach is often limited by the activation of regenerative MSCs on stiff culture surfaces, leading to a fibrosis-driving phenotype known as myofibroblast.

Rationale: Our lab has shown that culturing MSCs on substrates with a stiffness comparable to that of skin (1 kPa) preserves MSC regenerative properties. By comparing soft- and stiff-grown MSCs by RNA-sequencing (seq), we identified the spalt-like transcription factor 1 (Sall1). Sall1 overexpression enhances the anti-scarring properties of MSCs when transplanted into a rat model of hypertrophic skin scarring. We found the B-type natriuretic peptide (BNP) to be strongly upregulated and enriched in the secretome of Sall1 overexpressing MSCs compared to GFP overexpressing control MSCs.

Hypothesis: BNP expressed by mechanical and/or Sall1-manipulated MSCs reduces skin scarring.

Objective: To engineer MSCs that suppress scarring in skin wound-healing therapies.

Methods and Results: Umbilical cord-derived MSCs were stably transfected to overexpress HA-Sall1 and HA-GFP plasmids, and transiently knocked down using Sall1 siRNA. Changes in Sall1 and BNP expression were measured by qRT-PCR, Western blotting, immunofluorescence microscopy, and ELISA. The effects of MSC culture supernatants and recombinant BNP on TGF-β1-induced myofibroblast activation in primary human and rat dermal fibroblasts were evaluated by immunostaining and qPCR of various myofibroblast markers, including α-smooth muscle actin. Sall1 directly regulates BNP

expression, most likely by binding to an enhancer region of the BNP gene. Secreted BNP antagonizes the expression of genes promoted by pro-fibrotic factor TGF-β1 and reduces myofibroblast activation of dermal fibroblasts. Co-cultures with Sall1-GFP overexpressing MSCs, as well as control MSCs, resulted in complete inhibition of myofibroblast activation of dermal fibroblasts.

Conclusion: Our data support that BNP secreted by Sall1-overexpressing MSCs acts as an anti-fibrotic agent in the dermal environment. Because BNP is not expressed in homeostatic skin, it represents a promising future treatment strategy for preventing hypertrophic scarring.

ABSTRACT #3

Adjuvant Therapy Using Senolytic Drugs to Prevent Breast-to-Bone Metastasis

Eleane Hamburger[1,2], Lisbet Haglund[1,2,3], Derek Rosenzweig[1,2,4]

[1]McGill University; [2]McGill University Health Center; [3]Shriners Hospital for Children-Canada; [4]University of Connecticut School of Medicine

INTRODUCTION: The Canadian Cancer Society estimates ~30,000 new breast cancer cases annually. Bone metastasis is a major cause of mortality. Standard treatments for metastatic spine lesions include surgery, radiotherapy, and chemotherapy; however, recurrence is common. Chemotherapy is often administered at suboptimal doses to limit toxicity, which can promote therapy-induced cellular senescence and resistance. Senescent cells exhibit irreversible cell-cycle arrest and a pro-inflammatory, tumor-promoting phenotype that may enhance cancer progression. Targeted removal of senescent cells could reduce inflammation, improve treatment response, and support tissue repair.

METHODS: We developed a high-throughput in vitro 3D spine tumor microenvironment model in which breast cancer spheroids (MDA-MB-231, MCF-7, MDA-MB-453) were co-cultured with primary human spine osteoblasts embedded in collagen. Osteoblasts were isolated from vertebral bodies of organ donors in collaboration with Transplant Quebec with ethics approval (IRB#2019-4896) and family consent. Ki-67, caspase-3, p21 and p53 staining assessed proliferation, apoptosis, and senescence. Senolytics RG-7112 and o-Vanillin were tested in combination with Doxorubicin. Imaging, Alamar Blue, and Luminex cytokine analysis assessed spheroid growth, metabolic activity, and SASP factor secretion. Statistical significance was set at $p<0.05$.

RESULTS: Combination treatment significantly reduced spheroid area and metabolic activity in MDA-MB-231 and MCF-7 models, with parallel reductions in Luminex-measured SASP factors. Proliferation

decreased and apoptosis increased across co-cultures. Doxorubicin alone increased senescence, whereas combination therapy significantly reduced senescence levels.

CONCLUSION: Senolytic plus chemotherapy combinations may optimize breast cancer treatment by reducing tumor growth, metabolic activity, and senescence burden. Evaluating multiple breast cancer subtypes supports development of more tailored therapeutic strategies.

ABSTRACT #4

Contact with myofibroblasts activates macrophages into profibrotic states that depend on the proto-cadherins Dchs1 and FAT1/4

Li Diao[1], Fereshteh Younesi[1,2], Xinying Guo[1], Ronen Schuster[1,3], Boris Hinz[1,2]

[1]Keenan Research Centre; [2]University of Toronto; [3]QuantHealth, Tel Aviv, Isreal

Background: Macrophages (Mϕ) and fibroblasts are key in promoting the formation and remodeling of extracellular matrix (ECM) following organ injury. However, unduly prolonged or dysregulated crosstalk between Mϕ and fibroblasts contributes to the development of fibrosis. We published that direct contact with Mϕ activates fibroblasts into contractile myofibroblasts (MF) in 'scar-stiff' mechanical environment. Whether, in turn, the activation state of fibroblasts and physical contact regulate Mϕ polarization is not known.
Hypothesis: Mechanically activated MFs control distinct Mϕ functional states upon direct contact.
Methods: Mϕ were obtained by treating cultured mouse bone marrow-derived monocytes with M-CSF for 5 days. Subcutaneous fibroblasts were isolated from Col1a1 promoter-GFP reporter mice. Fibroblasts were cultured on skin-soft or scar-stiff gelatin-coated silicone elastomer substrates for 2 passages to establish fibroblast and MF populations, respectively. Mϕ were then co-cultured with fibroblasts or MFs on the respective substrates for 3 days, either in direct contact or sharing media only. Mϕ and fibroblastic cells from all experimental combinations were separately analyzed using immunofluorescence confocal microscopy and flow cytometry. F4/80-positive Mϕ and GFP-expressing fibroblastic cells were flow-sorted for bulk RNA sequencing (RNA-seq). Candidate genes identified by RNA-seq analysis were knocked down using respective siRNA pools to functionally validate their predicted roles. To translate the in vitro findings into an animal model, we compared full-thickness mouse dorsal skin wounds that were healing normally (relaxed) with those that were mechanically stressed with a splint.
Results: While mono-culture on scar-stiff surfaces activated fibroblasts into MFs, the RNA profiles of

monocultured Mϕ were not different on stiff versus soft surfaces. Co-culture with either fibroblasts or MFs produced Mϕ populations with significantly different RNA profiles. Unique Mϕ features were induced by direct contact with MFs, such as the activation of pathways associated with IL-17, TNF-α, NF-κB, and C-type lectin signalling. As potential mediators of this contact-dependent signalling, FAT1/4 was upregulated in Mϕ in tandem with high Dchs1 expression in MFs. FAT1/4 knockout in Mϕ and Dchs1 knockout in MF prevented contact-induced Mϕ signalling and activation. Histological and proteomic analysis of wound tissue collected from normal versus stressed wounds confirmed profibrotic activation of Mϕ through Dchs1-FAT1/4 ligand-receptor coupling.
Conclusion: Direct contact with MFs generates a unique Mϕ polarization state that features a combination of pro-inflammatory and pro-fibrotic 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 #5

Biocompatibility of a Technical-Grade SLA Resin: Mechanical and Biological Evaluation of 3D print samples for Tissue Engineering

Vincenzo Marchese[1], Jose Ignacio Contreras[1], Carola Millán[2], Heidi Lynn Ploeg[3], Juan Francisco Vivanco[1]

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Introduction
Osteoporosis increases fracture risk and highlights the need for improved scaffolds in bone tissue engineering. Current designs face limitations in geometry and material selection [1]. SLA printing offers precision and low-cost fabrication [2], but most resins are cytotoxic [3]. This study evaluated a resin in terms of mechanical performance and biological compatibility.
Methods
Specimens were printed using FormLabs 4B and Fast Model Resin V1. Mechanical properties were assessed through compression testing of 10 × 10 × 10 mm cubes for anisotropy (n=5) and porous scaffolds of 45%, 55%, and 65% (n=5). Biological assays used well-shaped specimens (5 mm diameter, 1.6 mm height, n=4). Human gingival stem cells (hMSC) were cultured in DMEM with SBF at 37 °C and 5% CO_2. Biocompatibility was evaluated through cell adhesion (fluorescence imaging) and viability using MTS assays on Days 1, 3, and 7.
Results
Compression testing revealed transversely isotropic behavior, with elastic modulus of 676±30.0MPa,

719±18.9MPa, and 605±26.8MPa for X, Y, and Z axes, with statistical significance in Z ($p < 0.00001$). Porosity was 26%, 45% and 58% for the 45%, 55% and 65% groups respectively and apparent elastic modulus decreased with increasing porosity: 435±14.0MPa (45%), 199±12.5MPa (55%), and 118±17.3MPa (65%). Fluorescence imaging of wells confirmed adhesion and proliferation, with nuclei increasing from ~1200 at Day 1 to 1700–2000 at Day 3. MTS activity rose from 0.26±0.13 at Day 1 to 0.52±0.174 at Day 7, supporting short term adhesion and biocompatibility.

Discussion

Mechanical results confirmed anisotropy and an inverse relation between porosity and elastic modulus. The higher modulus at 45% porosity is explained by trapped resin, highlighting limitations of post processing methods. Biological assays showed that MSC adhered and proliferated, suggesting non cytotoxicity. These results position the resin as an accessible model for in vitro evaluation. Limitations include short culture duration, single cell type, and assays restricted to wells rather than scaffolds. Future work will extend culture time, dead-live cytotoxicity and scaffolds tests, considering studies on effects of trapped uncured resin.

ABSTRACT #6

Nanodiamond-Conjugated Link N Peptide for Sustained Intra-Articular Delivery in Osteoarthritis

Fajer Yousef[1,2], Michael P Grant[1,2], Muskan Alad[1,2], Laura M Epure[1,2,3], John Antoniou[1,2,3], Fackson Mwale[1,2]

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Background:

Osteoarthritis (OA) is the most common form of arthritis worldwide and a leading cause of pain and disability. Current treatments primarily provide symptomatic relief and do not modify disease progression. Link N (LN), a peptide derived from link protein, promotes cartilage matrix synthesis and reduces inflammation. We developed a nanodiamond (ND)-based delivery system to provide sustained intra-articular LN release and maintain its therapeutic activity in inflammatory OA conditions.

Methods:

Carboxylated NDs were conjugated with LN peptide to generate ND–LN complexes. Physicochemical characterization included Fourier transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR), dynamic light scattering (DLS), and transmission electron microscopy (TEM). Release kinetics

were evaluated under physiologic and acidic pH conditions. Cytotoxicity was assessed in primary chondrocytes and HK-2 renal epithelial cells. Functional activity was evaluated in human chondrocytes stimulated with IL-1β to mimic an inflammatory OA environment. An enhanced formulation incorporating a tannic acid (TA) coating (ND–LN–TA) was developed to further extend peptide retention and release.

Results:

FTIR and NMR confirmed successful LN association with NDs surfaces, while DLS and TEM demonstrated a uniform nanoscale particle distribution. ND–LN exhibited sustained peptide release up to 25 days and high biocompatibility on both cell lines (>90% cell viability). In IL-1β-stimulated chondrocytes, ND–LN increased anabolic markers (COL2A1, ACAN), reduced catabolic enzymes (MMP-3, MMP-13, ADAMTS-4/5), and suppressed the inflammatory mediators TNF-α and IL-1β. ND–LN also reduced NF-κB activation. The addition of a TA coating increased particle diameter (~220 nm) and significantly extended-release duration from approximately 25 days to nearly 60 days while maintaining biocompatibility.

Conclusion:

ND–LN functions as a peptide delivery system that enables sustained intra-articular release while preserving the cartilage matrix and attenuating inflammatory signaling under OA-like conditions. The addition of TA further prolongs peptide retention, reinforcing the potential of ND-based peptide delivery systems as a strategy for long-acting disease-modifying therapies for osteoarthritis.

ABSTRACT #7

Bioengineering a 3D Intestinal Co-Culture Model for In Vitro Therapeutic Screening in Inflammatory Bowel Disease

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[1]Department of Surgery, McGill University, Montreal, QC H3A 0G4, Canada.; [2]Department of Medicine, Division of Gastroenterology, McGill University, Montreal, QC H3A 0G4, Canada.

Background: Inflammatory bowel disease (IBD), comprising Crohn's disease and ulcerative colitis, is characterized by epithelial barrier disruption, recurrent inflammation, and impaired tissue repair in the gastrointestinal tract. Despite advances in corticosteroids, immunomodulators, and biologic therapies, over 40% of patients exhibit primary non-response or secondary loss of response. This clinical heterogeneity highlights the need for predictive, patient-relevant platforms to model disease mechanisms and evaluate therapeutic response more effectively. Existing in vitro intestinal models have

important limitations in replicating the native microenvironment, as most lack extracellular matrix architecture and stromal components needed to support epithelial organization, and few incorporate patient-derived cells or organoids.

Objectives: This study aims to develop and characterize a patient-relevant intestinal 3D epithelial–stromal in vitro platform to study barrier organization, inflammatory responses, and therapeutic response in IBD.

Methods: HT-29 intestinal epithelial cells are seeded onto IMR-90 fibroblasts cultured within an electrospun poly(lactic acid) scaffold. Characterization includes cell viability, metabolic activity, and immunofluorescence staining. Inflammation is induced with lipopolysaccharide, modulated using dexamethasone, and quantified by cytokine ELISA. In parallel, primary intestinal epithelial and fibroblast cells from IBD patient biopsies are being isolated by a postdoctoral researcher in our group.

Results: Preliminary findings demonstrate sustained metabolic activity and high cell viability, indicating that the model supports a microenvironment suitable for long-term culture. Immunofluorescence staining for vimentin revealed a uniform distribution of fibroblasts throughout the scaffold, supporting successful stromal integration. Immunofluorescence analysis also showed that lipopolysaccharide stimulation increased Claudin-2, TNF-α, and IL-1β signal, whereas dexamethasone treatment attenuated these responses. In parallel, patient-derived organoids are being generated and fibroblasts are being isolated from donor biopsies, with preliminary findings supporting the feasibility of integrating these cells into the established 3D platform.

Conclusion: The 3D epithelial–stromal co culture platform shows potential as a more physiologically relevant in vitro model of IBD, capable of recapitulating key features of the intestinal microenvironment and inflammatory activation. Ongoing work will further refine the model through hydrogel incorporation, expanded molecular characterization, and integration of patient-derived cells. By addressing important limitations of existing intestinal models, this platform may provide new insights into disease pathogenesis and inter-patient variability in treatment response, while supporting predictive drug screening and the development of more personalized therapeutic strategies in IBD.

ABSTRACT #8

Role of laminin 211 in the muscle stem cell niche

Arianne Graveline[1], Simon Dumontier[1], Svenja Schüler[1], C. Florian Bentzinger[1]

[1]University of Sherbrooke

Skeletal muscle contains a specialized extracellular matrix (ECM) structure known as the basal lamina, which is enriched in laminin-211 and closely interfaces with muscle stem cells (MuSCs). However, the precise role of laminins in regulating MuSC function, as well as the cellular sources responsible for laminin production in homeostatic and regenerating muscle, remain poorly defined. Notably, we observed that the basal lamina undergoes extensive remodeling during skeletal muscle regeneration, accompanied by evidence of substantial de novo laminin synthesis. Here, we generated novel knockout mouse models to investigate the contribution of distinct muscle-resident cell types to laminin synthesis and to determine how these cells regulate MuSC function during skeletal muscle regeneration. To this end, we used cell type–specific inducible Cre recombinase drivers combined with a conditional allele of laminin gamma 1 (Lamc1) to disrupt functional heterotrimeric laminin-211 (Lama2, Lamb1, and Lamc1) in fibro-adipogenic progenitors (FAPs), muscle fibers, or MuSCs. Remarkably, we found that muscle fibers have only a minor contribution to laminin deposition in skeletal muscle, although their deletion induces feedback signaling that alters laminin subunit expression in other muscle-resident cell types. In contrast, Lamc1 deletion in FAPs results in a profound reduction of laminin levels across the tissue and leads to impaired regeneration and MuSC dysfunction. Preliminary results further suggest that MuSC-derived laminin contributes primarily to early stages of regeneration, but is dispensable for later phases. Collectively, our study reveals distinct cell type–specific contributions to laminin synthesis during skeletal muscle regeneration and highlights FAPs as a major source of laminin that shapes basal lamina remodeling and MuSC function.

ABSTRACT #9

Examining mechanical regulation of disseminated prostate cancer cells using an in vitro model of the bone marrow perivascular niche

Kimberly Seaman[1], Yu Sun[1], Lidan You[1,2]

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

Over 60% of primary prostate cancer patients harbor disseminated cancer cells (DCCs) that have spread to bone marrow (BM). DCCs enter a reversible, non-dividing state which enables metastatic recurrence. Studies have identified how cells within the BM perivascular niche regulate quiescence or reactivation of DCCs. However, the role of the mechanical environment in regulating prostate cancer cell quiescence in BM is undefined. The aim of this study is to investigate how osteocytes, mechanosensitive cells in bone, regulate prostate cancer cell quiescence under mechanical

stimulation, and how changes to the BM extracellular matrix (ECM) impact cancer cell growth.
BM mesenchymal stem cells (MSCs) and human umbilical vein endothelial cells (ECs) were mixed at a 5:1 ratio and seeded into 96-well-plates to induce organotypic vessel formation over 10 days. 400 PC-3 prostate cancer cells were seeded into each well on either Days 3, 7 or 10 during vessel formation. A laminin-rich hydrogel to mimic BM ECM was introduced to co-cultures at either 2 or 5mg/ml to modulate ECM protein concentration. Alginate at either 0.25 or 0.5% was incorporated with the hydrogel to modulate stiffness. Conditioned media collected from either static or oscillatory fluid flow (OFF)-stimulated MLO-Y4 osteocytes (1 Pa, 1 Hz, 2 hours) were added to co-cultures to determine effects of osteocytes on prostate cancer cell quiescence. Prostate cancer cells were co-cultured in BM microenvironments for an additional 10 days, and co-cultures were stained for CD31 to analyze EC vessel morphology.
Organotypic EC vessels formed 10 days after seeding. OFF-stimulated osteocytes supported the formation of microvasculature. In 2mg/ml hydrogels, seeding PC-3 cells on Day 3 resulted in elevated growth compared to later timepoints. Of interest, PC-3 growth was elevated in 5mg/ml hydrogels across all timepoints, however OFF-stimulated osteocytes exhibited an inhibitory effect when PC-3 cells were seeded on Days 7 or 10. Addition of alginate to co-cultures resulted in similar trends observed in 5mg/ml hydrogels, indicating that PC-3 growth within the BM perivascular niche is dependent on substrate stiffness. Notably, analysis of EC vessel after co-culture revealed that observed trends in PC-3 growth are dependent on several EC vessel parameters, specifically the total tube length and number of branching points.
We anticipate this study will provide first insights into the role of mechanical cues on cancer cell quiescence in BM, and motivate further studies on how mechanical alterations to BM, namely from aging or disease, may influence the proliferation status of DCCs.

ABSTRACT #10

Culture on skin-soft elastomer surfaces primes human mesenchymal stromal cells for scar-free wound healing therapies

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Rationale: Severe skin injuries such as burns often lead to hypertrophic scarring, which can impair function and reduce quality of life. Therapies with mesenchymal stromal cells (MSCs) are promising because MSCs can differentiate into fibroblasts and modulate immune responses. To obtain sufficient MSCs for therapy, they must be expanded. However, prolonged culture on stiff tissue culture plastic or bioreactor microcarrier beads can induce MSC-to-myofibroblast activation. This in vitro fibrogenesis potentially limits the therapeutic benefit of MSCs. We published that, instead, growth on skin-soft silicone elastomer substrates suppressed fibrogenesis in rat MSCs. Delivery of soft-primed MSCs improved skin wound healing in a rat hypertrophic scar model, whereas stiff-primed MSCs induced scar features. The effects of mechanical priming on MSC contributions to wound healing are unknown.
Hypothesis: Biomechanical priming of human MSCs on skin-soft substrates preserves MSC properties with unique secretory profiles that enhance wound repair following transplantation.
Objective: To adjust cell culture substrate mechanics for producing MSCs with anti-scarring properties in a rat model of hypertrophic skin wound healing.
Methods: MSCs were isolated from human umbilical cords and expanded for 4 passages on silicone surfaces that mimic soft-skin or scar-stiff mechanical conditions. MSCs were profiled by evaluating stem cell marker expression, fibrotic activation, and differentiation capacity. MSC-conditioned media were analyzed to assess secreted proteins and extracellular vesicles. Mechanically primed MSCs were transplanted onto a splinted rat full-thickness skin wound model that induce hypertrophic scar features. Wound tissues were collected after 2-9 days for analysis of immune cell populations, vascularization, and fibrotic tissue formation using immunohistochemistry and flow cytometry.
Results: Soft-primed MSCs maintained expression of stem cell markers CD44 and CD90 and preserved multilineage differentiation capacity. Compared to stiff-cultured MSCs, they showed lower levels of the myofibroblast marker α-smooth muscle actin. Analysis of MSC-conditioned media showed that secretome profiles and extracellular vesicle formation were influenced by the mechanical properties of the culture substrate. After transplantation into rat skin wounds, soft-primed MSCs were detected within the wound granulation tissue up to 4 days. Soft-primed MSCs induced earlier recruitment of $CD68^+$ and $CD206^+$ macrophages, increased vascularization by $desmin^+$ smooth muscle cells and reduced myofibroblast accumulation compared with wounds that received stiff-primed MSCs.
Conclusion: The mechanical environment used during MSC expansion can influence MSC phenotype and therapeutic effects. Culturing MSCs on soft substrates preserves MSC properties that promote non-scarring wound healing outcomes. Changes in cytokine release and extracellular vesicle signaling may contribute to these effects.

ABSTRACT #11

Effects of Diet-induced Metabolic Disease on Synovial Microvasculature in Knee Osteoarthritis

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Background and Rationale: Cardiometabolic disease is a strong risk factor for osteoarthritis (OA). Patients with both cardiometabolic disease and OA experience worse pain, worse joint damage, and faster progression to joint failure and arthroplasty. However, the underlying mechanisms remain poorly defined. The synovial microvasculature (SMV) is essential for maintaining joint organ homeostasis and supporting tissue repair processes. Recent studies indicate that patients with cardiometabolic disease and OA exhibit SMV pathology that is associated with pain. Here, we employed in vivo models combined with single-cell sequencing to investigate SMV remodeling and endothelial responses during OA under conditions of aging and metabolic disease.

Methods: 12-week-old (young) and 24-week-old (mid-age) male rats underwent joint destabilization surgery to induce OA and were studied at 4 and 12 weeks post-surgery. To assess the effects of metabolic disease, mid-age rats were fed either chow or a high-fat-high-sucrose diet for three months prior to OA-inducing surgery. Synovium was identified in H&E-stained joint sections and segmented into intimal and subintimal regions. Microvessel density and synovial morphology were quantified in both regions and analyzed using one-way ANOVA with Tukey correction within each time point. Synovial samples were harvested and digested into single-cell suspensions for single-cell RNA sequencing. Endothelial and mural cell populations were examined to characterize cellular responses within the SMV microenvironment. To model microvascular endothelial responses to joint stress in vitro, HMEC-1 cells were cultured in normal glucose (5 mM) or high-glucose (30 mM) media and stimulated with 5% OA synovial fluid (SF). Endothelial migration and tube formation assays were performed. Data were analyzed using linear mixed-effects models.

Results: Young rats with OA demonstrated synovial neovascularization, evidenced by increased microvascular density in the intimal region. This response was attenuated in mid-age rats and further blunted in the presence of metabolic disease. Single-cell transcriptomic analysis identified synovial endothelial cells undergoing endothelial-to-mesenchymal transition (EndoMT) in response to OA. Expansion of EndoMT cells was observed in OA models but not in those with metabolic disease. In vitro, SF stimulates endothelial migration and tube formation, whereas high-glucose conditions impaired these responses.

Conclusion: Using complementary in vivo and in vitro models of metabolic disease–associated OA, we

demonstrate that SMV functions supporting joint organ homeostasis during OA are impaired by metabolic disease. These findings suggest that SMV dysfunction mediates the effects of cardiometabolic disease on OA progression. Preservation of SMV function may represent a potential disease-modifying therapeutic strategy for OA.

ABSTRACT #12

A novel dual role of fibrillin-1 in adipose tissue early development and late maintenance

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Fibrillin-1 is an extracellular matrix protein organized in microfibrils that frequently intersect with basement membranes. Mutations in fibrillin-1 lead to Marfan syndrome, characterized by either a lipodystrophic or overweight body status, among other clinical symptoms. This study reveals a stage-specific novel dual role of fibrillin-1 in early adipogenesis and in late stages of adipose tissue maintenance.

To investigate the role of fibrillin-1 during early adipogenesis, we first used adipose-derived and bone marrow-derived mesenchymal stem cells. Treatment with a recombinant fibrillin-1 fragment containing an integrin-binding RGD motif (Fbn1-RGD) significantly inhibited adipocyte differentiation. It also resulted in downregulation of key adipogenic markers. In contrast, a control fragment harboring a non-functional RGA motif (Fbn1-RGA) showed no inhibitory effect, indicating specific involvement of integrin receptors. To further study the signaling pathways involved, we used adipogenic 3T3-L1 cells. Fbn1-RGD treatment increased phosphorylation of Fak, Src, and Erk. Selective pharmacological inhibition of these effectors restored adipogenesis in presence of Fbn1-RGD, indicating that fibrillin-1 inhibits early adipogenesis via the Fak-Src-Erk axis. To identify the specific integrins involved, we performed siRNA-mediated knockdown of relevant fibrillin-1 receptors, α5β1, αvβ3, and αvβ6. Only knockdown of αvβ3 restored adipocyte differentiation in Fbn1-RGD-treated cells, establishing αvβ3 as key mediator of fibrillin-1's inhibitory effect on early stages of adipogenesis.

To understand the role of fibrillin-1 during late stages of adipogenesis, an adipocyte-specific Fbn1 knockout mouse (Fbn1-AKO) was generated using Adipoq-Cre mice. The progeny mice exhibited ~25% reduction in body weight and fat mass by 30 weeks of age. Histological analysis showed adipocyte hypotrophy in ~90% of white adipocytes. Gene expression analysis of adipose tissue revealed a ~75-80% reduction in adipogenic markers. Despite maintaining normal glucose clearance, Fbn1-AKO mice

exhibited severe insulin resistance and dyslipidemia, with 2-fold increase in circulating triglycerides and 1.2-fold reduction in high-density lipoprotein levels. Single-nucleus RNA sequencing demonstrated decreased proportion of mature adipocytes and progenitor cells in Fbn1-AKO mice. Transcriptomic data highlighted downregulated insulin signaling genes and upregulation of integrin, beiging and mitochondrial fission genes in Fbn1-AKO mice. Western blot analysis showed significant reduction in phosphorylation of integrin pathway mediators Fak and Src, as well as Pi3k and Akt, key effectors in insulin signaling.
In conclusion, fibrillin-1 plays a stage-specific dual role in adipose tissue, inhibiting early adipogenesis via αvβ3-Fak/Src-Erk signaling while maintaining metabolic function in mature adipocytes via integrin-Pi3k/Akt signaling. Targeting this axis may offer therapeutic potential for adipose dysfunction in Marfan syndrome and related disorders.

ABSTRACT #13

Chondrocyte senescence in facet joint cartilage of adolescent idiopathic scoliosis and the therapeutic potential of senolytic drugs

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Objective: Facet joint osteoarthritis (OA) is increasingly recognized in adolescent idiopathic scoliosis (AIS) and may contribute to curve progression through impaired control of axial rotation. While cellular senescence is a key driver of age-related OA, its role in adolescent OA and the therapeutic potential of senolytics remain unexplored. This study aimed to characterize cellular senescence in AIS-associated facet joint OA and to evaluate the senolytic potential of o-vanillin in this context.
Methods: Facet joint cartilage was obtained from AIS patients undergoing elective posterior spinal fusion surgery and non-scoliotic organ donors. Cellular senescence was assessed using RNA sequencing (RNA-seq) and immunostaining for the senescence marker P16. Paired facet joints from the same spinal level were analyzed to examine side-to-side differences in degeneration and senescence burden by quantifying senescence-associated gene expression. The effects of the senolytic compound o-vanillin were evaluated in patient-derived chondrocyte and cartilage explant cultures by assessing senescence markers, SASP expression, matrix catabolism, and proteoglycan content.
Results: RNA-seq revealed significant enrichment of senescence-associated gene sets in AIS chondrocytes, with the SEN_MAYO signature exhibiting the strongest enrichment score (ES = 0.76).

Consistently, AIS cartilage exhibited a higher proportion of p16-positive chondrocytes compared with non-scoliotic control cartilage (AIS: 15.28%, 95% CI 11.75–18.81; NSC: 5.22%, 95% CI 3.47–6.96; $p < 0.0001$). In paired facet joints, increased OA severity was associated with elevated gene expression of cell-cycle inhibitors, RB (retinoblastoma) family members, and SASP factors, including P16ink4a, P21, RB1, RBL1, RBL2, ILs, CXCLs, CCLs, and MMPs. O-vanillin reduced senescence markers and SASP expression in isolated chondrocytes. In cartilage explants, o-vanillin further attenuated matrix catabolism and improved proteoglycan retention.
Conclusion: Cellular senescence is a prominent feature of AIS-associated facet joint OA and correlates with OA severity. These findings provide a rationale for further investigation of senolytic strategies as potential disease-modifying approaches in AIS.

ABSTRACT #14

Physioxic Preconditioning Improves Functional Maturation of hBM-MSC-Seeded Dense Aligned Collagen Gel Ligament Constructs

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Background: Ligament injuries are among the most prevalent musculoskeletal injuries, characterized by limited intrinsic healing capacity due to biomechanics, hypovascularization, and hypocellularity. The current gold standard, ligament reconstruction surgery, is associated with failure, complications, and frequent need for revision. Implantable bioengineered ligament grafts offer a promising alternative solution. Such constructs seeded with cells demonstrate greater regenerative potential compared to cell-free scaffolds. However, the optimal combination of cell source, scaffold architecture, and culture microenvironment remains a challenge. Human bone marrow-derived mesenchymal stem cells (hBM-MSCs) exhibit promising ligamentogenic potential, and dense aligned collagen gels (DACGs) mimic the native tissue architecture and composition and can be tuned to optimize mechanical properties. Another critical factor is the mismatch between standard in vitro culture conditions and the physiological environment of ligament tissues, which are characterized by relative hypoxia (tissue physioxia) and limited nutrient supply. Reproducing physiological oxygen and glucose levels may therefore improve the biofabrication of functional ligament grafts. This study aimed to develop bioengineered ligament grafts by maturing hBM-MSC-seeded DACGs under near physiological oxygen tensions and glucose concentrations, mimicking the intra-articular environment, and to evaluate the

impact of these conditions on graft development and functionality.
Methods: hBM-MSCs (RoosterBio, n=3 donors) were differentiated and primed for 10 days in ligamentogenic media under three oxygen tensions: atmospheric (20% O_2), intravenous (5% O_2), and intraarticular (2% O_2). Following priming, cells were embedded in collagen gels, which were compacted using gel aspiration-ejection on an automated biofabrication platform. Constructs were matured for 21 days under the same oxygen conditions. Cell viability and metabolic activity were assessed using Live/Dead and AlamarBlue assays. Scaffold architecture was examined using scanning electron microscopy. Immunofluorescence staining was performed to evaluate ligamentogenic marker expression, and mechanical properties were assessed by uniaxial tensile testing.
Results: Live/Dead assays demonstrated high cell viability across all culture conditions at both day 1 and day 21, with increased cell spreading and alignment over time. SEM confirmed highly aligned collagen fibres in all groups, with matrix remodelling under physioxia. Immunofluorescence analysis demonstrated higher expression of ligamentogenic markers under physioxia (5% and 2% O_2). Mechanical testing indicated significantly enhanced mechanical properties under physioxic conditions compared to their as-made counterparts and those matured under 20% O_2.
Conclusion: These findings highlight the critical role of oxygen tension in regulating structural stability and functional mechanical maturation during ligament tissue engineering and suggests that physiologically relevant culture conditions may enhance the development of bioengineered ligament grafts.

ABSTRACT #15

Laminin receptor Integrin β1 and Dystroglycan signaling regulates Muscle Stem Cell Fate and Enhances Repair

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Laminin α2 (Lamα2) is a key component of the basal lamina, a specialized extracellular matrix that surrounds individual muscle fibers and plays a critical role in skeletal muscle maintenance and regeneration. Mutations in Lamα2 cause Merosin-deficient congenital muscular dystrophy type 1A (MDC1A), a rare and severe congenital disorder characterized by progressive muscle degeneration and impaired regenerative capacity, accompanied by muscle stem cell (MuSC) dysfunction. To better understand disease progression in MDC1A and to investigate the regulatory role of the basal lamina on

MuSC function, we examined the impact of basal lamina remodeling as well as the roles of the Laminin receptors Dystroglycan (Dag) and Integrin β1 (Itgβ1) in MuSCs. Inhibition of basal lamina remodeling using a matrix metalloproteinase (MMP) inhibitor during early muscle regeneration transiently prevented MuSC exit from quiescence and reduced their proliferation. To directly assess the roles of Dag and Itgβ1, we generated inducible, MuSC-specific knockout models. Loss of either receptor impaired the ability of MuSCs to maintain quiescence and resulted in severe functional deficits following muscle injury. In homeostatic conditions, Itgβ1 deficiency led to premature activation of the myogenic differentiation program, depletion of the MuSC pool, and reduced myonuclear accretion. Following injury, Itgβ1-deficient MuSCs exhibited impaired lineage progression and failed to differentiate. In contrast, Dag-deficient MuSCs displayed spontaneous activation and proliferation in uninjured muscle, along with an impaired return to quiescence during later stages of regeneration. Importantly, MuSC dysfunction in a preclinical model of MDC1A recapitulated key pathological features observed in these knockout models. Furthermore, antibody-mediated activation of Itgβ1 stimulated MuSC function in MDC1A and significantly enhanced endogenous muscle repair. Together, our findings reveal previously unrecognized roles for Laminin receptors in regulating MuSC function and demonstrate the therapeutic potential of targeting these pathways to promote tissue repair in MDC1A.

Grant Acknowledgment
This work is supported by the Natural Sciences and Engineering Research Council of Canada (NSERC), the Fonds de recherche du Québec – Santé (FRQS), the Canadian Stem Cell Network, and the Canadian Institutes of Health Research (CIHR).

ABSTRACT #16

Endothelial Cells Drive Local Pericyte Activation in Response to Injury

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Background: Vascular endothelial cells (ECs) are ideally positioned to initiate lung repair as first responders to injury. In proximity to ECs are perivascular mesenchymal stromal cells, pericytes, which provide structural support for blood vessels. Following injury, pericytes are activated into myofibroblasts, which build the extracellular matrix during tissue repair. When injury persists, continued repair can turn into life-threatening pulmonary fibrosis. A key step in myofibroblast activation is the

extracellular presentation of latent TGF-β (L-TGF-β) by extracellular matrix- or membrane-bound tether molecules, enabling activation by pericyte αv integrins.

Hypothesis: ECs drive pericyte-to-myofibroblast activation by local delivery of TGF-β.

Objective: To reveal how ECs present and pericytes activate L-TGF-β1 to establish a perivascular fibrotic niche.

Methods: To evaluate whether lung ECs present L-TGF-β, we performed a meta-analysis of 6 single-cell RNA-sequencing datasets, collectively comprising 58 normal and 65 fibrotic human lungs. To investigate if EC-presented TGF-β1 activates pericytes into myofibroblasts, we immunostained co-cultures of primary ECs and pericytes for the EC marker CD31 and the myofibroblast marker α-SMA. Expression of molecular components involved in L-TGF-β1 presentation and activation was assessed after flow sorting of EC and pericytes for CD31 and CD90, respectively.

Results: In silico analysis showed that blood vascular ECs express higher levels of the TGF-β1 isoform than TGFB2 and TGFB3. Among all cell types in normal and diseased lung tissues, ECs are the primary sources of LRRC32, which encodes the L-TGF-β tether GARP. GARP is the primary L-TGF-β1-presenting tether in ECs, as confirmed by immunofluorescence staining of human lung tissue sections. GARP is also expressed in cultured human lung microvascular and umbilical cord-derived ECs, where it co-immunoprecipitates with L-TGF-β1. When co-cultured with pericytes, ECs form 'capillary-like' structures surrounded by pericytes. Only pericytes lining EC paths are activated into α-smooth muscle actin (α-SMA)-positive MFs in 2D and 3D cultures, a process blocked by inhibiting TGF-β receptors and αv integrins. Pericytes, flow-sorted from co-cultures with ECs, express α-SMA and the integrin subunits αv, β1, and β8. Flow-sorted ECs exhibit higher GARP expression compared to mono-cultured ECs.

Implications: Proximity is essential for ECs to present GARP-L-TGF-β1, facilitating pericyte-to-myofibroblast activation. Revealing pericyte-EC interactions will enhance our understanding of the mechanisms and pathways underlying fibrosis development. The molecules we identify, along with lung microvascular ECs, can serve as new targets for anti-fibrosis therapies.

ABSTRACT #17

Epigenetic Dysregulation in Adolescent Idiopathic Scoliosis

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Background. Adolescent idiopathic scoliosis (AIS) is a complex spinal deformity characterized by progressive spinal curvature and alterations in musculoskeletal tissues; however, the molecular mechanisms underlying disease pathogenesis remain poorly understood. Emerging evidence suggests that epigenetic mechanisms, particularly DNA methylation, may contribute to dysregulated gene regulatory programs involved in musculoskeletal development and degeneration. However, the functional consequences of DNA methylation alterations in AIS tissues remain largely unexplored.

Hypothesis. We hypothesize that epigenetic dysregulation contributes to altered gene expression programs in AIS musculoskeletal tissues and that these changes may be functionally modulated through targeted epigenetic perturbation.

Methods. To investigate this possibility, we performed pilot DNA methylation profiling of facet joint cartilage from AIS patients and non-scoliotic controls. To assess whether methylation differences influence gene expression, primary AIS chondrocytes were treated with the DNA methyltransferase inhibitors Decitabine and GSK-3484862. Gene expression changes were examined using a targeted qPCR panel focusing on pathways relevant to cartilage biology and inflammatory signaling.

Results. We identified 285 differentially methylated regions, indicating that AIS tissues exhibit distinct epigenetic signatures. DNMT inhibition was well tolerated and induced transcriptional changes in pathways related to cartilage signaling, inflammatory responses, and extracellular matrix regulation. Epigenetic modulation increased expression of several candidate genes, including FGFR2, suggesting that AIS-associated transcriptional programs may be partially reversible through DNA methylation modulation.

Conclusion. Together, these findings provide preliminary evidence that DNA methylation contributes to dysregulated gene expression programs in AIS musculoskeletal tissues and support further investigation of epigenetic modulation as a potential mechanistic pathway in AIS pathophysiology.

ABSTRACT #18

Fibronectin acts through alpha v beta 3 integrin in chondrogenic differentiation

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Fibronectin (FN) is a matrix glycoprotein that is often referred to as the 'master organizer' of the extracellular environment. FN is essential for vertebrate development, with its absence leading to prenatal death and/or severe embryonic defects. Mutations in FN cause rare corner-fracture-type spondylometaphyseal dysplasia (SMDCF). SMDCF patients suffer from a range of clinical symptoms affecting the skeletal system, including growth defects, scoliosis, coxa vara, abnormal ossification, and vertebral anomalies. The cell receptors and molecular mechanisms underlying fibronectin's role in chondrogenesis and subsequent skeletal development are still incompletely defined.

Here, we used the established ATDC5 mouse cell line as a model system for chondrogenic differentiation and siRNA knockdown of candidate integrins identified by bulk RNAseq of differentiated chondrocytes. Knockdown of the αv and β3 subunits individually, as well as both subunits combined, resulted in severe impairment of cell chondrogenesis after 10 d using collagen II as a readout. On the contrary, knockdown of the integrin α5 and β1, either single or combined, had little effect on chondrogenesis. Knockdown of FN also negatively affected chondrogenic differentiation. We further used a competition experiment in which we added to the ATDC5 cells a small (35 kDa) FN fragment containing the integrin-binding RGD site in the 10th FNIII domain, preceded by the synergy site. This fragment inhibited differentiation much more than a control fibrillin-1 fragment, demonstrating specificity for FN. We also analyzed the growth dynamics and maturation of FN and collagen II fibers, finding that these fibers promote matrix compaction, increased branching, and junction formation.

Together, the data identify the fibronectin-αvβ3 integrin interaction and the matrix fiber characteristics as essential aspects in ATDC5 chondrogenic differentiation. The data also provide potential new targets for future therapeutics in SMDCF.

ABSTRACT #19

UNCOVERING THE ROLE OF PROGESTERONE RECEPTOR IN SYNOVIAL PATHOLOGY DURING OSTEOARTHRITIS

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Purpose

Synovial inflammation and fibrosis contribute to joint pain and degeneration in osteoarthritis (OA). Our previous studies suggest that progesterone receptor (PGR) in synovial fibroblasts may act as an upstream transcriptional regulator of extracellular matrix (ECM) remodeling and fibrosis in knee (K)OA. This study aimed to characterize PGR expression and function in KOA synovium.

Methods

Immunohistochemistry was used to localize PGR expression in synovium of healthy donors (n=5) and advanced-stage (KL III/IV) KOA patients (n=8). PGR gene expression was reduced by siRNA transfection in primary fibroblasts from advanced-stage KOA patients (n=5), followed by gene profiling using a NanoString fibrosis panel. Chromatin accessibility and corresponding gene expression were examined using multiome approach combining single nuclei (sn)RNA-seq and snATAC-seq to assess chromatin accessibility and regulation of PGR and fibrosis/ECM-associated genes in advanced (n=8) KOA synovium. Fibroblast-selective Pgr conditional knockout (CKO) mice (Pgrf/f;Pdgfra-CreERT) were generated to evaluate the role of Pgr in osteoarthritis progression using the destabilization of the medial meniscus (DMM)-induced model of KOA.

Results

PGR expression was detected in synovial lining, sublining, and perivascular fibroblasts in OA synovium, while expression was limited to perivascular areas with almost minor staining of PGR found in healthy synovium. SiRNA-mediated knockdown of PGR in fibroblasts led to significant changes in the expression of multiple ECM and fibrosis-related genes relative to control siRNA. Multiome data analysis revealed four subsets of fibroblasts in advanced stage-OA synovium, each with unique transcriptomic features: Cluster_0 (top 2 features: AMTN, COL22A1); cluster 1 (BTBD11, KCNJ15); cluster 2 (PAMR1, AC093425.1); cluster 3 (SFRP2, C6). Motif and chromatin accessibility analysis identified open chromatin at the PGR locus across fibroblast subsets with Cluster 3 having the highest PGR gene expression. Gene ontology (GO) analysis of differentially expressed genes showed enrichment of biological pathways (BP) related to ERBP-2 and -4 signaling in cluster 0, several ECM and fibrosis related pathways in cluster 1, 2 and 3 and axonogenesis in cluster 3. Histopathological studies of Pgr CKO mice are ongoing.

Conclusion

Our findings suggest that PGR may play a role in OA pathology by impacting synovial fibrosis. Future studies are being directed towards comprehensively understanding the role of PGR in synovial pathology associated with OA.

Acknowledgement

This study is supported in part by the Canada Research Chairs Program & Tony and Shari Fell Platinum Chair in Arthritis Research. ND and PK are the recipient of the postdoctoral award from the Arthritis Society Canada.

ABSTRACT #20

Generating a high-resolution spatial transcriptomic atlas of the synovium during osteoarthritis

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Objectives: Osteoarthritis (OA), the most common form of arthritis, can affect multiple joint tissues leading to degeneration. The synovial tissue of OA joints undergo pathological changes within the lining and sublining layers. To visualize these changes, we sought to utilize Visium HD (VHD) to perform spatial transcriptomic analysis, at a single scale resolution, to create the first large-scale and high definition spatial atlas of the synovium of distinct joints including knee, hip, shoulder and spine affected by OA.

Methods: Synovial tissue obtained from 47 patients with advanced-stage knee (n=15), hip (n=14), shoulder (n=9) and spine (n=3) OA, and synovial knee tissue from cadaveric donors (control n=6) were subjected to spatial transcriptomic analysis using VHD. Samples with intact lining and sublining layers and an RNA integrity number > 6, were selected for spatial transcriptomics. Once spatial sequencing was performed, bioinformatic data analysis was conducted using SpaceRanger, which provided web summary reports, the Seurat R package and Loupe Browser.

Results: Spatial transcriptomics of 47 samples using VHD allowed us to sequence over 1 million cells at a single cell resolution. We have established a pipeline to map major cell populations located within the lining and sublining layers, including fibroblasts, macrophages, adipocytes, and endothelial cells, among others. Using the SpaceRanger analysis pipeline, we evaluated the quality of each sample from web summary reports to perform quality control checks. The Seurat R Package allowed integration of all the OA and control knee samples to identify cell clusters to facilitate comparison of synovial cell types across distinct OA joints, and within the control and OA knee joints. Furthermore, cell type annotation was performed using canonical markers to correlate spatial histopathology.

Conclusion: This is the first study to comprehensively use the VHD platform combined with advanced bioinformatics tools to carry out spatial transcriptomic profiling of multi-joint OA synovium at a single cell resolution.

ABSTRACT #21

Lung stiffness disrupts gremlin mediated fibroblast-AT2 interactions in pulmonary fibrosis

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Background: Idiopathic pulmonary fibrosis (IPF) is associated with dysregulated epithelial repair involving dysfunctional AT2 progenitor function. Our group has shown AT2 progenitor function involves intricately timed downregulation of the bmpr2 pathway initiated through transient increases in the bmpr2 antagonist gremlin released from activated fibroblasts, followed by bmpr2 restoration through AT2-bmp2 ligand production. Interestingly, gremlin levels are increased in IPF, especially in fibroblasts/myofibroblasts but the mechanism is unknown. IPF lungs are more stiff than normal lung from excessive ECM protein depositions from activated fibroblasts. Recently, the role of the increased lung ECM proteins and subsequent stiffness itself being a profibrotic environment and promoting excessive fibroblast activation through mechanical activation has gained traction. Few studies however, have explored if increased lung stiffness can dysregulate the fibroblast-AT2 bmpr2 signaling axis.

Methods: Sc-RNA-seq was performed on human tissue. Immunostaining was performed on IPF lungs for alpha smooth muscle actin (aSMA), vimentin and gremlin. Human lung fibroblasts were cultured in tissue culture plates simulating different stiffness and probed for gremlin and aSMA through immunoblotting. Alveolar organoids were generated from primary mouse epithelial progenitors isolated from C57 mice and treated with recombinant gremlin; organoids were counted and surfactant protein c (SP-C) expression was assessed. A549 cells were treated with conditioned media from fibroblasts and/or recombinant-gremlin; phosphorylated-SMAD1/5/8 (pSMAD1/5/8) is downstream of bmpr2 and used to assess bmpr2 signaling levels.

Results: Sc-RNA-seq showed fibroblasts can express gremlin and that fibrotic myofibroblasts express significantly more gremlin. Immunostaining showed increased co-localization between

vimentin/Gremlin and asma/gremlin in IPF lungs and immunoblotting showed increased gremlin from primary IPF fibroblasts suggesting activated fibroblasts are a source of gremlin. Immunostaining and immunoblotting revealed that fibroblasts grown on stiffer substrates expressed more gremlin and higher levels of aSMA. Mouse organoids revealed both higher number of organoids and increased SP-C+ cells when treated with gremlin in a dose dependent manner suggesting gremlin supports alveolar proliferation. A549 cells treated with fibroblast conditioned media showed decreased levels of pSMAD1/5/8 when treated with normal fibroblast media and even lower levels when treated with fibrotic fibroblast media suggesting a fibroblast-AT2 bmpr2 signaling axis that is altered when fibroblasts are activated in a fibrotic context.

Conclusions: In the IPF lung, accumulation of ECM proteins that increases lung stiffness could be a mechanism that sustains gremlin expression in fibroblasts, resulting in continuous bmpr2 suppression to epithelial cells along the bmpr2 pathway bmpr2 restoration and thus effective repair.

ABSTRACT #22

Distinct pathways underlie the senolytic and senomorphic effects of o-vanillin

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Objective: Low back pain is a leading global disability driver and is strongly associated with intervertebral disc (IVD) degeneration, a condition characterized by the accumulation of senescent cells (SCs) and their pro inflammatory senescence associated secretory phenotype (SASP). Senotherapeutics, including senolytics that selectively eliminate SCs and senomorphics that suppress SASP, represent a promising therapeutic strategy. This study defines the senolytic and senomorphic mechanisms of o vanillin in human senescent IVD cells, using RG 7112, a nutlin 3 analog that activates the p53 pathway, as a reference compound.

Methods: Because in vitro expansion reduces SC proportions, senescence was induced using combined inflammatory and oxidative stressors in primary cells from degenerated human IVDs. Cells were then treated with o vanillin, RG 7112, or left untreated. RNA sequencing (NovaSeq 6000), differential gene expression analysis, and gene set enrichment analysis (GSEA) were performed to identify differentially expressed genes (DEGs) and enriched pathways. Key DEGs associated with o vanillin–specific pathways

were validated by RT qPCR in additional samples, and protein level changes in molecular targets mediating senolytic and senomorphic activity were confirmed by Western blot.

Results: Following senescence induction, β galactosidase positive senescent cells increased by ~40% and p16INK4a positive cells by 47%. Transcriptomic profiling revealed 77 DEGs in o vanillin-treated cells and 127 DEGs in RG 7112-treated cells, with Gasdermin D as the only shared DEG, indicating distinct mechanisms of action. GSEA and pathway enrichment analyses showed that o vanillin significantly modulates TNF-NF κB, JAK/STAT, NOD like receptor, and NOTCH signaling, whereas RG 7112 selectively activates the p53-MDM2 axis. RT qPCR confirmed that o vanillin downregulates senescence and anti apoptotic markers (p16, BCL2, BCLxL) while upregulating pro apoptotic genes (BAX, BID, BAK1, BBC3). In senescent cells, o vanillin suppressed key survival pathways (JAK2, STAT3, PIM1, MTOR, KRAS, RAF1) and attenuated SASP associated inflammatory signaling through NF κB, with coordinated modulation of NOD2 and RBPJ (NOTCH). Western blot analyses validated the involvement of JAK/STAT and NOTCH pathways in o vanillin's senolytic activity and TNF-NF κB and NOD pathways in its senomorphic effects.

Conclusion: This study provides a comprehensive molecular characterization of o vanillin's dual senolytic and senomorphic mechanisms in human senescent IVD cells. By selectively inducing apoptosis in SCs while suppressing SASP driving inflammatory pathways, o vanillin emerges as a promising senotherapeutic candidate for mitigating IVD degeneration and improving outcomes in low back pain.

ABSTRACT #23

CD109 Regulates Fibroblast Activation and Macrophage–Fibroblast Crosstalk in Pulmonary Fibrosis

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Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive interstitial lung disease characterized by excessive extracellular matrix deposition, distortion of alveolar architecture, and progressive respiratory failure. Despite recent therapeutic advances, IPF remains associated with high morbidity and mortality, highlighting the need for a better understanding of the molecular mechanisms driving fibrotic

remodeling in the lung. Transforming growth factor-β (TGF-β) is recognized as the central profibrotic mediator that promotes fibroblast activation, myofibroblast differentiation, and extracellular matrix (ECM) accumulation. CD109, a glycosylphosphatidylinositol-anchored glycoprotein, has been identified as a negative regulator of TGF-β signaling and has demonstrated antifibrotic properties in several biological contexts; however, its role in pulmonary fibrosis remains unexplored.

To investigate the role of CD109 in pulmonary fibrosis, we determined how CD109 modulates lung fibroblast activation, profibrotic signaling pathways, and immune–fibroblast interactions using complementary in vivo, in vitro, and co-culture experimental approaches. Lung tissues from CD109 knockout and wild-type mice were analyzed using immunohistochemistry to evaluate ECM deposition, fibroblast activation, and inflammatory cell infiltration. Primary lung fibroblasts isolated from knockout and wild-type mice were stimulated with TGF-β to assess signaling responses and ECM production. In parallel, human lung fibroblasts derived from patients with IPF and healthy donors were used to examine the effects of CD109 using siRNA-mediated knockdown of CD109. Activation of key signaling pathways downstream of TGF-β was assessed by focusing on canonical and non-canonical pathways including SMAD, MAPK, and YAP signaling, together with the expression of profibrotic markers such as fibronectin, collagen, connective tissue growth factor, and α-smooth muscle actin.

To examine fibroblast–immune cell communication, a transwell co-culture system was used to evaluate macrophage polarization in response to fibroblast-derived signals. CD109-dependent regulation of fibrotic signaling was further analyzed using pharmacological inhibition of SMAD, MAPK, and YAP/TAZ pathways in IPF versus healthy fibroblasts.

Loss of CD109 markedly enhanced TGF-β-induced profibrotic signaling, as seen by increased activation of SMAD, MAPK, and YAP pathways and elevated expression of ECM proteins. In addition, CD109 deficiency altered fibroblast–macrophage communication and promoted macrophage polarization toward a profibrotic phenotype.

Together, these findings identify CD109 as a critical negative regulator of TGF-β-driven fibrotic signaling in the lung. By modulating multiple profibrotic pathways and influencing fibroblast–immune cell crosstalk, CD109 plays an important role in controlling fibroblast activation and extracellular matrix accumulation. Our findings provide new mechanistic insight into the molecular regulation of pulmonary fibrosis.

ABSTRACT #24

Role of PPARδ in tendon and ligament development, maturation and aging

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Background: Tendons connect muscle to bone and are essential for movement. They are composed of tenocytes embedded in a dense, type I collagen-rich extracellular matrix (ECM). Disruptions to this environment can lead to tendinopathy – a disease marked by collagen disorganization, altered crosslinking, increased cellularity and vascularity, and proteoglycan accumulation, often concomitant with site-specific ectopic chondrogenesis. Metabolic syndrome is a well-known risk factor for the development of tendinopathy, likely due to the impacts of systemic metabolic dysregulation and metabolite accumulation that compromise tendon homeostasis. One potential regulator of cellular metabolism in tendon is the nuclear receptor peroxisome proliferator activated receptor delta (PPARδ). PPARδ plays important roles in energy metabolism and tissue homeostasis in cartilage and bone; however, its role in tendon has yet to be investigated.

Hypothesis: We hypothesize that a tendon-specific deletion of PPARδ will accelerate the onset and severity of tendinopathic changes seen in risk factors such as aging.

Methods and Results: A tendon-specific Ppard cKO mouse model (cKO) was created by crossing the Ppardfl/fl mice with a tendon-specific Cre line (ScxCre). Male and female wildtype (WT) and cKO mice at 1-, 4- and 12-months of age were sacrificed and the following tissues were collected: knees, ankles, and various tendons, including tail, flexor digitorum longus (FDL), tibialis anterior (TA), Achilles, and patellar. Histopathological staining with Safranin-O/Fast Green was performed on paraffin sections of ankles (Achilles) and knees (patellar) to assess for tendinopathy-related changes in tendon tissue thickness, cell density, and ectopic chondrogenesis. Results for stained sections of 12-month male cKO Achilles tendon (N=4) showed an increase in percent of total tendon area exhibiting site-specific ectopic chondrogenesis (P=0.0222) compared to WT mice (N=5). Interestingly, no such change was found between WT (N=3) and cKO (N=3) groups for the 12-month male patellar tendon, or at our 1-month timepoint for either tendon.

Conclusions: These findings suggest that tendon-specific loss of PPARd does not impair early tendon or joint health but contributes to age-related, site-specific degeneration of the Achilles tendon compared to controls. The absence of changes in the patellar tendon points to region-specific roles of PPARδ in

tendon biology. Ongoing work will employ biomechanic analysis of Achilles and patellar tendons to define the molecular and biomechanical consequences of PPARδ loss and assess for potential sex-dependent effects. Together, this study positions PPARδ as a previously unrecognized regulator of tendon homeostasis.

ABSTRACT #25

Mapping pro-regenerative paracrine Apelin signaling in skeletal muscle tissue.

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Our previous work has shown that microvascular defects are closely linked to skeletal muscle stem cell (MuSC) dysfunction and fibrotic connective tissue accumulation in muscular dystrophy. We observed that, by promoting angiogenesis, the small peptide hormone Apelin-13 (AP-13) has a stimulatory effect on MuSCs, reduces fibrosis, and slows disease progression by enhancing endogenous tissue repair. While MuSCs do not express the AP-13 receptor APJ, the underlying mechanism involves the release of angiokines such as IGF2 from endothelial cells (ECs), which indirectly stimulate stem cell proliferation and tissue repair. Interestingly, muscle fibers also express APJ, although at lower levels than ECs. Thus, to better understand AP-13 signaling in skeletal muscle and characterize the involved pro-regenerative paracrine signals, we generated endothelial cell- and muscle fiber- specific APJ knockout mice. Loss of APJ in ECs leads to impaired MuSC function and a regenerative phenotype reiterating features of muscular dystrophy including impaired angiogenesis, fibrosis, and aberrant tissue regeneration. In contrast, muscle fiber-specific APJ knockout mice present a paradoxical gain-of-function phenotype manifesting only in slow-contracting muscle types including muscle fibers showing increased in mitochondrial activity and a denser microvasculature. These observations suggests that Apelin signaling in skeletal muscle tissue involves complex paracrine feedback loops with cell-type dependent effects, regulating both angiogenesis and myogenesis. Ultimately, our work will help to identify novel therapeutic targets for modulating apelin signaling in skeletal muscle disease.

ABSTRACT #26

Conditional overexpression of Mig6 accelerates cartilage damage in a post-traumatic murine model of OA.

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Introduction
Osteoarthritis (OA) is a complex, multifactorial disease of synovial joints characterized by pain, disability, and decreased quality of life. There are currently no disease-modifying therapeutics available, highlighting a critical need to better understand its underlying pathogenesis. The epidermal growth factor receptor (EGFR) has emerged as an important player in cartilage physiology and OA pathogenesis. Notably, EGFR exerts context-dependent effects on cartilage integrity and displays temporal changes in activity during OA progression. Accordingly, mitogen-inducible gene-6 (Mig6), the endogenous negative regulator of EGFR, has been identified as a potential therapeutic target in OA. Previously, our group demonstrated that cartilage-specific deletion of Mig6 resulted in increased cartilage thickness and chondro-osseous nodule formation, whereas Mig6 overexpression accelerated cartilage degeneration in aging mice.

Rationale and Hypothesis
Mig6 mRNA has been shown to be increased in canine cartilage in response to mechanical loading, suggesting a potential role in post-traumatic OA; however, this has not been investigated in vivo. Therefore, we hypothesized that skeletally mature mice with cartilage-specific Mig6 overexpression would experience accelerated cartilage degradation in a post-traumatic model of OA compared to wildtype and sham controls.

Methods and Results
Post-traumatic OA was induced in male cartilage-specific Mig6 overexpression (Mig6over/over) and wildtype mice at 16 weeks of age using destabilization of the medial meniscus (DMM) surgery. Behavioral outcomes were analyzed through Open Field Testing at 12 weeks post-surgery, however no significant behavioral differences were observed between groups. To assess joint pathology, mice were

sacrificed at 8- and 12-weeks post-surgery, and histopathological (OARSI) scoring was used to assess articular cartilage damage. While DMM surgery exacerbated cartilage damage compared to the sham surgery in both genotypes, cartilage damage was accelerated in Mig6over/over DMM mice compared to wildtype DMM mice. No differences were observed between genotypes following sham surgery, suggesting that altered mechanical loading is essential for the degenerative effects of Mig6 overexpression observed at both 8 and 12 weeks post-surgery.

Future Directions

This study provides evidence supporting a role for Mig6 in modulating cartilage responses to injury in vivo, further highlighting the complexity of the Mig6/EGFR axis in OA pathology. Future studies are required in both male and female mice to assess potential sex-specific differences. Additionally, investigating Mig6 activity in human cartilage will be important to evaluate the translational relevance of these findings.

ABSTRACT #27

Evaluating the Therapeutic Efficacy of Novel Cationic Polymers in a Preclinical Model of Osteoarthritis

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BACKGROUND: Osteoarthritis (OA) is a growing global health challenge, and available treatments remain limited to symptomatic relief or joint replacement, with no disease-modifying therapies capable of slowing or reversing structural damage in joint tissues such as articular cartilage. A major barrier to OA drug development is the rapid clearance of injected therapeutics from the joint space, which prevents sustained engagement with articular cartilage as well as other synovial joint tissues. Cationic drug carriers have emerged as a promising strategy to overcome this limitation because their electrostatic interactions with the negatively charged extracellular matrix (ECM) increase tissue retention and enhance drug delivery. Interestingly, several of these polymers have been shown to

improve cartilage mechanics and inhibit matrix breakdown, yet their therapeutic potential, independent of the delivered cargo, remains poorly understood.

HYPOTHESIS: We hypothesize that cationic macromolecules with high charge densities can directly slow OA progression by mechanically reinforcing cartilage and sterically blocking ECM-degrading processes.

METHODS & RESULTS: To test this, we have synthesized a panel of polymers composed of N-(2hydroxypropyl) methacrylamide and N-(3aminopropyl) methacrylamide at ratios of 1:9, 1:4, 1:2, 1:1, and 0:1. In vivo safety will be evaluated by intraarticular injection into healthy C57BL/6 mouse knees, followed by tissue assessment at 1, 7, and 28 days via high-resolution phase contrast μCT, histopathology, and fluorescence imaging to quantify polymer uptake, persistence, and effects on normal joint structures.

Therapeutic efficacy will then be assessed using the destabilization of the medial meniscus (DMM) mouse model. Male and female mice will undergo sham or DMM surgery, receive polymer injections two weeks post-surgery, and be evaluated at 8 and 16 weeks for pain and motor function. Joints will subsequently undergo phase-contrast μCT using a novel imaging modality under development by our group that enables the visualization of mineralized bone, tissue hydration of articular cartilage and other soft synovial tissues, and ultrastructural changes. Finally, histopathological scoring (i.e., OARSI scoring), bulk RNA-seq of joint tissues, and immunohistochemistry for articular cartilage proteins and catabolic enzymes will be performed to characterize differences in articular cartilage and synovium between groups.

FUTURE DIRECTIONS: This work introduces a novel, drug-free therapeutic strategy in which cationic polymers themselves function as disease-modifying agents, challenging the conventional paradigm that OA treatments must be small-molecule or biologic drugs.

ABSTRACT #28

TGFα drives metabolic reprogramming and mitochondrial membrane depolarization in primary articular chondrocytes

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Introduction

Osteoarthritis (OA) is a multifactorial disease of synovial joints characterized by pain, disability, and decreased quality of life. There are currently no disease-modifying therapeutics available, highlighting a critical need to better understand the OA pathogenesis. Transforming growth factor alpha (TGFα), a potent ligand of the epidermal growth factor receptor (EGFR), has emerged as an important regulator of cartilage physiology and OA pathogenesis, exerting context-dependent protective or destructive effects on the cartilage. While the effects of TGFα on anabolic and catabolic gene expression are well defined, its influence on cellular metabolism and mitochondrial function has yet to be investigated.

Rationale and Hypothesis

Activation and inhibition of EGFR signalling by other ligands have been shown to alter mitochondrial function in various tissues; however, this has not yet been studied in chondrocytes. We hypothesized that treating primary chondrocytes in vitro with TGFα would alter the transcriptional metabolic signature and mitochondrial function through EGFR signalling. This could provide a novel mechanism by which TGFα contributes to OA pathogenesis.

Methods and Results

Cultures of immature murine articular chondrocytes (iMACs) were isolated from 5-6-day-old male and female mice and treated with TGFα for 24-72 hours under serum-free conditions. Through bulk RNA sequencing and gene set enrichment analysis, we found that TGFα treatment induced differential expression of 1643 genes (1305 upregulated, 338 downregulated), with enrichment of processes related to Response to Reactive Oxygen Species, Glycolysis, and Inflammatory Response. Novel gene expression changes from 24-72 hours were validated by RT-qPCR, in which TGFα increased expression of genes related to antioxidation and ROS production (Sod3, Prdx1, Ucp2, Nox4), mitochondrial processes (Bnip3), metabolism (Ldha), and inflammation (Tlr4). Finally, fluorescent microscopy revealed a significant reduction in mitochondrial membrane potential following TGFα treatment. Overall, we found that TGFα exposure drives transcriptional changes associated with ROS, cellular metabolism, and inflammatory signalling, as well as mitochondrial membrane depolarization, supporting a link between TGFα signalling and mitochondrial dysfunction.

Future Directions

Our next steps will be to further explore the relationship between TGFα and mitochondrial function through measuring respiratory parameters from isolated primary chondrocytes using a Seahorse XFe-24 analyzer. These findings will be important for the field of OA research, as they will provide insight into the poorly characterized pathogenesis of OA, potentially leading to the identification of novel therapeutic targets.

ABSTRACT #29

Microfibril-associated glycoprotein 4 interaction with fibrillin-1

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Elastic fibers provide elasticity to tissues such as skin or blood vessels. The maintenance and proper function of elastic fibers depend on key accessory proteins, including microfibril-associated glycoprotein 4 (MFAP4). MFAP4 exists as an octamer under physiological conditions, as we reported using cryo-electron microscopy and single-particle analysis (Wozny et al., Nature Communications, 2024). MFAP4 colocalizes with fibrillin-1 containing microfibrils in elastic tissues, and it binds to fibrillin-1, tropoelastin, and cells. In Marfan syndrome mutations of fibrillin-1 induce dysregulation of the MFAP4 protein level and glycosylation patterns. Here, we characterized the MFAP4 interaction and complex formation with fibrillin-1 and tropoelastin. We used a series of recombinant fibrillin-1 truncation and domain-swapping fragments to locate binding sites for MFAP4. Surface plasmon resonance (SPR) determined a high binding affinity of MFAP4 to N-terminal fragments containing the hydrid domain 1 (H1) (KD = 1-3 nM). Deletion of the H1 domain or constructs swapping the H1 with the H2 domain abolished interaction with MFAP4. This demonstrates that the fibrillin-1 binding site for MFAP4 resides in the H1 domain. The smallest N-terminal fibrillin-1 fragment (rF38) spanning four domains was the strongest interactor with MFAP4 (KD = 0.8 nM). Dynamic light scattering measurements under physiological conditions of MFAP4, together with the fragments containing H1 and/or tropoelastin, showed larger particle sizes (Rh=7.8-16.0 nm) than those that did not contain H1 (6.5-8.0 nm), indicating that MFAP4 forms complexes with fibrillin-1 and tropoelastin. SPR and solid-phase assays with central fragments of fibrillin-1 revealed the presence of a secondary binding site on fibrillin-1 for MFAP4. We further characterized mixtures of MFAP4 with all the fibrillin-1 fragments (N-terminal and central region) and tropoelastin by atomic force microscopy and negative staining to visualize complex formation. Cryo-electron microscopy maps of the MFAP4/rF38 mixture show an octameric structure of MFAP4 with a shape compressed inward compared to that of MFAP4 alone. This may indicate alterations in the MFAP4 octamer structure upon interaction with rF38. Further assays are ongoing to determine which of the primary or secondary sites dominate the MFAP4 interaction with full-length fibrillin-1 and whether it involves synergistic binding. We foresee that this knowledge will contribute to the development of mechanistic models of MFAP4's involvement in elastogenesis, with direct relevance to understanding the pathogenetic mechanisms underlying Marfan syndrome.

ABSTRACT #30

Functions of lysyl oxidase-like 1 variants linked to pseudoexfoliation syndrome

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Lysyl oxidase-like 1 (LOXL1) is a critical enzyme for elastic fiber crosslinking. Pseudoexfoliation syndrome (XFS) is an age-related systemic disorder characterized by excessive production of abnormal elastic fiber components. Manifested predominantly in the eye, XFS is linked to LOXL1 variants. LOXL1 single-nucleotide polymorphisms (SNPs; denoted as nucleotide triplets below) leading to amino acid substitutions at p.141, p.153, and p.407 are associated with XFS, but their contribution to disease severity varies among geographic populations. Four LOXL1 variant combinations, identified as risk or protective factors in German and Japanese populations, were recombinantly produced in HEK293-EBNA cells: GGA (German, risk), TGA (Japanese, risk), GAA (German, protective), and GAT (Japanese, protective). Enzymatic activity assays using cadaverine and tropoelastin as substrates showed that all variants retain catalytic function. We hypothesized that the differences within populations are linked to how the respective variants interact with elastic fiber proteins, leading to variable deposition of anomalous elastic material. We applied solid phase and surface plasmon resonance spectroscopy to characterize the binding of variants to fibrillin-1, tropoelastin, fibronectin, fibulin-4, fibulin-5, LTBP4-L, LTBP4-S, and MFAP4. Both protective variants (GAA and GAT) bound more strongly to fibulin-4 (KD=3-10 nM) and tropoelastin (KD=3.5 nM) than their risk counterparts (GGA and TGA) (KD=4.5-202 nM). All variants interacted moderately with fibulin-5 (KD=192-252 nM). The protective GAA variant bound stronger to fibrillin-1 and fibronectin (KD=1.3-12 nM) than the risk variant GGA (KD=15-21 nM) in the German population. However, the TGA risk variant (KD=5.5-25 nM) had a higher affinity to these ligands than the protective GAT variant (KD=32-42 nM) in the Japanese population. All variants interacted strongly with tropoelastin (KD=11-25 nM), except TGA (KD=202 nM). Similarly, all variants bound LTBP4-L with high affinity (KD=2.2-3.8 nM), except TGA, which did not bind. Binding to LTBP4-S was weak (300-2400 nM) for all variants. The German variants bound MFAP4, but the Japanese did not. Atomic force microscopy and ProteoStat assays revealed that the GGA and TGA risk variants had a propensity to aggregate into 100-300 nm particles, but not the protective variants (10-20 nm). Overall, the SNPs significantly change LOXL1 binding behaviour to elastic fiber proteins. The functional changes are governed by the nature of the substituted amino acid residues and their combinations. The data reveal mechanistic insights into why, in some populations, disease risk is elevated, whereas in others it is not.

ABSTRACT #31

CD109 Restrains Fibroblast–Immune Crosstalk by Limiting NF-κB–Driven Skin Inflammation

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INTRODUCTION: Systemic sclerosis (SSc) is a complex autoimmune disease marked by chronic skin inflammation and progressive fibrosis. Unfortunately, it has a high morbidity and mortality rate, with treatment options only focusing on symptom management. It is very important to understand the molecular mechanisms involved in SSc to develop novel therapeutic strategies. While Transforming Growth Factor Beta (TGF-β) signaling is a well-established driver of fibrosis in SSc, inflammatory pathways such as Nuclear Factor Kappa B (NF-κB) also contribute substantially to disease morbidity. Our lab has identified CD109 as a co-receptor of TGF-β that inhibits TGF-β signaling and pro-fibrotic responses. However, its role in fibroblast inflammatory responses remains unclear. We hypothesize that CD109 functions as a fibroblast-intrinsic negative regulator of NF-κB signaling, and its loss enhances inflammatory activation and immune cell recruitment in the skin.

METHODS:
1) Human dermal fibroblasts were transfected with CD109-targeting siRNA and treated with TNF-α. NF-κB activation was assessed by Western blot. Cytokine secretion profiles were evaluated using a multiplex human inflammation array. CD109 interactions with TLR2, TLR4, TNFRI, and TNFRII were examined by immunofluorescence and co-immunoprecipitation.
2) Global CD109 knockout mice and tamoxifen-inducible fibroblast-specific CD109 conditional knockout mice were analyzed for inflammatory changes in dorsal skin. Immunohistochemistry was performed to assess immune cell infiltration.
3) Publicly available single-cell RNA sequencing datasets curated by the pan-disease skin fibroblast atlas were analyzed to assess CD109 expression in skin fibroblast subtypes. SSc fibroblasts were analyzed to evaluate subtype distribution and CD109 expression patterns.

RESULTS:
1) CD109 knockdown in dermal fibroblasts enhanced TNF-α–induced NF-κB activation, increased phosphorylation of IκBα, p65, and p50, and altered cytokine production, characterized by increased IL-6 and IL-15 and reduced IL-1ra and soluble TNFRII.
2) Both global and fibroblast-specific CD109 knockout mice demonstrated significantly increased immune cell infiltration, including macrophages, neutrophils, T cells, and plasmacytoid dendritic cells,

in the skin.
3) CD109 expression was enriched in structural and homeostatic fibroblast subtypes, while immune-interacting subsets exhibited lower CD109 levels.

CONCLUSIONS: CD109 acts as a negative regulator of NF-κB signaling in dermal fibroblasts, and its loss promotes fibroblast-driven skin inflammation and immune cell infiltration. These findings identify CD109 as a key regulator of fibroblast inflammatory signaling in skin diseases such as scleroderma.

ABSTRACT #32

Role of fibrillin-1 in adipocyte basement membrane formation and function

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Fibrillin-1 is a 350 kDa extracellular matrix (ECM) glycoprotein that assembles as characteristic beads-on-a-string microfibrils typically associated with elastic fibers. Mutations in the fibrillin-1 gene (FBN1) cause the genetic disorder Marfan syndrome (MFS). This connective tissue disorder presents with numerous clinical manifestations, including a paradoxical phenotype in adipose tissue. Younger patients tend to have an asthenic body habitus with very little body fat, whereas when individuals with MFS age, they typically accumulate fat and likely become obese. These data exemplify an important role of fibrillin-1 in adipose tissue development and maintenance. Each differentiated adipocyte is surrounded by a basement membrane, containing collagen IV, perlecan, and laminins. How fibrillin-1 interacts and is organized in the pericellular matrix is unknown. Here, we hypothesize that fibrillin-1 organizes the pericellular basement membrane, which in turn guides adipocyte differentiation and function.

We have generated conditional knockout mice of fibrillin-1 in white adipose tissue (Fbn1-AKO). These mice show, by transmission electron microscopy, that fibrillin-1 deficiency causes a thickened and disorganized basement membrane. Single-nucleus RNA-sequencing using white adipose tissue demonstrates upregulation of typical basement membrane genes, including collagen IV, laminins, and nidogen, likely representing a non-productive compensatory mechanism. These results are corroborated by immunofluorescence staining of white adipose tissue exhibiting a more robust expression of perlecan between adipocytes in Fbn1-AKO mice, along with a concomitant upregulation of elastin. Previous work with human adipose-derived mesenchymal stem cells revealed that fibrillin-1

mRNA is downregulated as adipogenic differentiation progresses. This is coherent with our cell culture model using 3T3-L1 pre-adipocytes differentiated over 7 days, where fibrillin-1 downregulation occurs simultaneously with the progressive formation of a basement membrane enveloping adipocytes, containing collagen IV and perlecan. However, in vivo analyses reveal strong fibrillin-1 expression between adipocytes, in stained whole-mount white adipose tissue isolated from 30-week-old wild-type mice. This observation highlights the difference in adipogenesis dynamics in mouse tissues compared to cell culture. The next step is to analyze the ultrastructure of the adipocyte basement membrane using super-resolution microscopic techniques such as focused ion beam scanning electron microscopy.

ABSTRACT #33

3D Printed Tissue-Engineered Heart Valves Using Light-based Printing Technology

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Heart valve disease remains a major global health burden, with current replacement options limited by complications such as thrombogenicity, calcification, and immune response. Tissue-engineered heart valves (TEHVs) offer a promising alternative; however, their fabrication is constrained by limitations in biomaterials and manufacturing techniques. In this study, we report the development of a novel hybrid bioink and its application in the high-resolution fabrication of human-sized TEHVs using digital light processing (DLP) 3D printing. The bioink was formulated by combining synthetic and natural biopolymers to achieve an optimal balance between printability, mechanical robustness, and biological functionality. The physicochemical properties of the resulting hydrogels were extensively characterized, demonstrating high swelling, slow degradation rates, and mechanical properties comparable to native valve tissues. Notably, the hydrogels exhibited nonlinear elastic behavior and high resilience under cyclic loading, critical for heart valve applications. The developed bioink enabled the fabrication of complex geometries with micron-scale resolution, including anatomically accurate, tri-leaflet heart valves. Micro-computed tomography confirmed high printing fidelity and structural integrity. Biological evaluation using human induced pluripotent stem cell-derived valvular interstitial and endothelial cells demonstrated excellent cytocompatibility, with sustained cell viability, attachment, and phenotype preservation over time. Further in vivo biocompatibility assessment revealed a foreign body response

characterized by early inflammatory infiltration followed by progressive fibrous encapsulation and reduced cellularity over time. Importantly, no evidence of necrosis, severe chronic inflammation, or systemic adverse effects was observed, indicating favorable host integration. Functional assessment under physiological conditions using a pulse duplicator system revealed that the printed TEHVs exhibited proper leaflet motion, complete coaptation, and stable hemodynamic performance without structural failure. Although slightly elevated pressure gradients were observed compared to bioprosthetic controls, the valves maintained effective unidirectional flow. Overall, this work presents a versatile platform for the fabrication of functional TEHVs, highlighting the potential of DLP-based bioprinting for next-generation cardiovascular implants.

ABSTRACT #34

Delivery of induced pluripotent stem cells in cell-instructive composite hydrogels for nucleus pulposus regeneration

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Chronic back pain is a leading cause of disability commonly associated with intervertebral disc (IVD) degeneration. IVD degeneration begins with degenerative changes in the nucleus pulposus (NP), the innermost gel-like structure. Limited endogenous repair mechanisms of the IVD and symptom-focused treatments motivate investigation of cell-based therapies targeting the NP to restore degenerated IVD. Human induced pluripotent stem cell (hiPSC)-derived populations are a promising clinically-translatable cell source due to their regenerative potential and feasibility of autologous sourcing. This study aims to develop in situ-forming mechanically-resilient hydrogels incorporating micronized decellularized nucleus pulposus (DNP) sourced from bovine IVD as a cell-instructive platform to guide hiPSC-derived notochordal-like (NC-like) or NP-like cell populations for IVD regeneration. We hypothesize that NC-like cells will exhibit enhanced regeneration compared to NP-like cells following encapsulation and culture under conditions mimicking the native human NP. We further hypothesize that regeneration will be augmented in hydrogels incorporating DNP particles. A hydrogel composed of 4-arm [poly(ethylene glycol)-b-poly(trimethylene carbonate)-acrylate](4a[(PEG-b-PTMC)-Ac] and thiolated chondroitin sulphate will be used as the basis of our platform. We will incorporate varying DNP content to establish formulations matching human NP tissue mechanical properties while supporting the viability and phenotype of encapsulated iPSC-derived NC-and NP-like cells. We developed a

detergent-free decellularization protocol that preserves the biochemical composition of the ECM while removing cells, yielding scaffolds rich in structural ECM components. Ongoing experiments are focused on differentiating hiPSCs to NC-and NP-like cells through a staged differentiation process mimicking embryonic development. Adapting published protocols [Laagland et al. Tissue Engineering Part A 2026], hiPSCs were differentiated through a mesendoderm progenitor stage, specified toward the NC lineage, and matured into NP cells. Cells were collected at defined stages of differentiation: day 2 (mesendoderm progenitor), day 7 (NC), and days 14, 21, and 31 (NP). Cell phenotype is being characterized by immunolocalization and RT-qPCR analysis of pluripotency (NANOG, SOX2, OCT4), mesendoderm progenitor (T, MIXL1), NC-associated (SHH, FOXA1, FOXF1), and NP (CCN2, CD24, DCN) genes. Next, effects of 3D culture in composite hydrogels on cell phenotype and ECM production will be characterized to validate formulations that maintain cell viability and phenotype. Physiological loading will be applied to composite hydrogels using a high-throughput bioreactor to identify the cell source (NC-like or NP-like) and platform (gel+DNP or gel alone) that enhances NP markers, matrix production, and biomechanics. This study will establish a cell delivery platform for NP regeneration and guide cell source selection using clinically relevant hiPSC-derived cells.

ABSTRACT #35

Uncoupling Matrix Production from Contraction During Fibroblast Activation

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Background: Skin fibrosis results from failed tissue repair, with myofibroblasts driving scar formation through the deposition and excessive contraction of collagen-rich ECM. The current dogma stipulates that myofibroblasts perform both functions simultaneously. However, recent findings suggest that ECM production and contraction are regulated by distinct, yet interdependent programs. Since both processes are energy-intensive, we investigated whether mitochondrial availability and distribution regulate their respective activity levels.

Hypothesis: ECM production and contraction are mutually exclusive myofibroblast activation stages coordinated by mechanical cues and cellular energy demands.

Objective: To explore feedback mechanisms governing ECM production and contraction.

Methods and Results: To model normal and fibrotic conditions, dermal fibroblasts from rat, mouse, and human skin were cultured on soft (1 kPa) or stiff (100 kPa) substrates ± profibrotic TGF-β1. Bulk RNA-seq of mouse fibroblasts showed that soft substrates reduced expression of contractility genes (e.g., Acta2) and increased ECM genes (e.g., Col1a1), whereas stiff substrates induced the opposite pattern. Analysis of single-cell RNA-seq datasets (publicly available) from healthy and fibrotic skin revealed that myofibroblast precursors expressing COL1A1 lacked ACTA2, and vice versa. To time-resolve the activation of ECM production and contraction programs, fibroblasts from dual-reporter mice expressing GFP under Col1α promoter and RFP under α-SMA promoter control were analyzed by live-cell imaging. Expression of GFP and RFP in these fibroblasts were inversely correlated (r=−0.74): highly contractile fibroblasts on stiff surfaces +TGF-β1 showed low Col1α (~0.5-fold) and high α-SMA promoter activity (~7.5-fold). This pattern reversed upon substrate switching, with stiff-grown cells transferred to soft surfaces showing decreased α-SMA and increased collagen promoter activity. Overexpression of α-SMA-GFP in rat fibroblasts reduced collagen expression, suggesting feedback regulation. Mechanistically, this feedback was probed using an α-SMA–disrupting peptide, contraction inducing agent-Calyculin, and inhibitors of FAK, Rho, MRTF, YAP, and TGF-βR. Of all mechanistic interventions, only inhibition of FAK mechanosensing uncoupled this feedback control. Analysis of the in vivo splinted fibrotic rat skin wound model showed increased α-SMA expression and elevated mitochondria per cell compared to a standard excisional skin wound. Mitochondrial morphometry analysis from 3D-rendered, sub-diffraction images showed that myofibroblasts on stiff substrates (α-SMA–high, collagen-low) have more fragmented mitochondria than fibroblasts on soft substrates (α-SMA–low, collagen-high), indicating distinct energetic states.
Conclusion: Our findings challenge the prevailing view that myofibroblasts simultaneously produce ECM and contract. Instead, substrate stiffness and TGF-β1 drive bifurcation into ECM-producing and contractile states, defining targetable myofibroblast subpopulations that may help restore balanced repair and reverse fibrosis.

ABSTRACT #36

Targeting the apelinergic system to regenerate skeletal muscle microvasculature for the treatment of muscular dystrophy

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Muscular dystrophies (MD) are genetically diverse disorders characterized by progressive muscle degeneration and a lack of effective therapies. Mutation-independent strategies are urgently needed to benefit a broad patient population. Recent studies have identified microvascular defects as a common feature across multiple preclinical MD models, which appear to contribute to skeletal muscle stem cell (MuSC) dysfunction and impaired regeneration. The angiogenic peptide Apelin has been shown to restore microvasculature, enhance MuSC function, reduce fibrotic connective tissue deposition, and promote muscle regeneration; however, its short in vivo half-life limits therapeutic potential. To address this, we screened Apelin analogs with improved pharmacokinetics (PK) and pharmacodynamics (PD) based on their capacity to stimulate endothelial cell proliferation. Two lead candidates were identified: AM02-123, a linear peptide with high APJ receptor affinity (0.02 nM) and a 50-minute half-life, and KT03-69, a macrocyclic peptide with lower affinity (396 nM) but enhanced stability and potent biological activity. We are assessing these analogs in vitro for myofiber proliferation and in MD mouse models to determine whether optimized PK/PD translates into superior regenerative outcomes. Additionally, BRET assays in endothelial cells will delineate the signaling pathways activated by Apelin and its analogs, providing mechanistic insight into their pro-regenerative effects. These studies aim to advance a mutation-independent therapeutic strategy for MD, potentially benefiting patients across diverse genetic backgrounds and offering a broadly applicable approach to restore muscle function.

ABSTRACT #37

Knee osteoarthritis patient-derived cartilage-on-a-chip model can evaluate mechanical and inflammatory clinical phenotypes

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Effective treatments for knee osteoarthritis (OA), a degenerative whole-joint disease, remain elusive due to limited research tools that can adequately capture patient heterogeneity and distinct phenotypes. Our objective is to mimic patient-specific mechanical overload and inflammatory clinical phenotypes using novel “organ-on-a-chip” engineering techniques.
To induce a mechanical overload phenotype, human OA cartilage biopsies were subjected to 30% compressive strain for 14 days, with 10% strain serving as a physiological control. To mimic a pro-inflammatory phenotype, human OA cartilage biopsies were treated with 5 ng/mL interleukin-1 beta (IL-

1B) and oncostatin M (OSM). Static unloaded biopsies were used as a baseline for comparison across all treatments. Cartilage degradation was assessed by sulfated glycosaminoglycans (sGAGs), Safranin-O staining, matrix metalloproteinase (MMP) and tissue inhibitor of MMP (TIMP) activity, and cartilage gene expression. Rescue effects were validated with 100nM dexamethasone, a glucocorticoid widely used to manage OA.

Physiological loading (10%) maintained cartilage homeostasis, whereas overloading (30%) triggered increased levels of extracellular matrix (ECM) degradation. Both mechanical overload and cytokine stimulation increased loss of proteoglycan content by Safranin-O staining and sGAG release, and increased MMP/TIMP levels compared to static controls. However, overload-induced changes did not reach the levels observed with cytokine stimulation. At the gene expression level, both overload and cytokine stimulation significantly upregulated catabolic (ADAMTS-4, ADAMTS-5, MMP-3, MMP-13) markers and downregulated cartilage-specific markers (ACAN, COL2A1) relative static controls, albeit to a lesser extent under overload conditions. Inflammatory (IL-6, IL-8) markers were significantly upregulated only in cytokine-driven conditions and showed non-significant increases under mechanical overload. Interestingly, only mechanical overload promoted cartilage matrix remodelling towards a putative fibrotic (COL1A1) and hypertrophic (COL10A1) state, suggesting distinct mechanical overload and inflammatory-driven phenotypes. Dexamethasone treatment rescued both matrix degradation gene expression and soluble factors, demonstrating the sensitivity of our end-stage OA explant model. Evidence of patient-specific responses to mechanical overloading and dexamethasone rescue thus validate the cartilage-on-a-chip model as an effective research tool for screening novel therapies.

In summary, our cartilage-on-a-chip model captures both OA clinical phenotypes and donor-driven treatment responses, highlighting its potential as a valuable tool for precision medicine applications.

ABSTRACT #38

Characterization of 3D bioprinted eggshell membrane-loaded alginate biomaterials for wound healing applications

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Wound healing remains a significant clinical challenge, underscoring the need for advanced biomaterials that facilitate active wound repair. Eggshell membrane (ESM), rich in collagen, glycosaminoglycans, and intrinsic antimicrobial proteins, is a promising biomaterial for wound healing applications. We hypothesize that particalized ESM can be incorporated into hydrogel-based bioinks to generate 3D printed constructs with defined gradients of ESM, for biocompatibility, antimicrobial activity, and structural stability. The objective of this study is to develop and optimize ESM-integrated hydrogel bioinks for 3D bioprinting of wound healing scaffolds. ESM particle dispersions were evaluated in water using OD600 kinetics and extrusion tests. Bioink formulations combining sodium alginate, ESM, and CaCl2 were screened to establish compositional limits of printability. Selected formulations were extruded into multilayered grids using the CELLINK BIO-X6 bioprinter, post-crosslinked in CaCl2 for gelation, and subsequently lyophilized for 48 hours to stabilize scaffolds. The architecture and topography of the ESM-based constructs were visualized after crystal violet staining. Stability of printed constructs was further evaluated in Dulbecco's Phosphate-Buffered Saline (DPBS), DPBS with Ca2+ and Mg2, and Simulated Wound Fluid (SWF). Wet weight loss was monitored over time to track the breakdown of the material. Group differences were analyzed using one-way ANOVA followed by Tukey post-hoc tests. The significance level was $P < 0.05$. ESM particle aggregation was only observed at the highest concentration. Printable bioink formulations were identified within defined alginate/ESM/CaCl2 ranges by calculating the spread ratio to determine shape fidelity. Degradation kinetics revealed progressive breakdown, with complete construct degradation after 3 days in DPBS and 5 days in DPBS with Ca^{2+} and Mg^{2} or SWF, suggesting suitability for short-term wound protection, with periodic reapplication required. Blank and low-concentration ESM-based scaffolds degrade more rapidly, while higher-concentration ESM-based scaffolds exhibit a slower degradation profile, suggesting stabilization at increased ESM concentrations. Rheological characterisation shows that ALG-ESM-CaCl2 (20mM) hydrogels ranging from low to high ESM concentration (0%-1.5%) possess strong shear-thinning behaviour at a constant temperature of 37°C. This is further supported by decreased viscosity as shear rate increases, classifying this bioink composition as non-Newtonian. Preliminary assessment demonstrates the potential of 3D-printed ESM-based constructs as sustainable biomaterials for next-generation wound healing applications. Future work will evaluate antimicrobial activity using skin-associated pathogens, including Staphylococcus aureus and Pseudomonas Aeruginosa, and biocompatibility in vitro, followed by acute in vivo assessment of wound closure (Supported by NSERC alliance, Egg Farmers of Canada, and Burnbrae Farms).

ABSTRACT #39

Toward Patient-Derived 3D Modeling of Epithelial-Stromal Interactions in Inflammatory Bowel Disease

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Inflammatory bowel disease (IBD) is characterized by chronic disruption of the intestinal epithelial-stromal interface, involving fibroblast activation, extracellular matrix remodeling, and progressive loss of epithelial barrier integrity. A major limitation of current in vitro models is their reliance on established cell lines, which fail to capture the patient-specific stromal reactivity and epithelial-mesenchymal dynamics implicated in IBD pathophysiology. Our group is developing a 3D co-culture platform incorporating intestinal epithelial cells and fibroblasts within alginate-gelatin hydrogels and electrospun mesh-based compartmentalized scaffolds, designed to model epithelial-stromal architecture in the gut. However, the isolation and long-term culture of primary intestinal epithelial cells and stromal fibroblasts from patient biopsies remains technically challenging, limiting the availability of disease-relevant cellular material for such systems. A central objective of the present work is therefore to introduce patient-derived primary cells into this platform, moving toward individualized modeling of the intestinal mucosa.

Toward this goal, we are pursuing two preparatory approaches. The first involves the isolation of intestinal epithelial cells and stromal fibroblasts from mucosal biopsies obtained during colonoscopic follow-up of IBD patients, from both inflamed and non-inflamed tissue sites. A protocol for the generation of patient-derived organoids (PDO), including serial passaging and cryopreservation, is currently being established, providing a biobanked source of primary intestinal epithelium for integration into the existing 3D platform. The second approach investigates invasin, a bacterial outer-membrane protein from Yersinia spp. that engages beta-1 integrins, as a matrix coating to promote epithelial adhesion and spatial self-organization across multiple configurations, including 2D substrates, alginate-gelatin hydrogels, and Matrigel.

Preliminary results include successful generation and passaging of PDO from donor biopsy material, with cryopreservation conditions under evaluation, and initial assessment of invasin coating across 2D and 3D matrix contexts, demonstrating promising adhesion and organizational responses in both HT-29 intestinal epithelial cells and PDO. In parallel, a complementary 3D co-culture system incorporating HT-

29 epithelial cells and IMR-90 fibroblasts on electrospun poly(lactic acid) scaffolds is under development and validation in our group, demonstrating sustained vsiability and inflammatory responsiveness. This scaffold platform, currently being extended to incorporate alginate-gelatin hydrogel compartments, represents the receiving system into which patient-derived epithelial and stromal cells will be introduced. Together, these preliminary findings support the feasibility of the proposed pipeline. This work lays the methodological groundwork for a patient-specific 3D co-culture system capable of modeling inter-patient variability in epithelial-stromal interactions and inflammatory signaling in IBD.

ABSTRACT #40

Topographical Modulation of Human Keratinocyte Behavior

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Skin wounds that fail to close after 3 months are defined as non-healing or chronic wounds, which represent an increasing clinical burden with no standardized or consistently effective treatments. Although keratinocytes in chronic wounds undergoing clinical management express markers associated with re-epithelialization, they exhibit misguided migration into the underlying dermis rather than across the wound bed (Tinney et al, in review). It was hypothesized that groove ridge topographies can correct the misguided migration by providing a physical substratum that promotes directed migration while maintaining a pro-healing keratinocyte phenotype. In this study, varying groove and ridge widths and depths were investigated to determine their effects on keratinocyte phenotype and migration. Topographies were fabricated from non-cytotoxic epoxy using negative impressions of topography containing master silicon wafers for in vitro experiments. Primary healthy human epidermal keratinocytes purchased from Lonza were used as a model system. Individual human keratinocytes recapitulated the normal human healing keratinocyte phenotype. Cytokeratin (CK) phenotyping of the primary keratinocytes on the groove ridge topographies demonstrated a basal, activated keratinocyte phenotype, with expression of CK14 (basal marker, Patel et al, 2006) and CK6/CK17 (activated keratinocytes, Zhang et al., 2019) across all conditions. No terminal differentiation was observed, indicated by the absence of CK10 expression (Patel et al, 2006). Proliferation occurred on all groove ridge dimensions. Keratinocytes formed integrin β4 adhesion plaques that were in contact with the substratum, as confirmed by confocal imaging. On 10 μm-deep grooves, integrin β4 plaques aligned

along groove ridge discontinuities, corresponding with significant cellular alignment to the long axis of the grooves. In contrast, shallow (1 µm) grooves induced alignment only when cells spanned multiple topographic features. All experimental topographies that induced alignment also promoted directed migration, confirmed with timelapse microscopy analysis. While groove ridge topographies stimulated directed migration, they did not alter overall migration propensity or persistence. Cells exhibited circular, oscillatory migration patterns; however, groove ridge topographies modulated this behavior in the direction of the groove long axis. Notably, 10 µm grooves and ridges with depths of 1 µm or 10 µm increased migration rates. Collectively, these findings demonstrate that 10 µm wide groove ridge topographies maintain a basal, activated, and proliferative keratinocyte phenotype while promoting directed migration. A scaffold containing these groove ridge topographies represents a rapidly translatable, clinically relevant chronic wound treatment strategy that may correct the misguided epithelial migration of chronic wounds.

ABSTRACT #41

Differential effects of extracellular matrix-derived fragments on adipocyte differentiation using a human biomimetic adipose tissue model

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Extracellular matrix (ECM) can release biologically active fragments (matricryptins) displaying functions that may differ from their parent molecules. These fragments influence important biological processes such as the terminal differentiation of stromal cells into specialized cells. Full-length Secreted Protein Acidic and Rich in Cysteine (FL-SPARC) is a matricellular protein involved in angiogenesis and reported to inhibit adipogenesis in murine cells in vitro. However, the role of SPARC113-130 peptide during human adipogenic differentiation remains unknown. We hypothesize that long-term supplementation with SPARC113-130 will not alter lipid accumulation, in contrast to FL-SPARC, during adipocyte differentiation of human adipose-derived stromal/stem cells (ASCs). ASCs from three female donors (36.3 ± 1.5 years) were treated in triplicate with adipogenic medium for 22 days using a highly

biomimetic human reconstructed adipose tissue (hrAT) model. During tissue engineering, cultures were supplemented with 250 µM ascorbic acid, SPARC113-130 (1, 5, and 10 µM), FL-SPARC (0.3, 3, and 11.8 µM). Endostatin (ES, 1.1 µM), a well characterized anti-angiogenic and anti-adipogenic matricryptin of collagen XVIII, was also evaluated. Metabolic activity and DNA content of the cultures were assessed using AlamarBlue™ and PicoGreen™ assays, respectively, and lipid accumulation using Oil Red O staining. Long-term supplementation with SPARC113-130, FL-SPARC or ES did not significantly impact the metabolic activity or DNA content in hrATs ($p>0.05$). SPARC113-130 and FL-SPARC did not have a statistically significant effect on lipid accumulation ($p>0.05$). In contrast, ES supplementation reduced lipid accumulation by 26.2 ± 0.1% ($p=0.04$) compared to the mock-treated control. Average lipid droplet size, as measured from mosaic images of all supplemented hrATs, did not differ from that of mock-treated controls ($p>0.05$). The anti-adipogenic activity of ES was previously described in monolayer cultures of murine preadipocytes (PMID: 25605807); but we report here, for the first time, its activity using a 3D human AT model. ES supplementation significantly decreased adipocyte-derived leptin and VEGF165 secretion, by 20.6 ± 0.1 and 13.8±0.4% respectively, as measured by ELISA in hrAT-conditioned media. In comparison, no significant differences were found for FL-SPARC and SPARC113-130-conditioned media ($p<0.05$). These findings establish that, under these experimental conditions and concentrations, FL-SPARC and its fragment do not modulate adipogenic differentiation of human ASCs in a biomimetic 3D AT model, while confirming the sensitivity of the system detecting ES-mediated effects. This highlights the potential of matricryptins as microenvironmental regulators of adipocyte differentiation. Future studies will evaluate the impact of these fragments on vascular network formation within the hrAT.

ABSTRACT #42

Design and testing stimulation parameters of in-vitro electrical stimulation (ES) device for SaOs-2 cells

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Electrical stimulation (ES) shows promise for enhancing bone cell growth. There is no consensus on stimulation parameters or how cells experience ES in a cell well. The purpose of this study was to characterize the electric field (EF) generated through different types of ES and electrode height in a cell well.

ES was generated using two L-shaped electrodes (platinum-iridium, PtIr – 10mm apart) in McCoy's 5A media in a single well of a 12-well plate. 22-gauge electrical wires (Cu) were inserted and glued through holes in the bottom of the well to create seven measurement electrodes in a line along the middle of the well (2.5 mm apart). We applied three waveforms at a targeted voltage [1V]: 1) constant held voltage [DC], 2) monophasic square wave [1Hz, 50% duty], and 3) biphasic square ware [1Hz, 50% duty]. PtIr electrodes were positioned at 1 or 5mm from the bottom of the well. Voltage in each of the seven Cu electrodes was measured relative to the PtIr ground electrode, and EF at each location was calculated based on distance from the ground electrode. Data were collected 10 times (1hour at 50 Hz) per parameter (3 waveform and 2 height). Average magnitude of raw voltage and calculated EF were compared using ANOVA. The EF at the locations of the electrodes were also modelled with a finite element model (ANSYS) for the constant voltage stimulation.

There were significant differences (pmonophasic>constant) for raw voltage and calculated EF. For raw voltage measurements, there was no significant difference among locations at the bottom of the well or between electrode height for any waveform type. For calculated EF values, EF was significantly different between electrode heights ($p<0.001$) and certain measurement locations, particularly Cu electrodes closest to the PtIr ground electrode ($p<0.001$).

Measured voltage exhibited uniform magnitude across the bottom of the well. The calculated EF was greatest near the ground electrode and lower at other locations. EF values more closely resembled the EF in the FEA model. These results informed the design of an in-vitro device to apply ES to SaOs-2 cells. It was important to measure and verify the stimulation that is applied to the cells to confirm results from in-vitro cell testing.

ABSTRACT #43

Mapping of extracellular matrix degeneration in human ascending thoracic aortic aneurysm using advanced MR imaging

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Objective

Thoracic aortic aneurysms (TAAs) carry a risk of catastrophic dissection. The main strategy to evaluate this risk is to measure aortic diameter. However, many patients suffer a dissection at sub-threshold diameters and strategies to evaluate aortic medial degeneration are needed. We sought to determine if the state of the extracellular matrix in TAAs could be determined by advanced magnetic resonance imaging (MRI).

Methods

Porcine and human ascending aortas were studied. The former were subjected to local enzyme microinjection and the latter harvested from TAA subjects undergoing ascending aortic replacement. Tissues were imaged ex vivo by diffusion tensor imaging (DTI) at a magnetic field of 9.4T. DTI metrics were correlated to histologically quantified elastin content, smooth muscle cell (SMC) density, and glycosaminoglycan (GAG) content. Human TAAs were also evaluated by chemical exchange saturation transfer (CEST) MRI at 9.4T and regional correlation between GAG-specific (gagCEST) signals and histologically quantified GAG content determined.

Results

Localized medial degeneration in porcine aortas was identified through tensor disorientation, disrupted diffusion tracts, and altered DTI metrics. Fractional anisotropy (FA) positively correlated with SMC and elastin content, and mean diffusivity (MD) and radial diffusivity (RD) negatively correlated with these components ($P<0.001$). Among 9 human TAAs, DTI metrics showed strong associations with regional elastin degradation, SMC loss, and GAG accumulation. Most striking were correlations with GAG accumulation, as captured by reduced FA ($R2=0.76$, $P=0.002$), increased MD ($R2=0.46$, $P=0.045$) and increased RD ($R2=0.60$, $P=0.015$). Furthermore, GAG-selective imaging with gagCEST MRI revealed heterogeneous GAG distribution within human TAAs, the ability to localize GAG hot-spots, and a positive correlation between CEST magnetization transfer ratio and histological GAG content within a given aorta ($R2=0.59$, $P<0.001$).

Conclusion

Medial degeneration in TAAs can be detected ex vivo by advanced MRI strategies. Notably, both DTI and gagCEST MRI provided a window into accumulation of GAGs, a critical determinant of aortic degeneration and risk. These findings offer prospects for personalized thoracic aortic disease risk assessment beyond measuring aortic diameter.

ABSTRACT #44

Natural history of adverse local tissue reactions around hip arthroplasty as identified by MARS MRI

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Purpose:
The clinical significance and management of adverse local soft tissue reactions (ALSTRs) following total hip arthroplasty (THA) continues to be controversial. The reported prevalence of ALTRs ranges between 1 % and 71%. The purpose of this retrospective study was to evaluate sequential MARS MRIs in patients with painful THA to establish management criteria.

Methodology:
Seventy-eight (78) hips in 78 patients were identified to have had sequential MARS MRIs for pain and were positive for ALTR. The group consisted of 31 metal-metal THA, 16 metal-cross-linked polyethylene THA, three ceramic on metal THA, four ceramic on ceramic, and 24 metal-metal resurfacing THA. A standardized form was used to evaluate the MRIs based on the 1) presence of adverse soft tissue reaction; 2) thickness of wall mass; 3) intensity on T1 and T2 weighted images; 4) contents of mass (fluid, solid or complex); 5) location relative to joint. Changes in the size of the lesions were evaluated between initial and final scans. Revisions were also documented.

Results:
MARS MRI evaluation revealed that 41 hips (51.9%) had cystic fluid-filled lesions, seven hips (8.9%) had solid masses and 31 hips (39. 2%) presented with complex mixed lesions. The mean follow up is 6.6 years (range: 4.68-286.8 months). The mean interval between the first and last MRI was 9.48 years (range: 2.52-402.12 months). The mean initial volume of the ALTRs was 302.85ml, increasing slightly to 309.81ml at the last follow-up. One outlier lesion that measured 808 ml and consisted of a large anterior fluid mass was completely resolved on follow-up MRI nine months later; this was presumed to represent a psoas bursal lesion. Nineteen cases (24.1%) increased in size, 28 cases (35.4%) decreased in size and the remaining cases showed no significant change on sequential MRIs. 13 cases required revision surgery; however, only five of these were revised for an initially large lesion or progressively increasing in size. No significant association was found between lesion classification and its progression.

Conclusion:
Serial MARS MRI imaging is essential for evaluating patients with painful hip arthroplasty in addition to metal ion level analysis. Larger lesions that increase in size over time tend to be more aggressive and can lead to more soft tissue and bony destruction. Stable ALTR should be followed and can be treated conservatively. Cystic fluid filled masses can resolve or decrease spontaneously.

ABSTRACT #45

Age-dependent fibroblast programs distinguish regenerative and fibrotic tendon healing

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Adult tendon injuries commonly heal through fibrotic scar formation rather than true tissue regeneration, leading to compromised mechanical integrity and incomplete functional recovery. In contrast, neonatal tissues exhibit an enhanced capacity for regenerative healing, suggesting that developmental repair mechanisms may be active early in life but become diminished in adulthood. However, the cellular populations and molecular programs that govern regenerative versus fibrotic tendon repair remain poorly understood. Fibroblasts are the primary stromal cells responsible for extracellular matrix production and remodeling during connective tissue repair. Increasing evidence suggests that fibroblasts exist in heterogeneous transcriptional states that can differentially regulate tissue organization, matrix remodeling, and scar formation. Understanding how these fibroblast programs vary with age may provide insight into the regulation of regenerative versus fibrotic healing. To investigate age-dependent differences in tendon repair, we compared neonatal and adult healing in a mouse Achilles tendon injury model. Genetic lineage tracing of fibroblast populations was used to assess contributions to the reparative tissue. Functional recovery was assessed using gait analysis, and spatial transcriptomic profiling was performed to characterize gene expression programs within the healing tissue microenvironment. Histological analysis across multiple timepoints demonstrated the contribution of the labeled stromal cell populations in the reparative tissue. Neonatal tissues exhibited gene expression patterns associated with tenogenic and extracellular matrix remodeling programs, whereas adult tissues displayed patterns consistent with more fibrotic cellular states. Together, these findings provide insight into differences in tendon healing across developmental stages.

ABSTRACT #46

A Bioprinted Bone-Mimetic Co-Culture Platform to Study Tumor Organization and Response to Cold Plasma Treatment

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Bone metastasis remains a major clinical challenge, particularly in the spine, where tumor-bone interactions are difficult to recapitulate using conventional in vitro models. Existing 2D systems and simple hydrogels fail to capture the structural, mechanical, and dynamic features of the bone microenvironment, limiting the study of tumor progression and treatment response.

This study aims to develop and characterize a 3D bioprinted tumor-stroma model based on an alginate (3%) and gelatin (7%) hydrogel (A3G7) supplemented with hydroxyapatite, designed to mimic bone tissue properties. The bioink was optimized for extrusion-based bioprinting and evaluated across a range of cell densities (1-10 × 10^6 cells/mL).

Rheological characterization demonstrated pronounced shear-thinning behavior, with viscosity decreasing under increasing shear rate. Increasing cell density modestly reduced low-shear viscosity; however, viscosity values converged at printing-relevant shear rates (~100-1000 s^{-1}), indicating preserved extrusion behavior across conditions. Frequency sweep analysis revealed increasing storage (G') and loss (G") moduli with frequency, consistent with a physically crosslinked and dynamically rearranging network. Temperature-dependent measurements showed a gel-sol transition, with the crossover temperature shifting linearly toward lower values with increasing cell density ($R^2 \approx 0.95$), indicating cell-induced modulation and partial destabilization of network formation. Incorporation of hydroxyapatite increased bioink stiffness and enabled tuning toward bone-mimetic properties.

Thixotropic recovery experiments demonstrated rapid viscosity reduction under shear followed by efficient structural recovery, confirming self-healing behavior and extrusion safety. Printability was further optimized using a cooled printbed (15 °C), which improved filament stabilization and shape fidelity compared to room temperature printing, consistent with gelatin thermogelation prior to alginate crosslinking.

Biological validation was performed using cocultures of MDA-MB-231 breast cancer cells and human bone marrow-derived mesenchymal stem cells (hbmMSCs). Repeated treatments applied weekly resulted in a dose-dependent decrease in metabolic activity, with sustained and cumulative suppression over time, particularly at higher treatment intensities, despite media renewal. At Day 14, cell migration remained limited; however, control constructs exhibited clear multicellular tumor

spheroid (MCTS) formation, whereas treated constructs showed reduced spheroid development, indicating modulation of early tumor organization and growth.
Overall, this A3G7-hydroxyapatite bioprinted model provides a reproducible and tunable platform integrating mechanical relevance and sustained biological response. This system enables the study of tumor organization and treatment effects in a physiologically relevant bone-like microenvironment and supports future investigations of tumor progression and therapeutic response.

ABSTRACT #47

Localized Cold Atmospheric Plasma Treatment Following Tumor Resection Improves Outcomes in a Rat Model of Breast Cancer Bone Metastasis

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Bone, particularly the spine, is a frequent site of metastasis for breast, lung, and prostate cancers and often requires aggressive treatments such as chemotherapy and invasive surgery. Complete surgical resection frequently extends into healthy tissue to reduce residual malignant cells, leaving large defects that necessitate reconstruction. Cold atmospheric plasma (CAP) offers a non-invasive approach by locally delivering reactive oxygen and nitrogen species (RONS) and could be applied directly to the tumor resection wound bed prior to reconstruction. While CAP has demonstrated promising anticancer effects, plasma-tissue interaction mechanisms and optimal treatment parameters remain under investigation.
This study evaluates CAP as a localized adjunct therapy following tumor resection in a model of breast cancer bone metastasis. An orthotopic xenograft model was established by implanting human MDA-MB-231 breast cancer cells embedded in an alginate/gelatin hydrogel (A3G7) into the caudal vertebrae of SRG (Sprague Dawley Rag2 knockout) immunodeficient rats. After 6 weeks, tumor growth was verified by bioluminescence imaging, and animals underwent surgical resection followed by CAP treatment (3 or 5 minutes). Outcomes were compared with resection alone and resection combined with doxorubicin. Tumor progression was monitored using bioluminescence imaging (Newton FT500) prior to surgery and at 4 and 10 weeks post-treatment.
Micro-CT analysis was performed to assess bone remodeling at the implantation site, with histological analyses ongoing. In vivo, consistent tumor formation in the caudal vertebrae validated the model. Survival at 10 weeks post-treatment reached 100% in both the doxorubicin and 5-minute CAP groups,

compared with 60% in controls and 50% in the 3-minute CAP group. Micro-CT analysis indicated enhanced bone regeneration (BV/TV) in treated groups compared to controls, suggesting a beneficial effect of CAP on both tumor control and bone healing.
Supporting in vitro studies using a bioprinted tumor-stroma model (A1G7 hydrogels containing MDA-MB-231 cells and human bone marrow-derived mesenchymal stem cells) demonstrated dose-dependent and selective CAP-induced cytotoxicity, primarily driven by oxidative stress mechanisms.
Together, these results demonstrate that localized CAP treatment following tumor resection enhances outcomes in a clinically relevant model of spinal metastasis. This work supports the development of CAP as a minimally invasive, localized adjunct therapy and provides a foundation for optimizing plasma-based treatment strategies for bone metastases.

ABSTRACT #48

Modulating Cold Atmospheric Plasma via Gas Composition: Differential Responses in Breast Cancer Cells and Osteoblasts

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Bone metastasis is a frequent and clinically challenging outcome of advanced breast cancer, particularly in the spine, where current treatments rely on systemic therapies and invasive surgery that can damage surrounding healthy tissue. There remains a need for localized approaches capable of selectively targeting cancer cells while preserving the bone microenvironment. Cold atmospheric plasma (CAP), which generates reactive oxygen and nitrogen species (RONS), has emerged as a promising non-invasive strategy. However, the influence of plasma gas composition on treatment response and selectivity remains insufficiently understood.
In this study, we modeled breast cancer heterogeneity using three human breast cancer cell lines representing distinct subtypes – hormone receptor-positive (MCF-7), triple-negative (MDA-MB-231), and HER2-positive (MDA-MB-453) alongside primary human osteoblasts to represent the bone microenvironment. Cells were exposed to CAP generated using argon supplemented with either nitrogen ($Ar+N_2$) or oxygen ($Ar+O_2$) for increasing treatment durations. Metabolic activity was assessed at Day 1 and Day 3, and dose-response curves were used to determine IC_{50} values.
Both plasma types induced a treatment time-dependent decrease in metabolic activity across all cell types. However, sensitivity varied depending on both plasma composition and cell type. MDA-MB-453

cells exhibited the highest sensitivity, with rapid loss of viability at shorter treatment durations, whereas MDA-MB-231 and MCF-7 cells showed more gradual responses. Comparisons between plasma conditions revealed cell-type-dependent response profiles, with variations in sensitivity observed across gas compositions. While both nitrogen- and oxygen-based plasma induced cytotoxic effects, the magnitude of response differed between cell lines and experimental conditions.
Analysis of IC_{50} values confirmed these trends and demonstrated a decrease in IC_{50} between Day 1 and Day 3 across conditions, indicating a sustained and progressive treatment effect over time. Osteoblasts exhibited reduced sensitivity compared to cancer cells at intermediate treatment durations, suggesting a degree of treatment selectivity depending on plasma composition and exposure time.
Overall, these results demonstrate that plasma gas composition is a critical parameter influencing cytotoxicity, cell-type-specific response, and temporal dynamics. This work highlights the potential to tailor CAP treatments by modulating gas composition to optimize anticancer efficacy while preserving healthy tissue across different cancer subtypes.

ABSTRACT #49

Evaluation of eggshell-based constructs for bone regeneration in rat calvaria critical-size defect model

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Introduction: Bone is a specialized mineralized connective tissue whose regeneration depends on coordinated extracellular matrix (ECM) deposition, mineralization, and remodeling. Development of bioactive materials that support these processes remains a key challenge in skeletal repair. This study evaluates the ability of eggshell (ES) and nanotextured eggshell (NTES)-based hydrogel scaffolds to support connective tissue regeneration in the calvaria critical-size defect (CSD) model.

Method: Scaffolds (fabricated from alginate, chitosan and glutaraldehyde) with or without incorporation of ES or NTES particles were processed via freeze-casting and evaluated in vitro through fluid swelling test, porosity test and pore-size measurement. In vivo assessment was conducted by implanting scaffolds into bilateral critical -size defects (6 mm) in 10-11 week old Wistar rats. One defect received

the scaffold implant, while the contralateral defect served as an empty control. At 12 weeks post-implantation, regeneration was assessed by micro-CT (bone volume, spatial tissue distribution) and histological analyses to evaluate ECM deposition, scaffold remodeling and host integration.

Results: In vitro, ES- and NTES-containing scaffolds exhibited increased porosity, enhanced fluid retention, and improved structural stability. Average pore diameters were 96.3 µm (blank), 97.6 µm (ES), and 113.6 µm (NTES, $p < 0.05$), and porosity values were 57% (blank), 74% (ES), and 82% (NTES, ****$p < 0.0001$). Crystal violet staining showed increased surface protein exposure in NTES versus ES scaffolds. Particle distribution within 1 mm thick scaffold discs was not significantly different between ES and NTES. After 12 weeks post-implantation, micro-CT analysis revealed average defect filling of 19.7% (Bio-Oss, n=4), 16.3% (NTES scaffolds, n=6), 14.8% (ES scaffold, n=1), 11.4% (blank scaffolds, n=4), 12.1% (NTES particles, n=2), and 5.8% (empty defects, n=20). H&E staining demonstrated no inflammation or immune infiltration. Empty defects showed thinner fibrous tissue and reduced osteoblast/fibroblast presence, whereas implant-treated defects had ~3-fold increased osteoblast numbers, indicating enhanced cellular activity. Ongoing micro-CT, histology, and immunohistochemistry (OPN, OC, ALP) analyses will further assess bone volume growth and the presence of bone remodeling cells and proteins.

Conclusion: ES-based scaffolds displayed improved porosity, pore size, with NTES scaffolds showing the highest values. In vivo, Bio-Oss and NTES scaffolds achieved the most defect filling, and all implants outperformed empty controls. Histology confirmed no inflammation and a ~3-fold increase in osteoblast numbers, indicating enhanced bone-forming activity. Overall, scaffold composition and structure strongly influence bone regeneration, and eggshell-derived biomineral particles serve as a sustainable bioactive cue for promoting mineralized tissue repair in non-load-bearing bone defects.

ABSTRACT #50

The 'Soft Skills' of Sall1-Engineered Mesenchymal Stromal Cells Prevent Hypertrophic Scarring

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Rationale: The severe scarring of large burn wounds causes significant morbidity and healthcare burden, which can be mitigated by transplanting mesenchymal stromal cells (MSCs). However, therapeutic efficacy is compromised by culture expansion on stiff culture surfaces, which induce MSC activation

into fibrogenic myofibroblasts. To identify myofibroblast preventive factors, we performed RNA-seq and ATAC-seq on MSCs cultured on soft and stiff surfaces. We identified the spalt-like transcription factor 1 (Sall1) gene to be highly upregulated and promoter-accessible in soft-grown MSCs. While Sall1 is a known transcriptional repressor maintaining stemness, its role in mechanically induced MSC-to-myofibroblast activation remains unexplored.

Hypothesis: Sall1 is a mechanoresponsive transcriptional suppressor of MSC-to-myofibroblast activation.

Objective: To elucidate how Sall1 suppresses myofibroblast activation and assess whether Sall1-engineered MSCs reduce hypertrophic scarring in a pre-clinical model.

Methods: Human umbilical cord-derived MSCs were explanted onto skin-soft (1 kPa) and scar-stiff (100 kPa) culture surfaces for 6 weeks/passages. Sall1 function was assessed by siRNA knockdown in soft- and overexpression in stiff-cultured MSCs. Myofibroblast activation (collagen I, α-SMA, fibronectin) and proliferation (ki67) were measured by immunoblotting and immunostaining. To identify signalling downstream of Sall1, RNA-seq was performed with: (i) MSCs on soft and stiff surfaces; (ii) Soft-grown MSCs after Sall1 knockdown; (iii) Sall1 overexpressing stiff-grown MSCs. Therapeutic performance was assessed by delivering Sall1-overexpressing MSCs in fibrin to splinted (hypertrophic) rat skin full-thickness wounds. Wound contraction, myofibroblast activation, collagen deposition, and vascularization (desmin, CD31) were measured and quantified 8 days post-wounding and compared to wounds receiving stiff-grown control MSCs.

Results: Sall1 expression was significantly higher in soft- than stiff-cultured MSCs. Sall1 knockdown reduced proliferation and increased myofibroblast activation of soft-grown MSCs. We identified the transcription factor WT1 as strongly downregulated upon Sall1 knockdown. Like Sall1, knockdown of WT1 enhanced MSC-to-myofibroblast activation. Conversely, Sall1 overexpression induced WT1 expression, enhanced proliferation, and reduced stress fibers in MSCs on stiff surfaces. Sall1 overexpression also resulted in higher expression of ANGPT1/2, ANGPTL4, HGF, PGF, and IGF1, and lower expression of SPARC and SPP1. When transplanted into splinted rat wounds, Sall1-overexpressing MSCs improved healing outcomes by enhancing tissue granulation and angiogenesis. Simultaneously, they reduced wound tension, myofibroblast activation, and collagen deposition. Our data support a VEGF-independent pro-angiogenic and anti-fibrotic paracrine profile of Sall-overexpressing MSCs.

Conclusion: We identify a Sall1-WT1 mechanosensitive transcriptional axis that governs MSC proliferation, myofibroblast activation, and production of pro-angiogenic factors. Sall1 overexpression programs pro-reparative MSCs for an enhanced therapeutic strategy to prevent hypertrophic scars.

ABSTRACT #51

The effects of P2 receptor signalling on gene expression, tissue remodelling, and maturation in murine tenocyte culture

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Rationale: Tendons are collagen rich connective tissues that connect muscles to bones, allowing movement through muscle contractions. Tenocytes, the cells in tendons, respond to mechanical loads by converting them into biological signals via mechanotransduction. Tenocytes release nucleotides such as ATP and UTP in response to mechanical stress, and our lab has shown that these extracellular nucleotides activate P2Y and P2X receptors on these cells, leading to calcium signalling and changes in gene expression, including inflammatory and tendon-specific genes. While these data establish that tenocytes respond to extracellular nucleotides, the effects of ATP breakdown products, specifically ADP and adenosine generated by cell-surface ectonucleotidases, on tenocyte signalling remain unclear. Moreover, whether any P2 receptor ligand or adenosine (via P1 receptors) influence tenocyte differentiation, maturation, and function has not yet been explored.
Hypothesis: We hypothesized that different P2 and P1 agonists drive both unique and overlapping transcriptional programs related to mechanotransduction and tendon homeostasis. We also hypothesized that these ligands promote tenocyte maturation in a 3D culture model compared to controls.
Methods and Results:
To examine the immediate early transcriptional responses to P2 ligands or their breakdown products (namely adenosine), we isolated primary tenocytes from male 1-month-old male wild-type mice and treated 2D cultures with 100μM ATP, ADP, UTP, and adenosine. RNA was then isolated at 1 h and 12 h post-treatment and subjected to bulk RNA-sequencing (RNA-seq). Interestingly, adenosine elicited the most pronounced transcriptional response at 1 h, with 162 differentially expressed genes (DEGs), followed by ATP, ADP and UTP. Gene set enrichment analysis revealed that adenosine uniquely enriched for TGF-β signalling, whereas ATP and ADP enriched for TNFα signalling via NF-kB.
Ongoing work is examining the effects of P2 and P1 receptor agonists on tenocyte maturation and function using a 3D culture system. Specifically, tenocytes were isolated from 1-month-old wild-type mice, combined with a type I collagen matrix, and suspended between two pins to generate a tendon-like construct. These 3D constructs are being cultured in the presence or absence of ATP, ADP, adenosine, or UTP, with gene expression and tissue architecture assessed by qPCR and histological

analysis.
Conclusions: This work will establish the importance of P2 and P1 receptor signalling in tenocyte cell signalling, maturation, and function. Understanding the cellular processes that regulate tendon mechanobiology is critical to recapitulating these conditions for tissue engineering applications, where regulation of tenocyte identity and tendon ECM remains a significant hurdle yet to be overcome.

ABSTRACT #52

Injury-Induced LTBP2 Promotes Adverse Remote Myocardial Remodeling After Myocardial Infarction

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Myocardial infarction (MI) triggers ventricular remodeling that extends well beyond the ischemic infarct scar into the remote, viable myocardium, where diffuse extracellular matrix (ECM) remodeling, fibrosis, and progressive stiffening contribute importantly to heart failure development. Although infarct expansion has been studied extensively, the molecular signals that drive adverse remodeling in remote myocardium remain incompletely defined, despite growing evidence that this compartment is a major determinant of long-term function and outcome. We therefore investigated whether latent transforming growth factor-β binding protein 2 (LTBP2), an injury-induced microfibril-associated ECM glycoprotein, contributes to maladaptive remote remodeling after MI. Interrogation of post-MI cardiac fibroblast proteomic datasets identified LTBP2 as a strongly induced ECM-associated and secreted candidate, and combined single-cell RNA-sequencing and spatial transcriptomic analyses localized Ltbp2 predominantly to injury-activated fibroblasts distributed across both infarct and remote myocardium early after injury. Immunofluorescence further demonstrated prominent LTBP2 deposition within Fibrillin-1-rich matrix structures in vivo. Functionally, global deletion of Ltbp2 significantly improved post-MI survival without altering infarct size or early injury responses, indicating that the benefit was not attributable to reduced initial damage. By 28 days post-MI, echocardiography and speckle-tracking strain imaging showed preservation of global systolic performance in Ltbp2-deficient hearts, including

improved ejection fraction and fractional area change, together with marked protection of regional mechanics in the remote basal and mid-ventricular segments. Ltbp2 deficiency also enhanced diastolic radial and longitudinal strain-rate dynamics throughout remote ventricular regions, consistent with improved relaxation and preserved myocardial compliance. Structurally, knockout hearts exhibited striking attenuation of remote interstitial fibrosis by picrosirius red staining and reduced tissue stiffness in both infarct and remote regions by atomic force microscopy, with especially strong preservation of compliance in remote myocardium. Unbiased proteomic profiling of remote left ventricular tissue at 28 days post-MI further revealed a distinct genotype-dependent remodeling signature, with wild-type hearts showing enrichment of fibrillar collagens, matricellular and microfibril-associated proteins, ECM organization programs, and TGF-β-responsive biological processes that were markedly blunted in Ltbp2-deficient hearts. Complementary in vitro fibroblast studies suggest that modulation of TGF-β bioavailability may be one indirect mechanism by which LTBP2 promotes a profibrotic phenotype and sustains maladaptive signaling within the injured heart. Collectively, these findings identify LTBP2 as an injury-induced, fibroblast-derived regulator of remote myocardial remodeling and support its potential as a novel therapeutic target for limiting long-term tissue remodeling and fibrotic responses after MI.

ABSTRACT #53

Calcification of In Vitro-Grown Cartilage Modulated by Static Mechanical Loading

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Osteoarthritis is the most common joint disease worldwide, affecting more than four million people in Canada alone. This disease causes joint pain, limitations to range of motion and reduced mobility for those affected and there is currently no cure or disease-modifying therapeutic that can halt or revert its progression. During the early stages of osteoarthritis, the zone of calcified cartilage between hyaline articular cartilage and the subchondral plate thickens. However, the causes for this change remain poorly understood. In articular cartilage, mechanical loading has been associated with maintaining tissue homeostasis or disease progression, with a range of static and dynamic loading regimens having been shown to elicit either catabolic or anabolic responses. In this work, an in vitro-grown cartilage tissue model that incorporates a zone of calcified cartilage was used to investigate whether static mechanical compression can modulate the extent of mineralization by chondrocytes.
Deep zone cartilage was aseptically harvested from the metacarpophalangeal joint of 18-30 month old

cows within 24 hours of death. Excised tissues were enzymatically digested to yield a suspension of deep zone chondrocytes, which were seeded at high density (1.67×10^6 cells / cm^2) onto tissue culture membrane inserts. The constructs were cultured in mineralization medium for 4 weeks and subjected to static compression for the last 3 weeks. Compression was achieved by placing stainless steel weights of 17.0 mN or 32.2 mN atop the growing tissues for 30 minutes five times per week. Unloaded tissues served as controls. At the end of the culture period, inserts were harvested, weighed, digested, and assayed for mineral (calcium and phosphate), ECM (sulfated GAG), and DNA content. When compared to controls, the mineral quantity per mg of tissue was decreased in stimulated tissues. A decrease in DNA content in the cultures in response to static compression was also observed, suggesting that the mechanical cue either caused reduced cell proliferation or increased cell death. The quantity of sulfated GAG deposited in the tissue constructs per cell, however was not seen to change significantly, suggesting that the specific cues administered did not result in an overall anabolic or catabolic response. During culture, the stimulated tissues were also observed to contain more uniform mineral deposition, compared with the larger nodules of more dispersed mineral in controls. This work suggests that mechanical cues can modulate mineralization processes at the zone of calcified cartilage; however, the mechanisms involved have yet to be identified.

ABSTRACT #54

Diabetes induces thinning and stiffening of native skin in a streptozotocin-induced rat model: structural and mechanical characterization

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In preclinical models of diabetic wound healing, metabolic parameters such as blood glucose and body weight are commonly monitored, yet the baseline structural and mechanical state of the skin itself is rarely characterized. A better understanding of how diabetes alters skin properties is essential to interpret tissue repair responses under diabetic conditions.
This study aimed to quantify structural and mechanical alterations of skin from streptozotocin-induced diabetic rats. It was hypothesized that diabetic skin would be thinner and less elastic.
Twenty-four young male Sprague Dawley rats received a high dose of streptozotocin (75 mg/kg), followed by additional doses (40 mg/kg) when required to induce hyperglycemia (diabetes defined when blood glucose ≥16.7 mmol/L). At euthanasia, intact dorsal skin samples of 1-1.5 cm2 were collected and

frozen at −80°C. After overnight thawing at 4°C, mechanical properties were assessed by microindentation to determine total skin thickness and the apparent instantaneous elasticity modulus (IM) according to the Hertz model. Then, paraffin tissue sections were stained with Masson's trichrome and used to measure living epidermis and dermal thickness.
Diabetes was induced in 21 of 24 rats (87.5%, n=21) while others remained non-diabetic (n=3). Hyperglycemia remained stable throughout the experiment, from post-induction levels (22.8 ± 4.8 mmol/L vs 6.1 ± 0.8 mmol/L, $p < 0.0001$) until the last measurement before euthanasia (23.7 ± 3.2 mmol/L vs 4.2 ± 0.3 mmol/L, $p < 0.0001$). Diabetic animals exhibited lower body weight at euthanasia (307 ± 41 g vs 400 ± 43 g, $p = 0.0013$), accompanied by classical clinical signs such as polyuria. Histological analyses revealed a significant reduction in living epidermal thickness (22 ± 6 µm vs 37 ± 2 µm, $p = 0.0006$) and dermal thickness (1.28 ± 0.31 mm vs 2.01 ± 0.27 mm, $p = 0.0009$) for diabetic skin. Similarly, total skin thickness measured using microindentation was reduced (1.89 ± 0.42 mm vs 2.74 ± 0.27 mm, $p = 0.0029$). Diabetic skin was significantly stiffer, as reflected by a higher instantaneous modulus (11.3 ± 2.6 kPa vs 7.6 ± 1.9 kPa, $p = 0.0274$).
Together, these complementary structural and mechanical analyses reveal that diabetes profoundly alters native skin architecture and biomechanics, leading to thinner and stiffer tissue. Microindentation provides a non-destructive method to quantify theses biomechanical changes, while histology identifies structural alteration underlying them. Characterizing these baseline skin alterations is essential for interpreting wound healing responses and evaluating therapeutic strategies in diabetic models.

ABSTRACT #55

Multiplexed Multi-Material 3D Bioprinted Platform to Model Mechanical Heterogeneity for Disease Microenvironments.

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Purpose: Mechanical heterogeneity is a hallmark of many diseases, including fibrosis across organs and tumors, where fibrotic and healthy regions coexist. Idiopathic Pulmonary Fibrosis (IPF) is one such example, characterized by spatially heterogeneous tissue stiffening in the lung. This mechanical heterogeneity plays a critical role in fibroblast activation and disease progression. However, current in vitro models frequently rely on rigid substrates or single-stiffness models, failing in capturing both the

spatial heterogeneity of fibrotic tissue and the independent contributions of stiffness and protein content. The objective of this project is to engineer a multiplexed, multi-material 3D bioprinted platform that mimics mechanical heterogeneity, and compare against conventional single-material models for mechanobiology studies and antifibrotic drug testing.

Methods: TissuGel inks (Tessella Biosciences) were used to match the mechanical properties of healthy and fibrotic lung tissue. Rheological characterization was used to measure storage and loss modulus and evaluate similarity to native healthy and IPF tissues. Using multi-material 3D bioprinting, heterogeneous constructs with defined soft and stiff regions were fabricated within 3D-printed multi-well plates, alongside homogeneous single-material controls. Constructs were crosslinked with 405 nm light and UV sterilized prior to seeding primary human lung fibroblasts. Cellular responses were assessed via immunofluorescence staining and western blotting for a-smooth muscle actin (a-SMA) and F-actin, with and without TGF-β1 stimulation conditions (1 and 3 ng/mL).

Results: Crosslinked inks exhibited mechanical properties matching healthy and IPF lung tissue. The construct stiffness influenced fibroblasts morphology and activation, with increased a-SMA expression and a spindle-shaped morphology observed in stiffer conditions, while softer microenvironments promoted a round cell phenotype. In heterogeneous multi-material constructs, fibroblasts exhibited spatially different activation patterns, with localized increases in a-SMA expression corresponding to the stiffer regions. In contrast, homogeneous single-material models showed different activation trends, highlighting their limited ability to capture the complexity of fibrotic microenvironments. TGF-β1 stimulation further enhanced a-SMA expression across all conditions, consistent with fibrotic phenotype.

Conclusion: This project introduces a reproducible and scalable 3D bioprinted platform that captures mechanical heterogeneity in a high-throughput format. By directly comparing multi-material and single-material systems, this work demonstrates the importance of incorporating tissue heterogeneity in vitro and provides a more physiologically relevant platform for tissue engineering research and antifibrotic drug screening.

ABSTRACT #56

The role of TRPV4 in age-associated intervertebral disc degeneration is mediated by biological sex

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Biological sex and cellular mechanoreceptors are important regulators in musculoskeletal diseases such as osteoarthritis and intervertebral disc (IVD) degeneration, but it is unknown how biological sex and sex hormone-associated mechanisms influence cellular mechanoresponses.

Previous work from our lab demonstrated that the calcium-permeable channel TRPV4 mediates mechanotransduction in the IVD. TRPV4 is ubiquitously expressed throughout all IVD compartments despite differences in their mechanical environment; the annulus fibrosus (AF) resists tensile strain while the nucleus pulposus (NP) resists compressive strain associated with normal spine movements. In mouse models where TRPV4 was removed from the NP and the majority of the AF (Col2CRE;TRPV4f/f), male mice demonstrated increased age-associated IVD degeneration compared to WT mice (24 months of age), changes not observed in female mice. In addition, TRPV4 gene expression was significantly increased in the IVDs of female compared to male WT mice at 24 months of age.

To further understand the role of TRPV4 in the IVD and its association with sex-dependent differences in IVD degeneration, TRPV4 was specifically deleted from only the NP using a novel mouse strain (NotoCRE;TRPV4f/f). Age associated degeneration was assessed by histopathology, gene expression, and tissue biomechanics. While no differences in histopathological measures of degeneration were detected at 18 months of age, NP-specific loss of TRPV4 protected from IVD degeneration in female mice compared to WT controls at 22 months of age. In contrast, male mice showed no difference. Expression of IVD extracellular matrix and matrix remodeling genes at 22 months of age were not changed by NP-specific loss of TRPV4. Mechanical testing of individual lumbar motion segments demonstrated a significant reduction in tensile stiffness in lower lumbar IVD from NotoCRE;TRPV4f/f female mice compared to age-mated WT at 22 months of age. To investigate potential molecular mechanisms underlying these sex-specific effects, we quantified the expression of sex hormone receptors. In WT mice, differences in sex hormone receptor expression between males and females were age-dependent (assessed at 12, 18, and 22 months of age).

Our results suggest that in both the AF and NP of the IVD, TRPV4 plays important roles in modulating age-associated degeneration that are sex-dependent. In additional, sex-dependent differences in TRPV4 and sex hormone receptor gene expression were dependent on age. Future studies will examine mechanistic associations between TRPV4 function and sex hormone signaling. A better understanding of sex-dependent mechanisms in musculoskeletal pathophysiology may inform more tailored therapeutic strategies to support different patient populations.

ABSTRACT #57

Integrated fate mapping and CITE-seq identify distinct temporal and transcriptional profiles of tissue-resident macrophages during experimental osteoarthritis

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Introduction: Osteoarthritis (OA) is the most prevalent form of arthritis and a leading cause of disability worldwide, yet effective disease-modifying therapies remain lacking. Synovitis is an early and predictive feature of knee OA (KOA) and is strongly associated with worsening clinical outcomes. Monocytes and macrophages are central drivers of synovial inflammation; however, their heterogeneity and temporal dynamics within the joint remain poorly defined. Conflicting findings from macrophage depletion studies, where transient depletion may be beneficial but global ablation exacerbates disease, highlight the need to better understand subset-specific macrophage functions. Methods: To investigate macrophage dynamics during OA progression, we combined fate mapping with high-dimensional single-cell analysis. Tamoxifen-inducible Cx3CR1CreER:Rosa26tdTomato mice were used to label macrophages prior to induction of post-traumatic OA via destabilization of the medial meniscus (DMM). Joints were collected at multiple time points to assess macrophage turnover, retention, and proliferation using flow cytometry and immunofluorescence, with EdU incorporation used to measure proliferation. In parallel, CITE-seq was performed on joint-derived immune cells to simultaneously profile transcriptomes and surface protein expression at single-cell resolution. Data were analyzed using Seurat to identify macrophage subpopulations and transcriptional states. Results: Fate mapping revealed distinct dynamics between monocyte-derived macrophages (MDMs) and tissue-resident macrophages (TRMs). MDMs exhibited rapid turnover, consistent with continuous recruitment from circulating monocytes during OA progression. In contrast, TRMs persisted as a stable population largely independent of monocyte input. Although TRMs retained proliferative capacity, their overall numbers did not expand following injury, suggesting maintenance through balanced proliferation and loss. Single-cell analysis identified multiple macrophage subclusters, including two transcriptionally distinct TRM populations with divergent responses to OA. One subset displayed a pro-inflammatory, metabolically stressed phenotype with reduced expression of homeostatic and regulatory genes, while the second subset maintained a reparative and immunoregulatory program. MDM populations exhibited gene signatures consistent with recent recruitment, including pathways related to cytoskeletal remodeling, migration, and inflammatory activation. Conclusion: These findings demonstrate that macrophage

responses in OA are highly heterogeneous and shaped by both cellular origin and functional state. The identification of distinct TRM subsets with opposing phenotypes provides a mechanistic basis for previously conflicting macrophage-targeting studies. Our results support the need for selective, subset-specific immunomodulatory strategies to effectively target macrophage-driven pathology in OA.

ABSTRACT #58

A Histological assessment of irradiated murine skin: effects of bioactive dressings engineered from adipose-derived mesenchymal cells

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Radiotherapy is used to treat solid tumours, but large doses of ionizing radiation passing through the skin can cause lesions (radiodermatitis). These lesions range from erythema to severe ulcers, significantly affecting patients' quality of life. Current treatments provide temporary pain relief but aren't curative. To address this clinical need, a biological dressing produced by tissue engineering has been developed using adipose-derived stem/stromal cells, which help to accelerate skin healing through the secretion of soluble bioactive factors. In our previous work, we showed that applying these cellularised dressings to wounds created in irradiated murine skin significantly improved global wound closure, reaching 77.0 ± 15.7% compared with 61.3 ± 11.0% in untreated irradiated skin. We hypothesize that repeated topical application of the dressings not only promotes faster closure but also improves the quality of the healed tissues.

The dressings were produced using the self-assembly method using a serum-free medium to ensure better clinical compatibility. Full-thickness excisional wounds were created on both sides of the dorsal skin of mice (n=12), with the left side previously exposed to a dose of 45 Grays (1 cm x 1 cm). The biological dressings were applied every seven days to provide optimal bioactive factor delivery and match clinical feasibility. At day 35, the end of treatment, scar tissues were harvested for histological and immunohistochemical analyses. In parallel, media conditioned by the dressings for 72 hours were

collected to quantify the secretion of pro-angiogenic factors using enzyme-linked immunosorbent assays (ELISA).

Histological assessment using a defined scoring system revealed improved healing outcomes in irradiated skin treated with the dressings. Untreated irradiated tissues had a low healing score of 2/12, whereas treated irradiated wounds reached a score of 6/12, approaching the quality observed in non-irradiated skin (9/12). Treatment improved the architecture of the neoepidermis and promoted the formation of a more uniform neodermis with better extracellular matrix organization. A 2.3-fold increase in microvascular density was measured in treated irradiated granulation tissue compared with untreated tissue by quantification of anti-CD31 labelling. These structural improvements correlated with an elevated secretion of major pro-angiogenic mediators in conditioned media, including VEGF, Ang-1, PAI-1, and HGF, which are known to stimulate vascular network formation and tissue remodelling.

Our preliminary results indicate that the application of cellularised dressings seemed to improve the quality of cutaneous wound healing in irradiated skin. This improvement could be attributed in part to the secretion of pro-angiogenic factors that stimulate angiogenesis.

ABSTRACT #59

Sex differences in radiation-induced skin lesions: a preliminary assessment

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Radiotherapy is a treatment for many localized cancers. Radiation dermatitis lesions are a common side effect developing over many weeks after exposure. Using our murine model, we hypothesize that sex influences the kinetic and severity of the lesions. In addition, we explored the impact of biological dressings applied in a preventive fashion, starting three days after irradiation, to assess their effects on the development of radiodermatitis. These dressings are produced using a self-assembly method in a

xenogen-free medium using Adipose-derived Stromal/Stem Cells (ASCs). ASCs exhibit a pro-healing secretome, making them promising for cell therapy. Previously, our team has shown the potential of ASC-based dressings to significantly improve global closure of excisional wounds created in irradiated skin.

Male and female mice received 45 grays of ionizing radiation to the left side of the dorsal skin (1 cm^2). Radiodermatitis development was monitored using photos taken over 28 days. After irradiation, female mice (n=2) received the ASC dressings which were changed weekly, and compared to untreated animals (n=4, males and females). At 28 days post-irradiation, 8 mm full-thickness biopsies were taken on both sides of the dorsal skin and analyzed following Masson's trichrome staining and immunolabeling.

The area of erythema developing post-irradiation was measured on macroscopic images. In both groups, a peak was observed on day 21, with a significant difference between male (70±48 mm^2) and female mice (181±20 mm^2, p=0.0053). This difference was maintained on day 28, with female mice displaying a 2.4-fold increase in erythema area and the skin appearing more swollen. Female mice that received dressings also showed a peak on day 21, however the erythema areas were smaller than for untreated females both at day 21 (124±4 mm^2) and 28 (41±49 mm^2). Histologically, a marked difference between the dermal thickness of tissues from male and female mice was observed, with female dermis being 50% thinner. Radiation mediated a similar increase in dermal thickness for both sexes, with a mean 1.4-fold increase. Finally, immunostaining for collagen IV on non-irradiated skin samples revealed that its expression at the dermo-epidermal junction was lost after irradiation, showing a diffuse staining throughout the dermis.

These preliminary results report the kinetic and extent of radiodermatitis development in our model. The severity of radiodermatitis in female mice would be consistent with a more reactive immune system, driven by hormones. Also, the ASC dressings applied to female mice seem to limit the extent of erythema.

ABSTRACT #60

Transcriptomic Profiling and Histopathological Assessment of Testosterone-Mediated Changes in Appendicular and Axial Tendons

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Background: Anabolic steroid use is rising in adults and adolescents. The mechanical properties, structure, and physiological responses to loading of synovial joint tissues, such as tendon and articular cartilage, vary by biological sex; however, the effects of exogenous anabolic steroid use on these tissues remain poorly defined. This study assessed the global transcriptional changes and histological alterations induced by exogenous testosterone administration in wild-type male mice and compared responses across axial and appendicular tendons and their associated muscles

Hypothesis: We hypothesized that exogenous testosterone administration would alter the expression of inflammatory and extracellular matrix (ECM) genes in axial and appendicular tendons, thereby increasing the incidence of histopathological signs of tendinopathy.

Methods: Male CD1® mice at 2.5 months of age were injected weekly with testosterone enanthate (15 mg/kg) or a vehicle control (n = 10 per group) for eight weeks. Following euthanasia, flexor digitorum longus (FDL), tibialis anterior (TA), and tail tendons, and associated muscles, were collected for bulk RNA sequencing (RNA-seq; Illumina NovaSeq X Plus). Ankles and knees were collected for phase-contrast μCT imaging and tendon histopathology. Raw reads were filtered for quality and aligned to the Mus musculus mm39 reference genome using HISAT2, and differential gene expression (DGE) and gene set enrichment analysis (GSEA) were performed using DESeq2 and GSEA, respectively. Simultaneously, tissue sections of the knees from each group were stained with Fast Green/Safranin-O and are being scored for tendinopathy.

Results: Testosterone caused greater transcriptional changes in skeletal muscle than in tendons. We found 1275 changed genes in the FDL muscle, nearly double the 643 identified in the FDL tendon and 558 in the tail tendon. Despite these different gene counts, both tendon sites showed similar activity in pathways related to muscle contraction, interleukin 17 (IL-17) signaling, and Hippo signaling. Ongoing work is assessing histological sections of the ankles (Achilles tendon) and knees (patellar tendon, cruciate ligaments) in the treatment group compared to controls for signs of tendinopathy. Based on the up-regulation of inflammatory markers such as interleukin-1 beta (Il1b) and components of the IL-17 pathway identified by bulk RNA-seq, we expect to observe increased tendinopathy in mice receiving testosterone enanthate.

Conclusion: Exogenous testosterone drives transcriptional programs in axial and appendicular tendons related to matrix remodeling and inflammation. Future work will reveal how these transcriptional changes alter tendons at a cellular and tissue level, offering clinical insights.

ABSTRACT #61

The histological characterization of cell-assembled decellularized adipose tissue bioscaffolds in a porcine wound model

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The progression of dermal wound healing relies on the development of a microenvironment that supports constructive tissue remodelling. When wounds persist in an inflammatory state, characteristic of chronic non-healing ulcers, regeneration is impaired, increasing the risk of infection and potentially necessitating limb amputation. There remains an unmet clinical need for new therapies that can promote consistent wound closure and the regeneration of tissues with similar structure and function to the non-injured state. To address this challenge, our team characterized tissue-engineered bioscaffolds generated using a cell-assembly approach that incorporates pro-regenerative adipose-derived stromal cells (ASCs) within a decellularized adipose tissue (DAT) framework. More specifically, we compared the wound healing response in a porcine wound model of scaffolds generated with porcine ASCs, to unseeded scaffold controls and wounds treated with ASCs delivered in saline at timepoints of 7, 30, and 38 days. The focus of this project was specifically on the blinded histological assessment of the healing response between the groups over time. While all treatment groups achieved full wound closure by day 38, there was variability in the quality of the remodeled tissue. Masson's trichrome staining and immunostaining for α-smooth muscle actin (α-SMA) was performed to visualize collagen abundance and myofibroblast persistence, respectively. High expression of both markers at day 30 in specific groups suggested a progression toward scar formation, which was supported by collagen fiber analysis using picrosirius red staining. Specifically, wounds displayed a rapid, but more reactive remodelling process, indicated by high collagen fiber alignment at the wound center and a broad distribution of fiber thickness across the entire wound bed. Other wounds with lower α-SMA expression displayed a similar abundance of collagen within Masson's trichrome staining. However, picrosirius red analysis of this treatment group at day 30 indicated a more narrow distribution of fiber thickness that gradually thinned at the wound's centre, and an alignment closer to the disorganized "basketweave" of unwounded dermis, potentially suggesting a more regenerative healing response. Further analysis of epidermal thickness revealed some treatment groups exhibiting a thick stratum corneum with absent rete ridges, while others demonstrated deep rete ridges anchoring the dermis, with a corneal layer similar to unwounded skin. Ongoing analyses are characterizing vascularization and macrophage presence and polarization in the wound bed, to assess whether there are differences in the time frame over which the

wounds transition from a more inflammatory to a more pro-regenerative state depending on the treatment group.

ABSTRACT #62

First-in-Human Clinical Trial of Bilamellar Gene-Corrected Skin Substitutes for the Treatment of Recessive Dystrophic Epidermolysis Bullosa

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Background: Recessive dystrophic epidermolysis bullosa (RDEB) is a genetic skin disorder caused by mutations in the COL7A1 gene, which encodes type VII collagen, a key structural protein of anchoring fibrils at the dermal–epidermal junction. Loss of functional collagen VII results in skin fragility and detachment after minor cutaneous trauma, chronic wounds, scarring, and a higher risk of aggressive squamous cell carcinoma. Current treatments in Canada are limited to palliative wound management, which is burdensome and costly. A topical treatment (Vyjuvek) has been recently approved by the US Food and Drug Administration (FDA). This gene therapy treatment requires repeated applications, and an extended period is necessary to achieve full re-epithelialization in larger wound areas.

Methods: Our strategy for the development of an RDEB treatment is to combine ex vivo gene therapy with tissue engineering to produce gene-modified epidermolysis bullosa-self assembled skin substitutes (GMEB-SASS). Following our demonstration of functional restoration in preclinical studies, a phase I/II clinical trial has been accepted by Health Canada and is conducted at the LOEX/CHU de Québec-Université Laval. Autologous keratinocytes and fibroblasts are isolated from a small skin biopsy from RDEB patients and transduced with a self-inactivating retroviral vector encoding for an optimized COL7A1 cDNA. After expansion, the corrected cells are used to generate GMEB-SASS that will be grafted onto the chronic wounds of RDEB patients.

Results: Preclinical studies comprised in vitro and in vivo (grafting on athymic mice) investigations. They have shown a stable vector integration, presence of epithelial stem cells, sustained expression and

adequate localization of type VII collagen at the basement membrane, as well as ultrastructural reconstitution of anchoring fibrils and increased dermal-epidermal adhesion strength. Biosafety assessments confirmed the absence of replication competent retrovirus or malignant transformation. The aim of the clinical trial is to assess safety, feasibility, and preliminary efficacy of GMEB-SASS. The measured clinical outcomes include durable wound closure, reduced blistering frequency, and enhanced dermal–epidermal stability. Secondary endpoints encompass pain reduction, improved mobility, and better overall quality of life. We have initiated the recruitment of patients.

Conclusion: GMEB-SASS clinical trial represents a highly innovative and promising therapy for an unmet clinical need for patients suffering from RDEB and could be a valuable treatment option to include within the Canada's health system after completion of the clinical trial.

ABSTRACT #63

TUNGSTEN EXPOSURE INDUCES ROS, INFLAMMATION AND CELLULAR SENESCENCE IN THE IVD LEADING TO INCREASES IN PAIN

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INTRODUCTION: Tungsten has been increasing in demand for use in manufacturing and recently, medical devices, as it imparts flexibility, strength, and conductance of metal alloys. Given the surge in tungsten use, our population may be subjected to elevated exposures. We recently determined that when mice are exposed to tungsten [15 ppm] in their drinking water, it bioaccumulates in the intervertebral disc (IVD) and vertebrae tissues leading to disc degeneration, fibrosis and increases in markers on inflammation and pain. This study was performed to determine the mechanism and physiological effects following tungsten exposure in the IVD.
METHODS: Bovine nucleus pulposus (hNP) were cultured in medium supplemented with tungsten in the form of sodium tungstate [0, 1, 5 and 15 ug/mL] or H2O2 [100 µm]. QPCR was performed to measure changes in the expression of matrix, inflammatory and pain markers. Assessment of oxidative stress was performed using H2DCFDA to measure ROS cellular levels. DNA damage was measured using γH2AX fluorescence. Cellular senescence was determined by measuring changes in β-galactosidase activity. IHC for MOMA staining was performed IVDs from C57BL/6 mice on water supplemented with 15 ppm tungsten for 4 weeks. Von Frey was performed on mice following 6 months of tungsten exposure.
RESULTS AND DISCUSSION: Tungsten and H2O2 significantly increased the expression of IL1B, TNFA,

NGF and COLII in NP cells ($p < 0.01$, n=4). On the contrary, the matrix protein aggrecan (ACAN) was downregulated suggesting a degenerative phenotype ($p < 0.01$, n=4). Oxidative stress was found to be increased in a dose-dependent manner in NP cells ($p < 0.05$; n=4). Interestingly, there were no changes in DNA damage nor the induction of senescence following tungsten exposure. Tungsten did, however, significantly increase the infiltration of macrophages at lumbar IVD sites ($p < 0.05$, n=4). These changes in disc cell and IVD characteristics were related to mechanical pain behaviour in the hind paws of mice exposed to tungsten ($p < 0.05$, n=5).
CONCLUSIONS: We provide evidence that chronic tungsten exposure induces disc degeneration and pain behaviour in mice and may play a role in disc degeneration disease.

ABSTRACT #64

How a mechanosensitive transcription factor potentially fits into the pathology of lung fibrosis

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INTRODUCTION: Pulmonary fibrosis (PF) is a life-threatening interstitial lung disease with limited treatment options that is caused by excessive deposition of extracellular matrix components. Recent work has linked the profibrotic activity seen in PF to the mechanotransduction pathway. Homeobox protein Nkx2.5, a transcription factor that has been identified as a mechanosensitive repressor or enhancer of α-smooth muscle actin expression, depending on the cell type and disease pathology.
AIMS: We aim to show this by characterizing current NKX2.5 expression and function in the context of the lung, seeing if NKX2.5 can be exploited in an overexpression system, and seeing if it can attenuate fibrotic progression in in and ex vivo systems.
METHODS: Baseline Nkx2.5 expression in the lung was quantified using primary human lung fibroblasts (PHLF) and primary vascular smooth muscle cells (PVSMC) grown in soft and stiff cell culture plates. A lentiviral overexpression system will be used in PHLFs to determine if transition to profibrotic phenotypes can be prevented, and normal function can be restored. Expression of α-smooth muscle actin and extra cellular matrix protein collagen was measured to quantify fibrosis progression and assess the role of Nkx2.5 in fibrosis.
RESULTS: Immunostaining shows a reduction in the amount of Nkx2.5 in fibroblasts when grown on stiff matrices compared to softer environments. Our work showed that Nkx2.5 expression did change in

response to varying environmental stiffness and that its role is dependent on cell type. Early results with the overexpression model suggest Nkx2.5 could be relevant in the context of PF and when overexpressed Nkx2.5 makes cells behave like they are on soft surfaces.
CONCLUSIONS: This research showcases a complex role that Nkx2.5 might play in PF, supporting opposing narratives in the literature that Nkx2.5 is both a driver of fibrosis and antagonistic to it. Further research of Nkx2.5 should be done to better understand how this protein functions outside the context of cardiac biology. Restoring proteins involved in normal, non-pathogenic function could also help to complement current interventions, but must be done carefully and with specificity.

ABSTRACT #65

Free calcium and the Extracellular Calcium-Sensing Receptor Regulate Intervertebral Disc Degeneration and Calcification

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INTRODUCTION: Degenerative disc disease (DDD) is a common cause of lower back pain. Calcification of the intervertebral disc (IVD) has been correlated with DDD, and is especially prevalent in scoliotic discs. The appearance of calcium deposits has been shown to increase with age, and its occurrence has been associated with several other disorders such as hyperparathyroidism, chondrocalcinosis, and arthritis. Trauma, vertebral fusion and infection have also been shown to increase the incidence of IVD calcification. Our data indicate that Ca2+ and expression of the extracellular calcium-sensing receptor (CaSR) are significantly increased in mild to severely degenerative human IVDs. In this study, we evaluated the effects of Ca2+ and CaSR on the degeneration and calcification of IVDs.
METHODS: Degenerative disc disease (DDD) is a common cause of lower back pain. Calcification of the intervertebral disc (IVD) has been correlated with DDD, and is especially prevalent in scoliotic discs. The appearance of calcium deposits has been shown to increase with age, and its occurrence has been associated with several other disorders such as hyperparathyroidism, chondrocalcinosis, and arthritis. Trauma, vertebral fusion and infection have also been shown to increase the incidence of IVD calcification. Our data indicate that Ca2+ and expression of the extracellular calcium-sensing receptor (CaSR) are significantly increased in mild to severely degenerative human IVDs. In this study, we evaluated the effects of Ca2+ and CaSR on the degeneration and calcification of IVDs.
RESULTS AND DISCUSSION: Ca2+ dose-dependently decreased matrix protein synthesis (proteoglycan

and Col II) in disc cells (n = 4). Ca2+ and cincalcet did not significantly increase the expression of catabolic enzymes save ADAMTS5. Similar effects were observed in whole organ cultures, as Ca2+ and cinacalcet decreased proteoglycan and collagen content. Although both Ca2+ and cinacalcet increased the expression of alkaline phosphatase (ALP), only in Ca2+-treated IVDs was there evidence of calcium deposits in NP and AF tissues. Biomechanical studies on Ca2+ and cinacalcet-treated IVDs demonstrated decreases in complex modulus ($p<0.01$ and $p<0.001$, respectively; n=5), however, only in Ca2+-treated IVDs were there significant increases in stiffness of NP and AF tissues (Figure 1; $p<0.001$ and $p<0.05$, respectively; n=3).
CONCLUSIONS: Our results suggest that changes in the local concentrations of calcium and activation of CaSR affects matrix protein synthesis, calcification and IVD biomechanics. Ca2+ may be a contributing factor in IVD degeneration. The effects of Ca2+ on IVD degeneration should be considered when investigating repair strategies of the disc.

ABSTRACT #66

A Novel Rat Caudal Vertebra Metastasis Model Demonstrates Suppression of Tumor Recurrence and Preservation of Bone by Doxorubicin-Releasing PMMA Cement

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Introduction: Spinal metastases frequently lead to pain, instability, and neurological compromise, with high rates of local recurrence following incomplete surgical resection. While polymethylmethacrylate (PMMA) bone cement provides mechanical stabilization, it does not eliminate residual tumor cells. Local chemotherapeutic augmentation offers a strategy to combine structural support with targeted tumor control. We previously developed a nanoparticle-functionalized, doxorubicin (DOX)-releasing PMMA cement with sustained drug release. This study aimed to establish a clinically relevant rat caudal vertebral metastasis model and evaluate the efficacy of DOX-PMMA in preventing tumor recurrence.

Methods: Luciferase-expressing MDA-MB-231/Luc breast cancer cells were encapsulated in alginate-gelatin hydrogel plugs and implanted into the caudal vertebrae of SRG OncoRats (n = 12), with sham controls (n = 2). Tumor burden was monitored using bioluminescence imaging (BLI), and bone structure assessed by CT. At 6 weeks, tumors were surgically debulked and defects filled with control PMMA (n = 6) or DOX-PMMA (n = 6). Drug release kinetics were quantified in vitro over 28 days. Animals were

followed for 10 additional weeks before endpoint analyses including microCT, histology, hematology, and serum biochemistry. Tumor burden was quantified as Δlog10(radiance) pre- and post-resection, with group comparisons using Welch's t-test and nonparametric sensitivity analyses.

Results: Nanoparticle-functionalized PMMA demonstrated sustained DOX release over 28 days (2.04 ± 0.18 nmol/implant). Tumors established reliably and remained stable between weeks 2 and 5 ($p > 0.05$), validating the model. Following resection, control animals exhibited increased tumor burden, whereas DOX-PMMA-treated animals showed suppressed progression. Mean Δlog10(radiance) was +0.81 in controls versus -0.22 in DOX-treated animals, corresponding to an approximately 10.8-fold difference in tumor burden change. This represented a large effect size (Cohen's $d = 1.23$), with a strong trend toward significance ($p = 0.076$), supported by nonparametric analysis. An aggressive regrowth phenotype (≥5-fold increase) was observed in 3/6 control animals and 0/6 DOX-treated animals. MicroCT demonstrated improved trabecular bone parameters in DOX-treated vertebrae, indicating partial restoration of bone architecture. No significant differences in hematological or biochemical markers were observed, indicating absence of systemic toxicity.

Discussion: This study establishes a reproducible rat caudal vertebral metastasis model enabling controlled tumor implantation, resection, and longitudinal evaluation of local therapies. DOX-PMMA demonstrated sustained drug release, suppression of tumor recurrence, and preservation of bone microarchitecture without systemic toxicity. These findings support nanoparticle-functionalized PMMA as a promising dual-function biomaterial to improve local tumor control following spinal metastasis surgery.

ABSTRACT #67

O-vanillin Reduces Senescence and Improves Disc and Bone Quality in Aging Wild-Type Mice

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INTRODUCTION: Age-related musculoskeletal disorders, including intervertebral disc (IVD) degeneration and osteoporosis, are leading contributors to disability and reduced quality of life in aging populations. A key driver of these conditions is the accumulation of senescent cells, which promote inflammation and tissue degeneration through the senescence-associated secretory phenotype (SASP).

These processes are regulated by pathways including NF-κB and p53/MDM2, which contribute to chronic inflammation and impaired tissue homeostasis. Senolytic therapies that selectively eliminate senescent cells have emerged as promising strategies to mitigate age-related tissue degeneration. We have previously shown that o-vanillin, a natural compound with senolytic and senomorphic properties, modulates these pathways and improves degeneration in the sparc$^{-/-}$ mouse model, a model of accelerated IVD degeneration, low back pain, and early bone loss. Here, we evaluate whether o-vanillin can similarly improve tissue health in naturally aging wild-type (WT) mice.

METHODS: WT mice (9-12 months old) received weekly oral administration of o-vanillin, RG7112, or their combination for 12 weeks. Spinal micro-CT was used to quantify IVD volume and bone quality, assessed as bone volume fraction (BV/TV). Immunohistochemistry was performed to evaluate senescent cell burden using p16, a well-established marker of cellular senescence, in IVDs.

RESULTS: Aging WT mice exhibited reduced disc volume and BV/TV, along with increased p16-positive senescent cells compared to younger animals. Treatment with o-vanillin significantly improved disc volume and bone quality and reduced senescent cell burden. While RG7112 and combination therapy also reduced senescent cells and increased bone quality, they did not provide additional improvements beyond o-vanillin alone in WT mice, in contrast to prior findings in the sparc$^{-/-}$ model.

CONCLUSIONS: This study highlights age-associated increases in senescent cell burden and declines in disc and bone parameters in WT mice up to 12 months of age. Moreover, these results demonstrate that o-vanillin effectively reduces p16-positive senescent cells and improves IVD and bone integrity in aging WT mice. This work supports the potential of o-vanillin as a therapy for age-related degeneration and highlights its translational relevance beyond accelerated disease models. To further evaluate the impact of senolytic treatment, ongoing studies will extend this work to older animals, while optimization of dosing strategies and structural analogs of o-vanillin may enhance therapeutic efficacy.

ABSTRACT #68

AFM nanomechanical characterization of collagen fibrils

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Introduction: Collagen is the fundamental building block of connective tissues such as skin, tendon, and bone, where it forms a hierarchical structure from triple helical molecules to nanoscale fibrils and

larger fibers. The mechanical properties of collagen fibrils play a critical role in determining tissue-level function and performance. Atomic force microscopy (AFM) is the gold standard for nanoscale mechanical measurements. However, accurately characterizing collagen fibril mechanics remains challenging because the experiment is highly sensitive to factors such as fibril hydration, zone-specific heterogeneity, and experimental parameters including AFM cantilever stiffness. These factors lead to significant variability in reported mechanical properties of collagen and limit consistent interpretation across studies.

Objective: Here, we address current limitations in AFM-based nanomechanical characterization of collagen fibrils by systematically examining how hydration, intrinsic fibril heterogeneity, and imaging parameters influence collagen nanomechanics, and by establishing a framework for accurate and reproducible characterization.

Methods: Native collagen fibrils extracted from rat tail tendons were analyzed using the AFM PeakForce Nanomechanical Mapping (PFQNM) mode. Degree of fibril hydration was tuned through imaging in dry, rehydrated, and relative humidity conditions. Zone-specific analysis was performed by defining gap and overlap regions from quantitative height profiles of the fibrils. The effects of cantilever stiffness were evaluated using AFM probes with varying spring constants.

Results: Our results show that hydration strongly influences collagen fibril nanomechanics, where increasing hydration reduces D-banding visibility, significantly lowers collagen fibril stiffness, and diminishes mechanical contrast between gap and overlap regions. Rehydrated fibrils imaged at 100% relative humidity maintain intrafibrillar hydration while limiting the presence of loosely bound and free water, providing a reliable condition that closely reflects the native tissue environment. In addition, mechanical properties differ significantly between overlap and gap regions, highlighting the importance of a reliable method for defining these regions for zone-specific analysis. Finally, measured collagen fibril modulus vary drastically with AFM cantilever stiffness, ranging from ~500MPa to ~20GPa, emphasizing the need for appropriate probe selection and validation using force-distance curve fitting.

Conclusions: Together, our results define key factors required for accurate and consistent AFM-based characterization of collagen fibril nanomechanics.

Significance: These findings resolve key limitations in AFM-based characterization of collagen fibrils, where measurements remain highly variable. By identifying sources of variability and establishing a framework for more reliable nanoscale measurements, our work enables more consistent investigation of collagen mechanics and can be extended to other biological materials.

ABSTRACT #69

Regenerative Bioinks: Material-Intrinsic Properties Drive Immune-Guided Bone Repair

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Carrageenan, a sulfated polysaccharide similar in structure to glycosaminoglycans, is a promising material for bone tissue engineering. However, its tendency to trigger inflammation through TLR4 activation limits its potential. To address this, we modified kappa carrageenan (κC) through esterification, creating two derivatives: carboxymethyl-κC (C-κC) and methacryloyl-κC (M-κC). These modifications were designed to reduce inflammatory signaling and promote a more regenerative immune response. Both C-κC and M-κC successfully downregulated TLR4 signaling and promoted anti-inflammatory, pro-repair macrophage phenotypes (marked by CD206/Ly6Clow expression). Both materials enhanced osteogenic signaling, although C-κC achieved a better balance between immune modulation and bone regeneration. In vivo, C-κC triggered a transient immune response resembling native tissue regeneration, which led to robust osseointegration and active tissue remodeling. M-κC, in contrast, promoted fibrous encapsulation and achieved only partial, non-uniform integration. These findings demonstrate that controlled immune activation, not suppression, is essential for effective regeneration. Modified κC offers a scalable, bioprinting-compatible, and immuno-instructive scaffold platform for bone and soft tissue repair.

ABSTRACT #70

Novel Storage Medium for Extended Preservation of Osteochondral Allografts in Canada: Translation to Human Tissue

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Background: Cartilage has limited intrinsic healing capacity, making cartilage damage a major cause of disability. Osteochondral allograft transplantation (OCAT) is a surgical approach for treating focal cartilage defects by replacing damaged tissue with healthy osteochondral (bone and cartilage) grafts from a cadaveric donor. Prior to transplantation, grafts must be stored for microbiological testing and surgical scheduling. Successful OCAT is related to maintaining a minimum of 70% viability during the storage period. In Canada, grafts are approved for storage in Lactated Ringer's solution (LRS)-based media for up to 14 days, a limited time frame that results in unused grafts. Using a rabbit model, our lab has developed a novel storage medium, which includes Dulbecco's Modified Eagle Medium, hyaluronic Acid and doxycycline, that extends viability to 56 days. The goal of this study is to validate the novel medium in human osteochondral tissues to support clinical translation.
Methods: Twelve human distal femurs from six healthy donors will be obtained through a Canadian tissue bank. Osteochondral grafts from knee joint surfaces will be divided in a pair design, with each donor providing samples for both storage conditions. Samples will be stored at 4°C in either the novel medium or an LRS-based medium and will be evaluated at 0 (fresh control), or after 42, 56, or 63 days of storage. Chondrocyte viability will be assessed using live/dead fluorescence staining (Calcein AM and Ethidium Homodimer-1) followed by confocal microscopy. The extracellular matrix (ECM) will be characterized through biochemical assays, mechanical testing and histological analysis. Repeated-measures ANOVA will be employed to compare media performance over time.
Anticipated Results: The novel media is expected to preserve viability at levels sufficient for transplantation to at least 56 days in human cartilage, as observed in rabbit models. Compared to rabbits, human cartilage is thicker with lower cellular density and metabolism, which is hypothesized to improve outcomes during prolonged storage. The novel medium is also expected to maintain viability at the articular surface, which is important for graft survival following transplantation. Maintenance of the ECM is expected to be more variable in human cartilage compared to rabbit, with collagen content and organization maintained throughout storage and possible loss of proteoglycan over time.
Conclusions: This study represents a critical validation step toward clinical translation of the novel media for storage of osteochondral allografts in Canada. Extending preservation has the potential to increase graft availability and improve access to OCAT for patients.

ABSTRACT #71

Thrombin-Induced Dose-dependent Effects of Aspirin on Endothelial Cell Function

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Avascular necrosis (AVN) is a debilitating condition characterized by focal bone tissue death due to impaired blood supply, with endothelial dysfunction recognized as a key underlying mechanism. Disruption of endothelial homeostasis contributes to impaired vascular support of connective tissues, highlighting the importance of targeting endothelial health in AVN. While Aspirin is widely used for its antiplatelet and anti-inflammatory effects, its direct, dose-dependent impact on endothelial function within clinically relevant ranges remains incompletely defined.

To address this, human umbilical vein endothelial cells (HUVECs) were treated with Aspirin across a dose range (0–500μM) with and without thrombin stimulation to model pro-thrombotic endothelial stress. Endothelial responses were assessed using immunocytochemistry for apoptosis (Caspase-3), proliferation (Ki-67), and senescence (p16), alongside a tube formation assay to evaluate angiogenic function. In parallel, quantitative Polymerase Cell Reaction (PCR) was performed to assess a panel of pro-thrombotic and adhesion-related genes, including Tissue Factor (TF), E-selectin, Vascular Cell Adhesion Molecule-1 (VCAM-1), Plasminogen Activator Inhibitor-1 (PAI-1), Von Willebrand Factor (VWF), and Thrombomodulin (TM).

Aspirin demonstrated a clear dose-dependent effect on endothelial cell behavior. At a clinically relevant concentration (90μM), endothelial viability and proliferative capacity were preserved, with reduced apoptotic and senescence-associated staining compared to thrombin alone. In contrast, higher concentrations (≥250μM) induced apoptosis, senescence, and impaired endothelial integrity, consistent with cytotoxic stress. Functionally, low-dose Aspirin preserved the organization of the angiogenic network under thrombin stimulation, whereas higher doses disrupted tube formation.

At the transcriptional level, thrombin robustly induced the expression of pro-thrombotic and adhesion-related genes. Low-dose Aspirin modulated this response in a time- and dose-dependent manner, characterized by dynamic early-phase responses, divergent regulation at intermediate timepoints, and a shift toward attenuation of endothelial activation markers with prolonged exposure. These effects were gene-specific, with TF, E-selectin, VCAM-1, PAI-1, and VWF showing differential temporal patterns, while TM remained relatively stable.

Collectively, these findings demonstrate that Aspirin modulates endothelial function in a dose- and time-dependent manner, with dynamic and context-specific effects under thrombotic stimulation. Low-dose Aspirin preserves endothelial integrity and angiogenic capacity under thrombotic stress, whereas higher concentrations promote endothelial dysfunction. These results define a clinically relevant endothelial protective window and support a potential role for low-dose Aspirin in preserving vascular function in connective tissue pathologies such as AVN.

ABSTRACT #72

Understanding the mechanism of arterial calcification

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Arterial calcification is a major clinical burden worldwide and is strongly associated with increased cardiovascular morbidity and mortality. Despite its prevalence, there is no FDA approved therapy that could stop or reverse this disease. The available treatments are limited to surgical or interventional approaches due to an insufficient understanding of the underlying mechanisms driving arterial calcification. Up to now, multiple pathways have been proposed to explain mineral formation in arteries. On the one hand, elastin degradation, which exposes binding sites for calcium phosphate nucleation, leads to calcification. On the other hand, several studies have reported that mineral deposits are predominantly located in vicinity of collagen, with little to no association with elastin. Therefore, the relative contributions of elastin- versus collagen-driven mineralization remain unclear. In addition, studies on calcified human arteries have reported that minerals deposited in arterial calcification are composed of different calcium phosphate phases, such as amorphous calcium phosphate (ACP), octacalcium phosphate (OCP), dicalcium phosphate dihydrate (DCPD), hydroxyapatite (HA), carbonated hydroxyapatite (CHA), and whitlockite (WL). However, these studies were performed only on arteries in the upper body (thoracic aorta, carotid arteries). Since it is known that calcification patterns in upper and lower body differ, this limitation is particularly critical because lower body arteries are the primary sites affected in peripheral artery disease (PAD), a condition in which medial calcification could induce impaired blood flow and increased risk of limb amputation. In this study, we aim to elucidate whether elastin- or collagen-mediated is the main extracellular matrix where minerals nucleate and grow in arterial calcification focusing on lower body arteries, and whether mineral phases present in the lower body arteries differ from those found in previous studies in the upper body. To do so, we analyze iliac calcified arteries from body donors using histological staining and advanced materials characterization techniques including confocal Raman microscopy, scanning electron microscope (SEM) and X-ray Absorption Near Edge Structure (XANES). These techniques allow mapping the spatial relationships between mineral deposits, elastin, and collagen, thereby identifying dominant nucleation pathways, and enable precise mineral phase identification in complex calcium phosphate mixtures. In the future we plan to extend this analysis to tibial arteries from patients undergoing limb amputation. By clarifying the nature of the minerals and the driving forces of mineralization in medial calcification, this

study contributes to identifying potential therapeutic targets as well as developing models for further studies on calcification reverse treatment.

ABSTRACT #73

Phase-Resolved Mapping of Cartilage Calcification Reveals Sex-Specific Mineral Signatures in Osteoarthritis

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Pathological deposition of calcium crystals, such as calcium pyrophosphates (CPP) and calcium phosphates, contributes to the progression of osteoarthritis (OA), a leading cause of disability worldwide. These minerals accumulate in articular cartilage, where they disrupt extracellular matrix (ECM) integrity, alter cellular behaviour, and promote inflammation. While mineral phase and composition are increasingly recognized as key determinants of pathology, their spatial distribution and phase-specific identity in human cartilage remain poorly defined. Despite the higher prevalence of OA in women, the role of sex-specific biological and hormonal factors in regulating mineral formation and maturation is not well understood.

We developed an integrated, multi-modal characterization framework to investigate the composition, distribution, and morphology of calcium-containing minerals in human articular cartilage. Histological staining localized calcified regions and assessed matrix integrity; micro-XRF mapped elemental distributions; and SEM/EDS characterized mineral morphology and Ca/P ratios. Ca K-edge XANES enabled phase-specific identification and quantification of crystalline and amorphous mineral species.

Analysis of 8 human cartilage samples revealed heterogeneous mineral phases with distinct compositional profiles. Ca K-edge XANES identified CPP phases predominantly in male samples, consistent with SEM/EDS findings, while whitlockite was observed exclusively in female samples. These findings suggest potential sex-dependent differences in mineral phase distribution.

Ongoing work expands this framework to larger sample cohorts and integrates complementary analyses to investigate hormonal regulation, particularly in postmenopausal OA. By linking mineral phase distribution to biological context, this work provides a foundation for mechanistic insight and informs

targeted therapeutic strategies. Beyond OA, this framework offers transferable tools for studying pathological biomineralization.

ABSTRACT #74

Anatomy-Registered Multimodal Characterization of Calcification in the Human Optic Nerve Head

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Pathological calcification is the abnormal deposition of calcium-containing minerals in soft tissues that do not normally mineralize. It features in multiple age- and disease-related conditions, including cerebral, ocular, vascular, and valvular disorders, in which mineral deposits alter tissue biomechanics, disrupt cellular function, and impair organ performance. Mineral characteristics, such as phase, crystallinity, hetero-ion inclusion, morphology, and spatial context, encode local formation conditions; variation in these signatures distinguishes mineralization pathways and, by extension, the biological mechanisms that drive them. Because extracellular matrix composition, ion clearance kinetics, and the local balance of mineralization inhibitors and promoters differ across anatomical compartments, mineral-focused analyses are needed to link deposit chemistry to tissue-specific drivers of calcification.

The optic nerve head (ONH) is a posterior ocular structure through which unmyelinated retinal ganglion cell axons exit the eye, traversing a connective tissue plate, to form the myelinated optic nerve. Calcification of the unmyelinated ONH, known as optic disc drusen (ODD), occurs in up to 2% of the population and causes visual field defects in roughly 25% of affected individuals. ODD exhibit a consistent spatial preference for the nasal and superior disc regions, implying that compartment-specific conditions selectively favor mineral nucleation. No treatments exist, in part because the mechanisms permitting precipitation in neural tissue remain undefined. We hypothesize that systematic characterization of mineral properties across ONH quadrants will reveal environmental discriminants that explain why certain anatomical zones are preferentially affected.

To test this, we examine ONH tissue from body donors whose advanced age produced a range of calcification severity despite causes of death unrelated to mineralization. Cadavers are fixed by carotid infusion of an embalming solution (isopropanol 24%, propylene glycol 4.5%, phenol 4.5%, formaldehyde 0.1%, methanol 2.5%) and stored one year prior to collection. Following eye enucleation, quadrant orientation is marked by an expert anatomist (G.V.) and eyes are rehydrated through graded phosphate-

buffered saline/ethanol dilutions. The ONH is isolated with a 6-mm trephine and embedded in optimal cutting temperature compound within a custom 3D-printed insert that locks tissue orientation relative to the cutting plane. Serial sagittal sections, 4 consecutive 10-µm sections every 100 µm, are collected across the entire tissue, and provide depth-registered sampling across the ONH. Histology, electron microscopy, and spectroscopy are used to localize and characterize mineral deposits within defined anatomical compartments. As such, this method allows for direct investigation of spatial variations in calcification signatures of ODD.

ABSTRACT #75

Senotherapeutics Prevent Disc Degeneration and Back Pain

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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 often associated with intervertebral disc (IVD) degeneration. 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 cancer, and osteoarthritis. They adopt a so-called senescent associated phenotype (SASP) and produce high levels of inflammatory and pain-mediating factors. The goal of this study is to evaluate the potential of senolytic components as a preventive approach for IVD-related low back pain.
METHODS: Treatment of sparc -/- mice began at 3-4 months of age when the animals start to show signs of IVD degeneration and back pain. The animals received weekly oral gavage treatments for five months with RG-7112, o-vanillin, or a combination of both, to evaluate if treatment prevented the progression of IVD degeneration. Axial pain was assessed using the grip strength test, while radiating pain was measured using the acetone-evoked test and the Von Frey stimulus. Micro-CT scans of the spine were conducted to evaluate IVD volume and vertebral bone health. Immunofluorescence staining was employed to quantify senescent p16Ink4a and p21-positive cells in treated and untreated IVDs, endplates and vertebral bone.
RESULTS: Animals treated with o-vanillin and RG-7112 for five months experienced significantly reduced pain compared to vehicle-treated group. The combination treatment further improved analgesic effects

on grip strength and von Frey compared to single-drug treatments but did not additionally enhance acetone-evoked behavior test. Micro-CT analysis demonstrated lower bone quality and disc volume in sparc -/- mice compared to wild-type. Treatment with individual drugs improved bone parameters, including bone volume, bone/total volume ratio, trabecular thickness, and trabecular number. However, senolytic treatments reduced endplate bone/total volume and increased porosity, with combination therapy exhibiting an additive effect. Disc volume improved with single-drug treatment and significantly enhanced with the combination treatment. Immunofluorescent staining indicated lower p16Ink4a and p21-positive senescent cells in the intervertebral discs, endplates, and vertebral bone of treated animals compared to untreated sparc -/- controls.
CONCLUSIONS: Our findings suggest that senolytics can enhance disc volume, improve bone, and endplate health, reduce the number of senescent cells in the IVDs, endplates and vertebral bone, and alleviate pain in sparc -/- mice.

ABSTRACT #76

Nanometrological Characterization of Mechanically Aligned Collagen Scaffolds: Quantifying the Structure-Function Relationship

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Engineered collagen scaffolds that mimic the anisotropic architecture of the extracellular matrix (ECM) provide a controlled platform for investigating structure–function relationships relevant to connective tissue disorders, such as Ehlers-Danlos Syndrome. However, quantitative metrics linking fibrillar organization to mechanical behavior remain limited.
In this study, we developed a scalable platform to induce uniaxial collagen fiber alignment through controlled mechanical stretching (13%, 18%, and 23% strain). The resulting micro- and nanoscale architecture was characterized using high-resolution Atomic Force Microscopy. To enable quantitative analysis, a custom MATLAB pipeline was implemented to compute an alignment order parameter, $S = \langle \cos(2(\theta_i - \theta\_mean)) \rangle$, alongside the full width at half maximum (FWHM) of the fibril orientation distribution.
We observe a highly significant ($p < 0.0001$) strain-dependent increase in alignment, with S rising from ~0.66 in control samples to ~0.82 at 23% strain, accompanied by a reduction in FWHM, indicating a narrower distribution of fibril orientations. To assess the mechanical consequences of this

reorganization, nano-indentation mapping (~2,400 points per sample) was performed, revealing systematic variations in local elastic modulus with increasing alignment.
Ongoing work includes correlative imaging using Scanning Electron Microscopy and Transmission Electron Microscopy to resolve fibrillar organization further and packing across scales.
Together, these results establish a quantitative framework linking collagen architecture to mechanical phenotype in engineered ECM systems, providing a basis for future studies of structure–function alterations relevant to Ehlers-Danlos Syndrome.

ABSTRACT #77

Cold Plasma Medicine to Treat Spine and Bone Metastatic Lesions

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INTRODUCTION: Cancer remains a leading cause of death worldwide. In Canada, approximately 30,500 women were diagnosed with breast cancer in 2024. While current treatments achieve response rates of about 90%, survival falls below 30% when distant metastases develop, particularly in bone. The spine is a common metastatic site, where osteolytic lesions cause bone loss, fractures, spinal cord compression, pain, and reduced quality of life. Although surgery, radiotherapy, and chemotherapy have improved outcomes, local recurrence persists, often requiring repeated interventions. This highlights the need for localized therapies that reduce tumor burden, limit metastasis, and spare healthy tissue. Plasma medicine shows anti-proliferative and wound-healing effects, but its mechanisms in breast cancer bone metastases remain unclear.

Objective/Hypothesis: To investigate the efficacy of cold atmospheric plasma medicine in selectively targeting triple-negative breast cancer (TNBC) cells, as well as MCF7 cells, within primary (co-cultured with fibroblasts) breast and spinal metastatic (co-cultured with osteoblasts) environments. We hypothesize that cold plasma exposure will induce apoptosis in cancer cells while sparing surrounding stromal cells, thereby reducing tumor migration and invasion.

METHODS: To test this hypothesis, we will use a patented 3D compartmental tumor–stromal interface model that recapitulates primary breast tumors and bone metastatic niches. IRM90 lung fibroblasts and spinal osteoblasts (from breast reduction surgeries and organ donors) will be seeded onto PLA

electrospun scaffolds. TNBC cells will be embedded in an alginate/gelatin hydrogel and layered over these scaffolds to generate a 3D co-culture construct.
Treatment groups will include vehicle (negative control), 0.5 μM doxorubicin (positive control), and escalating cold argon plasma exposures (15, 30, 45, 60, 120 s). Post-treatment, invasion and migration will be quantified via fluorescence microscopy, and viability assessed using metabolic assays and LIVE/DEAD staining. Parallel monoculture experiments (stromal or cancer cells alone) will evaluate treatment selectivity. These studies will define the minimum plasma exposure that maximizes tumor cell killing while preserving healthy tissue.

RESULTS: Plasma exposure under 60 seconds consistently reduced cancer cell viability in fibroblast–MCF7 mesh models at Days 3 and 7, exceeding doxorubicin (0.5). This reduction was significant versus control at both timepoints, while stromal cells were largely preserved. Plasma also decreased tumor invasion and migration across conditions.

CONCLUSION: Cold physical plasmas are effective in wound healing and diabetic foot ulcers, but their anticancer mechanisms, especially in bone malignancies, remain unclear. Clinical translation requires stronger mechanistic insight and better preclinical models. Our in vitro system helps bridge this gap.

ABSTRACT #78

Collagen hybridizing peptide-functionalized chitosan as a selective sealant for annulus fibrosus repair

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Introduction: Lower back pain is a leading cause of disability worldwide, with intervertebral disc (IVD) degeneration and disc herniation as major contributors. A common surgical treatment for persistent symptomatic disc herniation is a partial discectomy (nucleotomy), which decompresses neuronal elements by removing nucleus pulposus (NP) tissue through the annulus fibrosus (AF) defect, but fails to restore the mechanical integrity of the AF. Consequently, post-operative complications including re-herniation occur in up to 25% of cases, underscoring the need for a novel, selective bio-adhesive strategy to seal AF defects and reduce reoperation rates.
Collagen comprises approximately 70% of IVD dry weight and undergoes molecular-level denaturation from degeneration. Collagen hybridizing peptide (CHP) is a synthetic peptide with a collagen-mimetic

GlyXaaYaa structure. It selectively binds with denatured collagen by inserting into exposed single collagen strands and re-forming the native triple-helix structure – a structure unique to collagen. Chitosan is a biocompatible polymer that binds to tissue via electrostatic interactions and physical entanglement with collagen fibrils, but has insufficient adhesion strength for load-bearing AF tissue. Functionalizing chitosan with CHP enhances its adhesion strength by incorporating peptides that wind directly into additional hierarchical structures, the damaged collagen fibers.
Methods: Chitosan hydrogel (2% w/v) was functionalized with rhodamine B-tagged CHP via EDC-NHS conjugation chemistry, with unmodified chitosan serving as a negative control. Adhesion strength was evaluated by bonding healthy and degenerated human AF tissue to a alginate-polyacrylamide (Alg-PAAm) tough adhesive substrate. Tissue degeneration, primarily age-related, was assessed via radiographic disc height measurements and visual grading of dehydration, ossification, and vascular invasion.
Results: Successful CHP functionalization is confirmed by rhodamine B fluorescence and NMR spectroscopy. Functionally, CHP-chitosan significantly enhanced the adhesion energy between degenerated tissue and the tough gel substrate when compared to chitosan primer alone. Further, CHP-chitosan did not enhance the adhesion energy to healthy AF tissue when compared to chitosan alone, consistent with less collagen denaturation in the tissue. The Alg-PAAm substrate alone exhibited negligible AF adhesion, while unmodified chitosan showed consistent adhesion across degenerative conditions, comparable to CHP-chitosan on healthy tissue but significantly inferior on degenerated tissue.
Conclusion: Functionalizing chitosan with CHP selectively enhances adhesion to degenerated AF tissue through re-trimerization with damaged collagen fibers, while preserving the material's biocompatibility. This peptide-based modification strategy yields a targeted and strong sealant for AF defect repair following nucleotomy, with the potential to reduce re-herniation, lower reoperation rates, and improve long-term IVD recovery outcomes.

ABSTRACT #79

BIOMENIX – Bioadhesive Platform with Arthroscopic Device for Meniscal Repair

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Meniscal injuries are common and represent a major challenge in orthopedics, often accelerating the development of osteoarthritis. Current surgical interventions, partial meniscectomy or suturing, remain suboptimal: meniscectomy removes damaged tissue, compromising long-term joint function and increasing the risk of osteoarthritis, while suturing is technically demanding, limited to certain types of tears, and often associated with incomplete healing or reoperation. Despite decades of improvements, no reliable method currently restores meniscal integrity across all types of lesions.
The BIOMENIX project proposes an innovative approach: a bioadhesive applied arthroscopically to enable suture-free meniscal repair. The adhesive consists of a biocompatible hydrogel designed to replicate the mechanical, structural, and biochemical properties of native meniscal tissue. In parallel, an arthroscopic delivery device is being developed to allow precise and minimally invasive application directly at the tear site.

ABSTRACT #80

Decoupling plasma-induced redox chemistry via spatially-resolved microfluidics for tissue response modulation

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Cold atmospheric pressure plasmas generate highly reactive oxygen and nitrogen species (RONS) that interact with biological targets via redox pathways, modulating key tissue responses such as wound healing and fibrosis regulation. Understanding which specific RONS drive which biological effect is critical for developing targeted plasma therapies. However, decoupling the individual contributions of plasma-produced RONS remains an essential, yet unmet, challenge [1]. Tumor spheroids offer a sensitive readout to probe dose-response mechanisms, as cancer cells are selectively killed by plasma redox therapy [2]. Insights gained from these models ultimately inform broader applications, including the modulation of connective tissue responses.

To address this gap, we propose a plasma-microfluidic platform designed for spatially-resolved treatment of biological models [3]. This system exploits the spatiotemporal evolution of plasma-induced chemistry within microchannels, exposing cells to distinct chemical environments depending on their position along the channel. The microscale ensures well-characterized physical forces, making the system both reproducible and amenable to numerical modeling [4]. A COST reference jet is employed as

the plasma source to ensure inter-laboratory comparability [3].
To characterize the chemical environment delivered to cells, plasma-treated liquid is characterized using optical and colorimetric techniques, integrating experimental and modeling data: hydrogen peroxide and hydroxyl radical concentrations are quantified as a function of plasma power. A dual-phase flow of plasma effluent bubbles and liquid enhances species transfer and mixing, with no significant impact on cell viability under tested conditions.

Preliminary results demonstrate a dose-dependent response of MDA-MB-231 spheroids (cultured, treated, and analyzed entirely on-chip) to plasma-treated media. This work provides a modular framework for investigating the mechanisms underlying selective plasma-liquid-cell interactions, and opens perspectives for studying plasma interactions with connective tissue models.
This work was supported by Québec-Flanders Bilateral Research Cooperation Program.

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