Canadian Connective Tissue Society
::: CCTS :::

PROGRAM BOOK
CCTC 2015

http://connective-tissue-canada.com/cctc-2015/

Canadian Connective Tissue Society
::: CCTS :::

Welcome to the 21th Canadian Connective Tissue Conference held at Université Laval, Québec from May 28th to May 30th, 2015.

The mission of the CCTC is to strengthen contacts among connective tissue researchers and in particular to help foster young Canadian scientists. Therefore trainees at the conference are given the opportunity to present their work, to chair scientific sessions and to network with the participants from other institutions in Canada.

This year's annual meeting includes a tribute to Dr. Cy Franck presented by Hani El-Gabalawy in memory of the vision and leadership of the first Scientific Director of the Institute of Musculoskeletal Health and Arthritis.

On a happier note, the gala dinner on Friday night will feature a talk given by François A. Auger in celebration of the 30th anniversary of the LOEX, one of the first laboratories in the world to perform organ reconstruction by tissue engineering using normal human cells.

The program features 8 sessions of oral presentations and 2 poster presentation sessions. The two poster sessions will take place during the lunch hours and the posters will also be available for viewing during the coffee breaks.

This year's research themes are: Connective tissue remodeling, musculoskeletal system, tissue engineering and biomaterials, and cell-extracellular matrix interactions.

The Canadian Connective Tissue Society will hold its general assembly during the conference.

We are very grateful for the support provided by our sponsors. This educational program and the availability of numerous awards for students would not have been possible without their contribution. On behalf of the CCTS, we thank you for joining us and supporting connective tissue research in Canada.

We look forward to seeing you and hope that you will enjoy the program and social activities of the 21st Canadian Connective Tissue Conference in Québec.

Véronique J Moulin, PhD Julie Fradette, PhD
2015 CCTC chairs

Department of Surgery, Faculty of Medicine
Université Laval
CHU de Québec Research Center
Centre universitaire LOEX

Canadian Connective Tissue Society
::: CCTS :::

Dear Canadian Connective Tissue Society (CCTS) members,

As of today, all attendees of this conference are automatically members of the CCTS Society. This 2015 Canadian Connective tissue Conference (CCTC) is the 21st annual conference. The aim of the conference and of the society is to advance our current scientific knowledge and clinical understanding of connective tissue in health and diseases. Another aim is to provide a platform for graduate students, post-doctoral and clinical fellows as well as scientists and clinicians to share their science and to network in order to develop new collaborations and new opportunities.

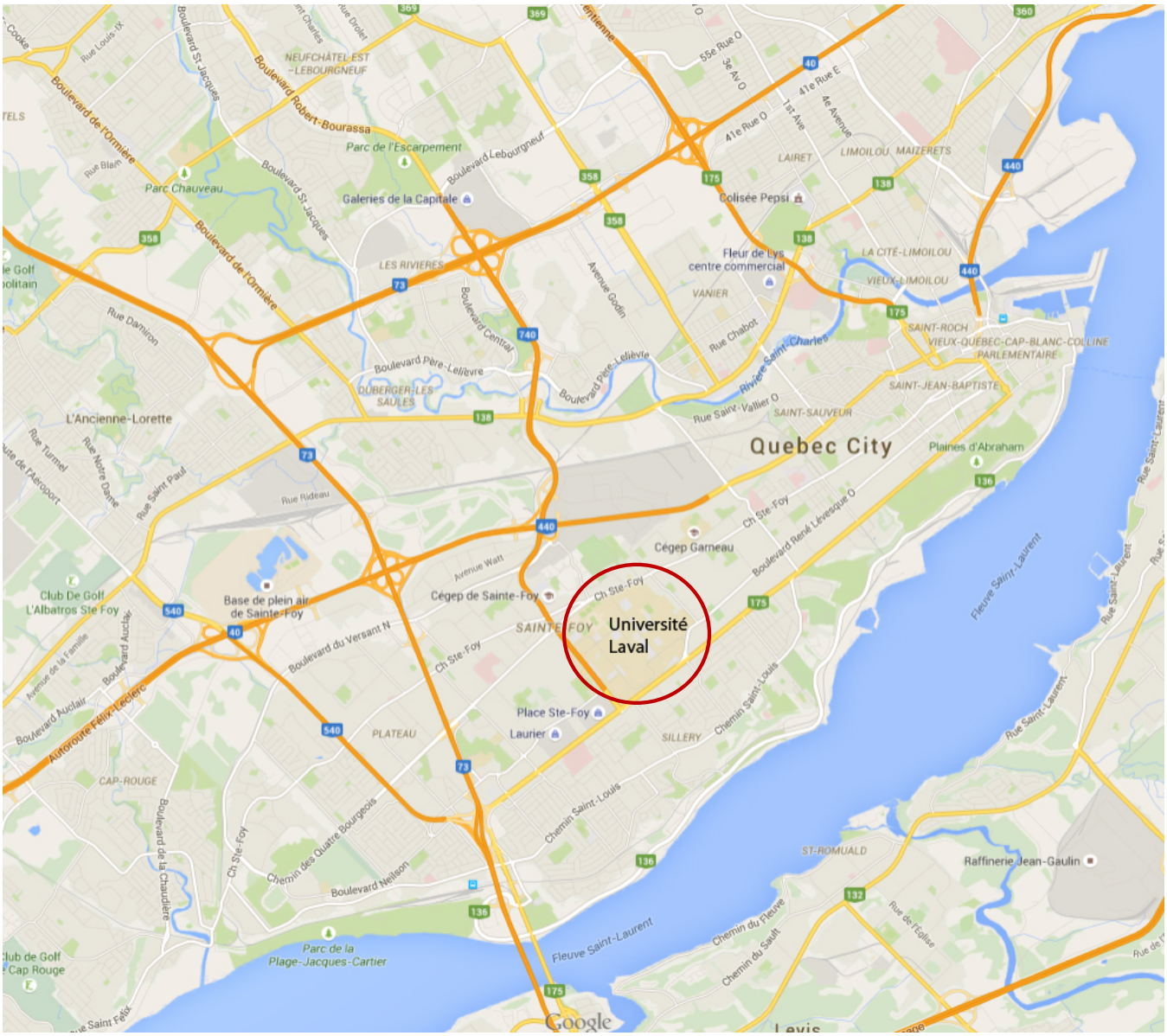
Over the last decade, the CCTC has contributed to the development of a new generation of Canadian scientists by allowing them to present their research and to interact with leading scientists in the connective tissue field.

Drs. Veronique Moulin and Julie Fradette as well as their team have put together an outstanding programme of basic, translational and clinical research for this conference. I hope that you come away from this conference with new ideas to advance your research. The future of this Society is in your hands.

Best regards,

Marc Grynpas, Ph.D.
President of CCTS
Director Bone and Mineral Group, University of Toronto
Professor, Department of Laboratory Medicine and Pathobiology
Institute for Biomaterials and Biomedical Engineering, University of Toronto
Senior Scientist, Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto

Directions to Université Laval



Bus stop: RTC # 1561 « de l'Université / des Sciences-Humaines ». Tickets are 2,85$, 1-day pass 7,50$ and 2-day pass 13,50$.

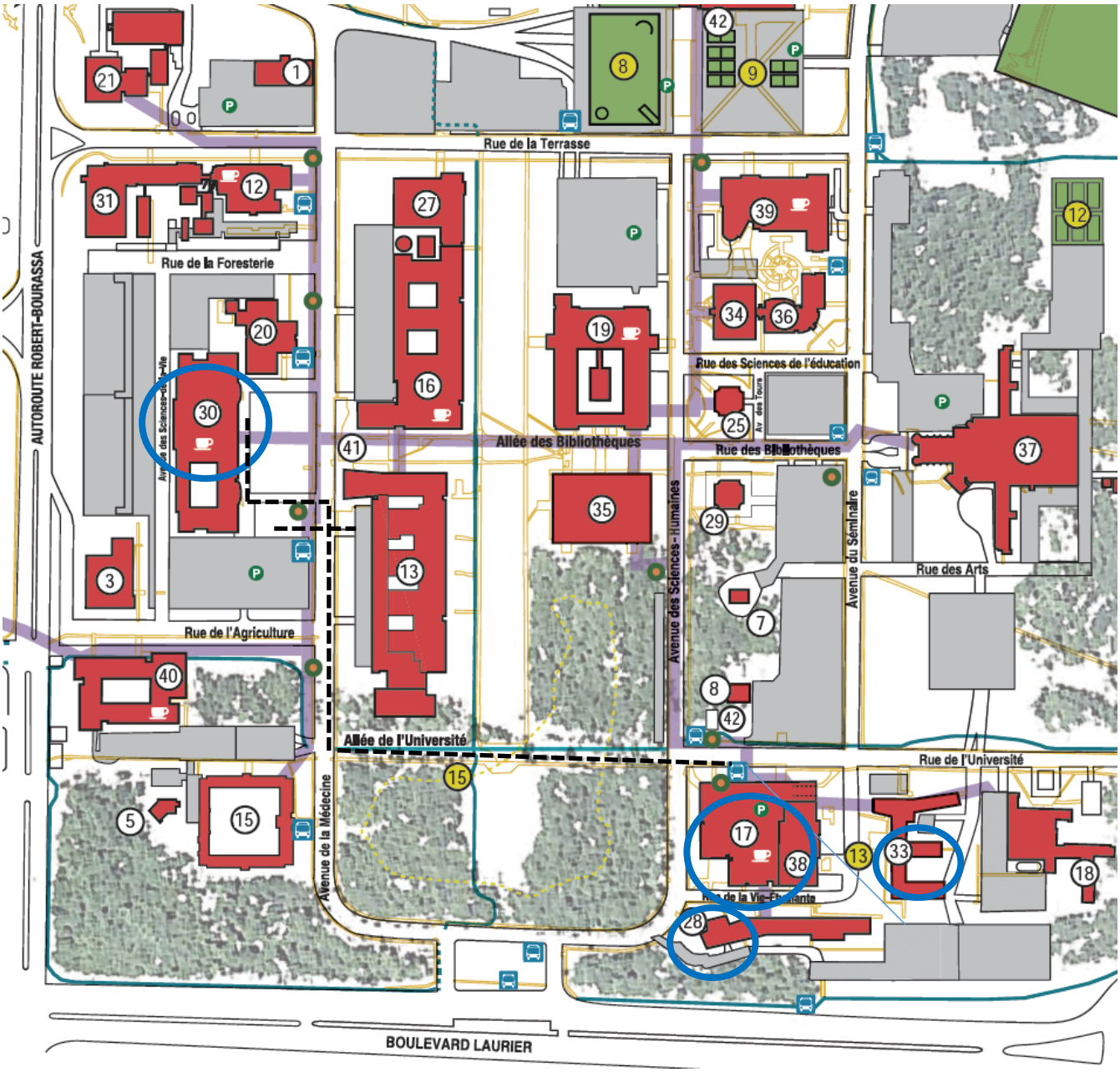
From **Gare d'autocars de Sainte-Foy** 3001, Chemin-des-Quatre-Bourgeois Québec (Sainte-Foy), take line 800 in direction of Beauport or 801 in direction of Charlesbourg (Est).

From **Gare de train de Sainte-Foy**, take line 13 in direction Du Versant-Nord/Sainte-Foy Centre/Saint-Louis or by taxi.

From **Gare de train de Québec** (downtown**)**, take line 800 (Arrêt 2558 Jean-Lesage / de la Gare-du-Palais) in direction of Pointe de Sainte Foy or line (5601 - de la Gare du Palais / Abraham-Martine Foy) in direction of Université Laval

From **Aeroport International Jean Lesage** by Taxi. There is a taxi line up at the terminal.

Map of the Campus of Université Laval



17: Pavillon Alphonse-Desjardins (the bus stop RTC No 1561 is right in front of it)

30: Pavillon Ferdinand-Vandry

28, 33: Buildings of Residence at University Laval (for accommodations booked through the CCTC 2015 website)

Paid parking is available on the visitor parking lots (follow the indications) 3,75$ per hour for a daily maximum of 17,25$ (weekdays only)

Foot-path between the buildings -----

Map of the Pavillions

<table>
<tr>
<td> 
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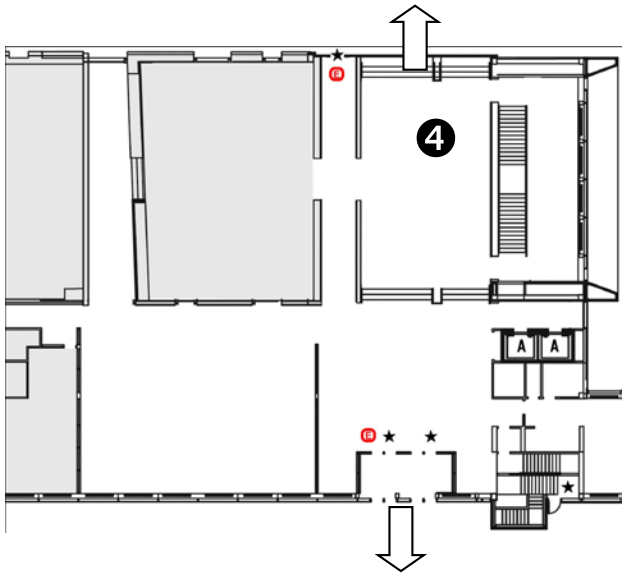
<td>Avenue de la médecine
 

Autoroute Robert Bourassa</td>
</tr>
<tr>
<td>Pavillon A. Desjardins 2nd floor
Conference Friday and Saturday
❶Amphitheatre Hydro Quebec
❷Grand Salon
❸ Elevators to the Cercle on 4th floor (gala dinner)</td>
<td>Pavillon Vandry
❹Cocktail Event Thursday May 28</td>
</tr>
</table>

Pavillon A. Desjardins: from the entrance level, take the elevator or central stairs to the 2nd floor. Registration table is at the top of the stairs on 2nd floor.

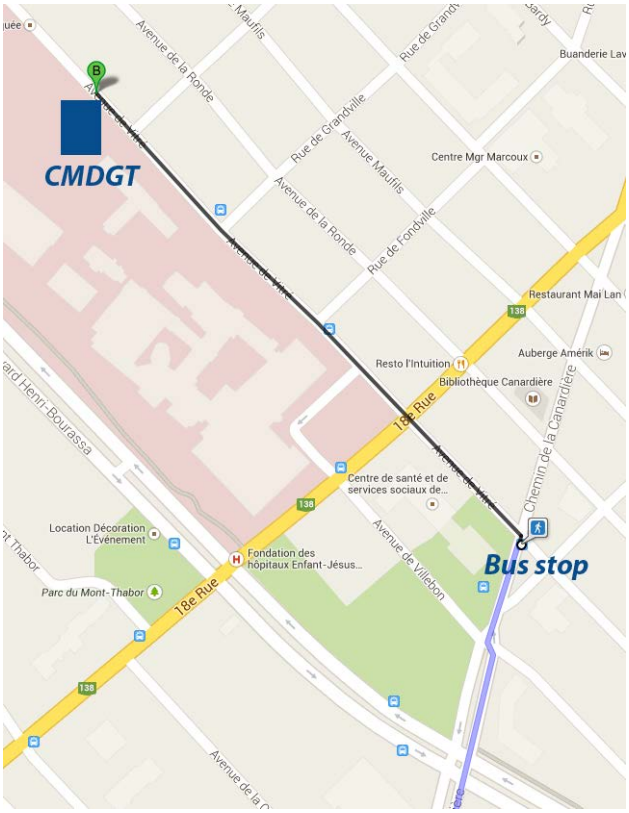
Map to the LOEX

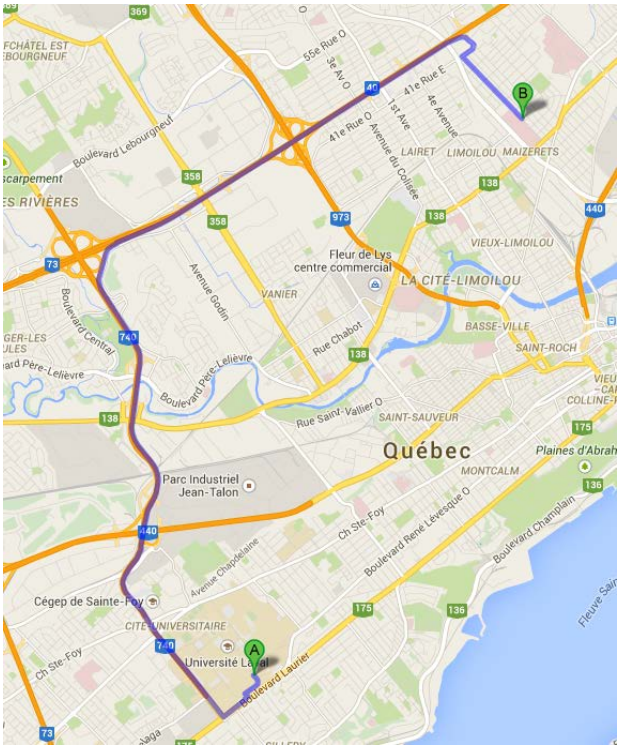
Web site: http://www.loex.qc.ca/
Address:
LOEX-Hôpital Enfant-Jésus, Aile R
CHU de Québec
1401, 18e Rue, Québec, G1J 1Z4
From Université Laval,

By bus: line 800 (just in front of Desjardins building at Université Laval), in direction of Beauport, stop at de la Canardière/Vitré (Enfant-Jesus Hospital) (around 35 min), walk on de Vitré ave (see map).

By car: Take Boulevard Laurier W/QC-175 S to Robert Bourassa Hwy/autoroute 740N; Follow Robert-Bourassa Hwy/autoroute 740N and autoroute 40E to Champfleury ave. Take exit 316 from highway 40E; Continue onto Champfleury ave; Turn right onto rue Antoine Silvy; Take the 3rd left onto de Vitré ave.

Free parking is available in adjacent streets (for time limitation, please check parking signs in the streets).





Event Sponsors

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UNIVERSITÉ LAVAL
Faculté de médecine
Département de chirurgie

The Arthritis Society

The Travel Awards have been made possible thanks to the generosity of the Arthritis Society and the organizers of CCTC 2014

Scientific Evaluation and Travel Awards Committee

François A Auger
Mari Kaartinen
François Berthod
Diego Mantovani
Cheryle Seguin
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Guillaume Grenier
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Laurence Cantin-Warren

General Information

<u>On-site registration:</u>

Cheques will be accepted for onsite registration up to the start of the conference. 250$ CAD for trainees/assistants and 125$ CAD for researchers. A limited number of tickets are available for late inscriptions to the gala dinner.

Invited Speakers:

Sylvie Ricard-Blum (IBCP, Lyon, France)

Guillaume Grenier (Université de Sherbrooke, Sherbrooke, QC)

Robert Gauvin (Université Laval, Québec, QC)

Chris Overall (University of British Columbia, Vancouver, BC)

Darius Bägli (University of Toronto, Toronto, ON)

In Memory of Dr. Cy Frank

A highly personal tribute to my friend and colleague, Cy Frank

It is with a heavy heart and much sadness that I write this month's Scientific Director's message. The sudden passing of our dear friend and colleague, Cy Frank, has shaken all of us to the core.

I have probably known Cy longer than most who have had the privilege of knowing him and working with him over the years. In the early 70's, we were medical students together at the University of Calgary. Cy was in the class ahead of mine. We were literally kids in a medical school that was itself in its adolescence. This forward-thinking school was the perfect breeding ground for "out of the box" thinkers and problem solvers like Cy. We shared fun times and serious times together. We played a few hockey games together. I remember he had what is often referred to by goalies as a "heavy" slap shot. In other words, if you had the misfortune of being in the net facing him, you knew it when his shot hit your glove!

I lost track of Cy after we graduated. He pursued a brilliant career in academic orthopedic surgery, establishing himself as a leading researcher in a field that most would agree is not known for producing scientists of his caliber. He was as equally comfortable discussing mesenchymal cell biology with his basic science colleagues as he was discussing models of care for joint arthroplasty with Ministers of Health. He helped establish an exemplary multidisciplinary musculoskeletal research centre, the McCaig Bone and Joint Centre at the University of Calgary, that seamlessly brought together clinical researchers, basic scientists, and biomechanics experts, to name a few.

He was the ideal inaugural Scientific Director for the Institute of Musculoskeletal Health and Arthritis in the newly-established Canadian Institutes of Health Research. He was given the daunting task of delivering strategic funding opportunities for a mandate that included arthritis, bone, muscle, musculoskeletal rehabilitation, oral health, and skin disease! "Here Cy…see what you can do with these…"

And he did. He developed the famous IMHA cube (see page 3). He put these mandated areas of research together into themes: physical mobility and health, inflammation and tissue repair, pain disability and health. He He gave it all a fresh new perspective that all could embrace.

He sought advice everywhere he could. Everyone wanted to work with Cy because he respected them and their perspectives. If he did not think you had a good idea, he did not embarrass you publicly; he would just point out why it was not a good idea without making it a personal attack. He got things done by consensus!

It was at this stage that I reconnected with Cy. I had the privilege of serving on IMHA's Institute Advisory Board (IAB) under his leadership. We had lots of serious things to do, but also had a lot of fun doing them. The devil's tail was established: if you made a particularly outrageous statement at a meeting, you got to

wear it (along with a set of horns) until someone else made an even more outrageous statement. Why did Denis Morrice seem to wear them most of the time!

One of Cy's favorite tools for setting research directions and priorities was the consensus conference. One of the first things he did as Scientific Director of IMHA was to collaborate with The Arthritis Society and the Canadian Arthritis Network Centres of Excellence to host an Osteoarthritis Consensus Conference that set the stage for major collaborations and strategic funding in this area. This was followed by a similar consensus conference in Inflammatory Joint Diseases, which I had the privilege of co-chairing. I still recall that after the conference was over, and we had numerous notebooks and flipcharts full of information, Cy insisted on keeping the key organizers behind for an extra half day on Saturday to make sense of the input. That made all the difference. We made sense of the chaos, and out of it we developed funding opportunities.

Two years ago, I had the privilege of being appointed the Scientific Director of IMHA. No sooner had I hung up the phone after speaking with Alain Beaudet and accepting the position, than I picked it up again and called Cy: "What do you think? Where should we go with IMHA now?" True to his name, he was "frank". In his typical clear yet diplomatic manner, he let me know the strengths and weaknesses of the Institute, and areas that may be the most productive. He agreed to continue to help in any way he could, and I took him up on his offer!

In 2013, in conjunction with IMHA's dedicated IAB, we began to develop a new Strategic Plan for the Institute. We organized a terrific brainstorming session in Vancouver in March of 2014 and brought together stakeholders from all over Canada, along with a number of international experts. At the end of the day, as he has always done so well, Cy brought it all together. He talked about one of his favorite topics: return on investment and performance measurement. He had chaired a committee for the Canadian Academy of Health Sciences that produced a thoughtful and timely report on this topic. The principles outlined in the report have since been adopted by CIHR as a whole in its evolving performance measurement regime. He gave us great advice as we developed our strategic plan and debated how we were to assess success down the road: "Be careful what you measure," he said, "because that is what you will get."

A few weeks ago when I heard that Cy had passed away suddenly, I was incredulous. I wandered around trying to understand this, but I still really cannot…how could he be gone when we can still feel his presence and impact all around us? There is an old Egyptian saying when someone passes away… "The rest is in your life." This saying gives me comfort in knowing that the rest is truly in our lives. It will be up to us to carry on his legacy and his vision.

Cy, we will miss you.

Hani El-Gabalawy MD FRCPC
Scientific Director
CIHR Institute of Musculoskeletal Health and Arthritis

http://www.cihr-irsc.gc.ca/e/49104.html

Gala dinner, celebration of the 30th anniversary of the LOEX

Centre de recherche de l'Université Laval

In 1985, the first research group dedicated to the reconstruction of human tissues by cell culture was established in Québec in the Hôpital Saint Sacrement. One year later, this unique initiative allowed the first autologous transplantation of reconstructed epidermis for the treatment of extensively burned patients in Canada. This success was the result of a fruitful collaboration between our dedicated plastic surgeons and the emerging team of Dr François A. Auger. The possibility of creating a complete skin equivalent *in vitro*, including both epidermal and dermal components, became the laboratory's central research theme. Later on, the LOEX adapted the methods developed for skin culture and applied them to the production of different tissues and organs: cultured blood vessels, ligaments, cornea, ... Thus, the LOEX was one of the first laboratories in the world to get involved in the field of organ reconstruction through **tissue engineering**.

In 2010, the Multidisciplinary Center for the Development of Tissue Engineering was built to give room to the expanding activities of the LOEX's research and to facilitate the application of tissue engineering methods to treat patients. The building at Hôpital de l'Enfant-Jésus du CHU de Québec houses approximately 125 persons including 9 full-time researchers, 15 associate researchers, 41 graduate students and postdoctoral fellows along with additional scientific and administrative staff.

François A Auger, MD, FRCP (C), CQ, FCAHS

Since 1985, François A. Auger, MD has been the director and founder of the Experimental Tissue Engineering Laboratory (LOEX), which is affiliated with Université Laval in Quebec City. Dr. Auger has completed his medical training at Laval University and was board certified in microbiology and infectious diseases at the University of Montreal. As a fellow of the McLaughlin Foundation of Canada,

he completed two years of postdoctoral training at the University of Maryland Cancer Center and then at the NIH National Institute of Allergy and Infectious Diseases in Bethesda. He is presently full professor of surgery at Laval University. Various granting agencies support the work of Dr. Auger and he has published more than 180 peer-reviewed publications. In 2003, Dr. Auger was appointed to the title of Chevalier of l'Ordre national du Québec. His expertise includes, but is not limited to, tissue engineering, especially for skin, cardiovascular substitutes and tissue microvascularization.

Thursday, May 28, 2015

10:00 am – 12:00 am	Optional visit of the LOEX Research Center – registration needed, transport is not provided (located outside the campus of Université Laval, see map)
6:00 pm - 8:00 pm	**Registration** - Vandry Building, Université Laval
7:00 pm - 10:00 pm	**Cocktail event** - Vandry Building, Université Laval

Friday, May 29, 2015

8:00 am - 17:00 pm	**Registration** - Desjardins Building, Université Laval
8:00 am - 8:45 am	**Breakfast** (Grand Salon – Desjardins Building)
8:45 am – 9:00 am	**Welcome Remarks** (Veronique Moulin – Julie Fradette, chairs of CCTC 2015) (Amphitheatre HydroQuébec – Desjardins Building)
9:00 am – 10:33 am	**Session 1: Connective Tissue Remodeling I** Session chairs: Boris Hinz & Pardis Pakshir
9:00 am – 9:45 am	**Invited Speaker: Sylvie Ricard-Blum (IBCP, Lyon, France)** *"Endostatin, a multifunctional matricryptin at the crossroads of several interaction networks*"
9:45 am	**Brandon H Kim** (Western University, London) *"M-CSF induces redistribution of ezrin-radixin-moesin proteins in migrating osteoclasts.*"
9:57 am	**Sotcheadt Sim** (École Polytechnique, Montreal) *"Wound Healing Revealed by a Novel Automated Indentation Technique.*"
10:09 am	**Jake Bedore** (Western University, London) *"Assessing the effect of notochord-specific CCN2 deletion on intervertebral disc degeneration and behaviour associated with back pain.*"
10:21 am	**Julia M Pasquale** (University of Toronto, Toronto) *"Why do only some women develop postmenopausal osteoporosis?*"
10:33 am – 11:03 am	**Coffee Break and Poster viewing** (Grand Salon)
11:03 am – 12:15 pm	**Session 2: Connective Tissue Remodeling II** Session chairs: Stephen Sims & Noelle Ochotny
11:03 am	**Matthew Tsang** (Western University, London) *"Investigating the mechanisms of formation and myofibroblastic differentiation of skin-derived precursors.*"
11:15 am	**Ryan J Beach** (Western University, London) *"Role of subcellular calcium elevation in polarization of osteoclasts toward the chemoattractant M-CSF.*"

11:27 am	**Nuno M Coelho** (University of Toronto, Toronto) *"Discoidin Domain Receptor 1 Mediates Collagen Remodeling By Compaction and Internalization.*"
11:39 am	**Yongqiang Wang** (University of Toronto, Toronto) *"Adseverin Mediates Alveolar Bone Loss Associated with Inflammation.*"
11:51 am	**Sally Esmail** (University of Toronto, Toronto) *"Elucidating where and why the V-ATPase "a" subunit isoforms a1, a2, and a4 are glycosylated.*"
12:03 pm	**Heena Kumra** (McGill University, Montreal) *"Synergistic effects of cellular and plasma fibronectin in tissue organization in vivo.*"
12:15 pm – 2:00 pm	**Lunch and poster session I** (evaluation for even number posters only) (Grand Salon)
	Canadian Connective Tissue Society board meeting - Room 3425
2:00 pm – 2:30 pm	**In memoriam to Dr. Cy Franck** : Hani El-Gabalawy (Scientific Director of the Institute of Musculoskeletal Health and Arthritis, Canadian Institutes of Health Research) *"Cy Frank's legacy and IMHA strategic plan"*
2:30 pm – 4:00 pm	**Session 3: Musculoskeletal System I** Session chairs: Lisbet Haglund & Emerson Krock
2:30 pm – 3:00 pm	**Invited Speaker: Guillaume Grenier (Université de Sherbrooke, Sherbrooke, QC)** *"Microenvironment influence on skeletal muscle progenitor cells*"
3:00 pm	**Derek H Rosenzweig** (McGill University, Montréal) *"An ex vivo model for dynamic culture and tissue repair of human intervertebral disc degeneration.*"
3:12 pm	**Sam Si-Hyeong Park** (University of Toronto, Toronto) *"Intramedullary Wire Fixation of Bone Allograft for Reconstruction of Critically-Sized Radius Segmental Defect in a New Zealand White Rabbit Model.*"
3:24 pm	**Noelle Ochotny** (Western University, London) *"Fluid Flow-Induced Migration of Osteoclasts: Crawling Against the Tide.*"
3:36 pm	**Juliana Marulanda** (McGill University, Montreal) *"Role of Matrix Gla Protein in Craniofacial Development.*"
3:48 pm	**Gabrielle Martel** (Université de Montréal, Montreal) *"Epiphyseal cartilage vascular and matrix changes at sites predisposed to osteochondrosis detected by 3T MRI, second harmonic generation microscopy and histology.*"
4:00 pm – 4:30 pm	**Coffee Break** (Grand salon)

4:30 pm – 5:54 pm	**Session 4: Musculoskeletal System II** Session chairs: Jeffrey Dixon & Yara Hosein
4:30 pm	**Yara Hosein** (Western University, London) *"Comparison of Mechanical Measures Used to Assess Orthodontic Mini-Implant Stability.*"
4:42 pm	**Emerson Krock** (McGill University, Montréal) *"Toll-like Receptor 2 Regulates Nerve Growth Factor via NF-κB Signalling in Intervertebral Disc Cells.*"
4:54 pm	**Nicolas Anne-Archard** (Université de Montréal, Montreal) *"Histological characteristics of new-born foals metacarpal growth cartilage: to a better understanding of the pathophysiology of osteochondrosis.*"
5:06 pm	**Michael P Grant** (McGill University, Montréal) *"Ca2+ Regulates the Expression of Type II Collagen and Aggrecan in Intervertebral Disc Cells by Activating the Extracellular Calcium-Sensing Receptor.*"
5:18 pm	**Nicholas Mikolajewicz** (McGill University, Montréal) *"Role of exosomes released by breast cancer and myelogenous cells in stimulation of osteoclast formation by cancer-derived factors.*"
5:30 pm	**Ryan Marinovich** (Western University, London) *"Role of Bone Sialoprotein in the Tendon-Bone Insertion.*"
5:42 pm	**Yoon Chi** (McGill University, Montréal) *"CD109 inhibits TGF-beta signaling and ECM protein production in human chondrocytes.*"
7:30 pm	**Gala Dinner** – at "the Cercle" (fourth floor of Desjardins Building) **Guest speaker: François A Auger** (Director, Centre de recherche en organogénèse expérimentale de l'Université Laval /LOEX)

Saturday, May 30, 2015

8:00 am - 9:00 am	**Breakfast** (Grand salon)
9:00 am – 10:06 am	**Session 5: Tissue Engineering and Biomaterials I** Session chairs: Julie Fradette & Meryem Safoine
9:00 am – 9:30 am	**Invited Speaker: Robert Gauvin (Université Laval, Québec, QC)** *"Functional tissue engineering: Optimization of the ECM architecture through microfabrication and bioreactor technologies*"
9:30 am	**Maxime Picard-Deland** (Université Laval, Québec) *"Self-Assembled Tubular Heart Valves from Human Fibroblasts.*"
9:42 am	**Corey Sermer (**University of Toronto, Toronto) *"Platelet rich plasma enhances the integration of bioengineered articular cartilage with native tissue.*"
9:54 am	**Amélie Morissette** (Université Laval, Québec) *"A Reconstructed Urethra by Tissue*

	Engineering: In Situ Grafting in Rabbits for Proof of Concept."
10:06 am – 10:48 am	**Coffee Break and Poster viewing** (Grand salon)
10:48 am – 12:00 am	**Session 6: Tissue Engineering and Biomaterials II** Session chairs: Jean-Francois Beaulieu & Jake Bedore
10:48 am	**Derek H Rosenzweig** (McGill University, Montreal) *"Nucleus pulposus and chondrocyte cell growth and tissue deposition on 3D-printed scaffolds.*"
11:00 am	**Bastien Paré** (Université Laval, Québec) *"Early detection of extracellular matrix disorganization and cytoplasmic accumulation of TDP-43 in tissue-engineered skins derived from ALS patients: an optimized approach to recover secreted proteins for biomarker discovery.*"
11:12 am	**Vanessa Bianchi** (University of Toronto, Toronto) *"Articular Cartilage Tissue Engineering with Redifferentiated Human Chondrocytes.*"
11:24 am	**Caroline Loy** (Université Laval, Québec) *"Tri-Culture of Vascular Cells from Cellularised Collagen-based Scaffolds Promotes Vascular Tissue Remodeling.*"
11:36 am	**Julien Tremblay Gravel** (McGill University, Montréal) *"The culture of Nucleus Pulposus Cells on a continuously expanding culture surface enhances their phenotype for tissue engineering purposes.*"
11:48 am	**Kiefer Thouin** (Université Laval, Québec) *"Study of the impact of glycation in wound healing using a tissue-engineered skin.*"
12:00 am – 2:00 pm	**Lunch and poster session II** (Evaluation for odd number posters only) - Grand Salon
2:00 pm – 3:30 pm	**Session 7: Connective Tissue Remodeling III** Session chairs: Dieter Reinhardt & Heena Kumra
2:00 pm – 2:30 pm	**Invited Speaker: Chris Overall (University of British Columbia, Vancouver, BC)** *« New Biological Roles for MMPs Revealed by Proteomics of the ECM in vivo»*
2:30 pm	**Beatriz Noé** (Université de Montréal, Montreal) *"Development of a biomarker for osteoarthritis: Cathemsin K degradation of cartilage.*"
2:42 pm	**Ali Virk** (McGill University, Montreal) *"LPS-Induced Inflammation Inhibits Bone Repair.*"
2:54 pm	**Abdualltef Alrashoudi** (McGill University) *"Calcium suppresses parathyroid hormone-related peptide-induced synthesis of aggrecan and type II collagen in osteoarthritic chondrocytes.*"
3:06 pm	**Muhammad Naji I Albesher** (McGill University, Montreal) *"Degeneration of Articular Cartilage is mediated by Calcium and the Extracellular Calcium-Sensing*

	Receptor in Osteoarthritis."
3:18 pm	**Rahul Gawri** (University of Toronto, Toronto) *"Enhancing Nucleus Pulposus Tissue Formation In-Vitro: A Novel Approach.*"
3:30 pm – 4:00 pm	**Coffee Break** (Grand salon)
4:00 pm – 5:30 pm	**Session 8: Cell – Extracellular Matrix Interactions** Session chairs: Anie Philips & Yoon Chi
4:00 pm – 4:30 pm	**Invited Speaker: Darius Bägli (University of Toronto, Toronto, ON)** *« Urinary bladder smooth muscle-matrix interactions: molecular and epigenetic insights »*
4:30 pm	**Ingrid Saba** (Université Laval, Québec) *"Development of a three-dimensional (3D) cell culture model that mimics female genital mucosae.*"
4:42 pm	**Iris Boraschi-Diaz** (McGill University, Montreal) *" Regulation of osteoclast formation by collagen type I degradation products.*"
4:54 pm	**Salah Boudjadi** (Université de Sherbrooke, Sherbrooke) *" Pro-proliferative α1 integrin subunit over-expression is regulated by the c-MYC oncogenic factor in colorectal cancer cells.*"
5:06 pm	**Dieter P Reinhardt** (McGill University, Montreal) *"Fibrillin-1 interaction with cells regulates microRNAs.*"
5:18 pm	**Husam Almajed** (McGill University, Montreal) *"Comparison between osteoarthritic and osteonecrotic bone marrow-derived mesenchymal stem cells: Differences in the expression of the extracellular calcium-sensing receptor and osteogenesis.*"
5:30 pm – 6:00 pm	**CCTS General Assembly Meeting** (for all members) **Oral presentation, poster and travel awards announcements** **Closing Remarks**

Sessions 1&2: Connective Tissue Remodeling

Invited Lecture

Endostatin, a multifunctional matricryptin at the crossroads of several interaction networks

Sylvie Ricard-Blum (UMR 5086 CNRS - University Lyon 1, France)

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The extracellular matrix (ECM) determines the shape and organization of tissues, provides them with mechanical properties and regulates cellular processes. ECM is remodeled in many diseases such as fibrosis, cancer and diabetes and its remodeling releases bioactive fragments, called matricryptins, which have biological activities of their own (*Ricard-Blum and Salza, Exp Dermatol 2014 23:457-63*). We are currently studying endostatin, a matricryptin of collagen XVIII, which has anti-angiogenic and anti-tumoral properties. Since most biomolecules exert their biological activities by interacting with other molecules, we have built the interaction network of endostatin in order to determine if it has further molecular functions and/or regulate further biological processes. Using protein and glycosaminoglycan arrays probed by surface plasmon resonance imaging, we have identified 20 new partners of endostatin including transglutaminase-2 (*Faye et al. Biochem J 2010*), procollagen C-proteinase enhancer-1 (*Salza et al. Biochem J 2014 457:137-49*), glycosaminoglycans (heparan sulphate, hyaluronan), matricellular proteins (SPARC, thrombospondin-1), the ectodomains of membrane collagens (XIII and XVII) and the □-amyloid peptide. We have shown *i*) that endostatin is targeted, together with other regulators of angiogenesis, by pathogens and contributes to the interactions of these pathogens (*Leishmania* parasites) with the ECM of the host (*Fatoux-Ardore et al. Infect Immun 2014 82:594-606*), and *ii*) that endostatin binds to the □-amyloid peptide and to several ECM proteins expressed in brains, that it is present in the cerebrospinal fluid of patients with Alzheimer's disease (AD) and that the measurement of its concentration relative to established markers of AD improves the discrimination of AD from other neurodegenerative diseases (*Salza et al. J Alzheimers Dis 2015 44:1253-61*). We are currently studying the molecular mechanisms used by endostatin to regulate angiogenesis in infectious diseases and in Alzheimer's disease and the molecular interconnections of its interaction network with the networks of other ECM proteins involved in these processes, namely procollagen C-proteinase enhancer-1 and collagens XIII and XVII. This integrative approach mimics what happens *in vivo* where interactions influence each other and will allow us to identify the interactions, which are specific of a molecular step/mechanism in angiogenesis and/or Alzheimer's disease and are potential therapeutic targets.

M-CSF induces redistribution of ezrin-radixin-moesin proteins in migrating osteoclasts

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Introduction: Osteoclasts are dynamic cells that mediate bone resorption, playing a crucial role in bone remodelling. Osteoclasts migrate along the bone surface, while releasing acid and enzymes to resorb bone. Migrating osteoclasts assume a polarized morphology, with outgrowth of the lamellipod at the leading edge and retraction of the uropod at the rear. Ezrin-radixin-moesin (ERM) proteins are a family of actin binding proteins, which, when activated by phosphorylation, link actin filaments to integral membrane proteins. Ezrin and moesin are suggested to be key regulators of uropod organization in migrating cells. However, the role of ERM proteins remains poorly understood in osteoclasts.\\ Objective: Our goal is to understand the role of ERM proteins in osteoclasts. We hypothesized that directed migration of osteoclasts is accompanied by localization of activated ERM in the uropod.\\ Methods: Osteoclasts were isolated from long bones of neonatal rats and were allowed to adhere to the substrate for 30 minutes. Migration was induced by the chemoattractant, macrophage-colony stimulating factor (M-CSF). Osteoclasts were fixed, blocked with 10% bovine albumin, and rabbit anti-ERM (Inactive) or rabbit anti-phospho-ERM (Active) primary antibodies were applied, followed by goat anti-rabbit secondary antibody conjugated with Alexa Fluor 555. Controls included processing without primary antibodies as well as with a rabbit monoclonal isotype control antibody. Cells were visualized using a Zeiss Axiovision Imager M2M upright microscope.\\ Results: In unstimulated osteoclasts, inactive ERM was generally localized throughout the cytosol and we did not observe any change in localization following treatment of osteoclasts with M-CSF. In contrast, activated ERM was present in the cytosol, but also showed patchy localization at the perimeter of unstimulated osteoclasts. When we induced directed migration of osteoclasts by focal application of M-CSF, we observed marked localization of activated ERM. Intense labeling of activated ERM was apparent in the uropod of migrating osteoclasts, whereas there was virtually none associated with the actin-rich ruffles at the leading edge of the lamellipodia.\\ Conclusion: Emerging evidence points to involvement of the protein family ERM in polarization of migrating cells. Here we show for the first time that activated ERM redistributes to the uropod of migrating osteoclasts, implicating these proteins in the establishment of cell directionality.

Wound Healing Revealed by a Novel Automated Indentation Technique

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Introduction: Mechanical characterization of wound healing in skin samples mostly relies on uniaxial tensile rupture tests, which provide local information along the wound and are disruptive for samples (Chao et al., 2011). In this study, we wanted to test the ability of a novel automated indentation technique to non-destructively characterize mechanical properties of the entire wound and its integration with the surrounding skin. //Methods: Wounded pig skin samples (4x8cm centered 3 cm incision length) were placed skin surface up on a flat platform of a multiaxial mechanical tester equipped with a 6.35mm diameter spherical indenter under its load cell (Mach-1v500css, Biomomentum Inc., Canada). Following top-view photodocumentation, a position grid (>130 positions) was superimposed over the image. At each position, the tester was programmed to precisely measure overall skin thickness through detection of contact with the surface and to perform an indentation ramp of 1.5mm at 200µm/s. Subsequently, the sample was reshaped in two adjacent dumbbell-shaped strips (perpendicularly to the wound), mounted in tension grips and tensile rupture tests were performed at 2mm/s. //Results: High-resolution mapping of maximum load and thickness were generated (about 30s per position). These mappings revealed significant spatial variation of the mechanical properties and thickness over the wound region compared to the uniform properties of the intact skin observed at least 1cm away from the incision site. Also, quantitative information about the hypertrophy/atrophy of the wound could be calculated where a sample revealed a 12.25 mm2/mm of wound hypertrophy. Considering the load at rupture in the tension tests, a correlation could be observed with the maximum load in indentation (or thickness) measured at the rupture site. //Conclusion: Despite the low number of samples (n=2), these results indicate that the novel automated indentation technique can provide a novel assessment of mechanical properties revealing 2-dimensional distribution over the wound and its surrounding areas. These measurements can complement the rupture force, which provides insight on the basic mechanical function of the wound along its surface (maintaining the wound closed). Since the technique is non-destructive for the sample, it allows additional analyses (tensile, histology or biochemical assessment) to be performed at matched positions. Moreover, analysis of the relaxation curve could provide viscoelastic parameters. This high spatial resolution and non-destructive technique, provides new opportunities when studying wound healing where the number of animals involved could be significantly reduced.

Assessing the effect of notochord-specific CCN2 deletion on intervertebral disc degeneration and behaviour associated with back pain

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Introduction: Currently, our ability to treat intervertebral disc (IVD) degeneration is hampered by an incomplete understanding of the pathways regulating disc aging and the proteins contributing to symptomatic vs. asymptomatic disc degeneration. In particular, the specific function of matricellular proteins including CCN2, and their ability to modulate these pathways remains enigmatic. Recently, we reported that a mouse strain with targeted deletion of CCN2 from the notochord and nucleus pulposus (NP) (referred to as CCN2-deficient) demonstrated aberrant matrix content during development and accelerated age-related degeneration. Herein, we further characterize the mechanism underlying IVD degeneration observed in these mice by examining markers of inflammation and matrix breakdown. Moreover, we investigate whether these mice show behaviours indicating discogenic back pain. Methods: IVD tissues were assessed in notochord-specific CCN2-deficient mice and littermate controls at 13 and 18 months of age using gene expression and immunohistochemical analyses. Behaviours associated with axial back pain were also evaluated using established grip force, tail suspension and locomotor assays to measure stretch-induced axial discomfort. Results: Compared to control mice, CCN2-deficient mice showed increased gene expression of the proinflammatory cytokines Tnf-α and Il-1β at 13 months of age, and increased expression of matrix-metalloproteinase (Mmp)3 and brain-derived neurotrophic factor (Bdnf) at 18 months of age. Immunolocalization showed a striking increase in the accumulation of MMP-mediated aggrecan cleavage products in the NP of CCN2-deficient mice compared to controls at 18 months of age. CCN2-deficient mice showed reduced resistive force during the grip force test relative to controls at both 13 and 18 months of age. 13-month-old CCN2-deficient mice also show decreased time spent in full extension and an increased time spent self-supported in tail suspension, indicative of increased discomfort induced by axial stretching. Lastly, the locomotor activity of CCN2-deficient mice at 13 months of age was significantly reduced compared to controls immediately following tail suspension, indicative of increased movement-evoked discomfort. Significance: We demonstrate that CCN2 expression by nucleus pulposus cells regulates IVD tissue health, as accelerated degeneration in CCN2-deficient mice was associated with inflammatory cytokine expression and MMP-mediated matrix degradation. Furthermore, our findings suggesting that CCN2-deficient mice demonstrate symptomatic disc degeneration. The ability of CCN2 to regulate the composition of the intervertebral disc suggests that it may represent an intriguing target for the treatment of disc degeneration. Further, our CCN2-deficient mouse model may be useful to probe the connection between disc degeneration and associated pain.

Why do only some women develop postmenopausal osteoporosis?

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Background: Postmenopausal osteoporosis develops due to a decrease in estrogen and a subsequent increase in bone turnover rate, which leads to net bone loss. However, only 25-30% of postmenopausal women develop clinical osteoporosis (fractures). The increased presence of advanced glycation endproducts (AGEs) has been demonstrated in both postmenopausal osteoporotic patients and in ovariectomized (OVX) animal models. AGEs are also known to enhance resportion and inhibit bone formation. When AGEs bind to the Receptor for Advanced Glycation Endproducts (RAGE), signaling pathways are activated and pro-inflammatory factors are released, which may stimulate resportion. There is natural variation in postmenopausal women in the amount of estrogen lost and the amount of AGEs in their bones; therefore these factors together could determine why only certain women develop postmenopausal osteoporosis. We hypothesize that OVX rats have increased osteoclast RAGE expression, and that the amount of bone lost due to OVX is higher when bone AGE content is higher.\\ Methods: Female Wistar rats were divided into six groups of ten. Three groups were fed a high fructose diet to induce AGE formation, and three groups were fed an isocaloric control diet. After three months one group from each diet were OVX, sham-operated or sacrificed. The surgical groups continued on a maintenance diet and were sacrificed three months later. Bone architecture and density was assessed through imaging of the femur and vertebrae using micro-computed tomography. Blood serum was analyzed for AGEs and soluble RAGE content using ELISA, while HPLC assays were used to determine AGE content in bone. Histology techniques including immunohistochemistry and histomorphometry are currently being employed to investigate tissue-level bone remodeling as well as the content and localization of membrane-bound RAGE and AGEs within the bone.\\ Results: MicroCT showed a decrease in trabecular bone volume, number and density due to OVX, while cortical bone density and microarchitecture remained unchanged. OVX also caused an increase in the soluble RAGE detected one month after surgery. HPLC and ELISA did not show a significant difference between the surgical groups in circulating AGE levels, although AGE content was detected in both bone and serum.\\ Conclusions: OVX showed significant trabecular bone deterioration and increased soluble RAGE content, while AGEs were present at similar levels in surgical groups. The increase in RAGE suggests the receptor is up regulated due to OVX; however tissue-level analysis will further determine whether RAGE has a role in postmenopausal bone loss.\\

Investigating the mechanisms of formation and myofibroblastic differentiation of skin-derived precursors

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Skin-derived precursors (SKPs) are multipotent spheroid-forming cells cultured from the dermis that express markers of neural crest progenitor cells including Nestin and Sox2. Recent evidence suggests that fibrosis in multiple tissues can occur through the differentiation of progenitor cells – including those which express neural crest markers – into myofibroblasts. CCN2 (CTGF) is a marker and mediator of fibrosis, and is highly expressed by cells during myofibroblast differentiation. In this study, we attempt to elucidate the cellular origin of SKPs, and identify the role of CCN2 in the differentiation of SKPs into myofibroblast-like cells. We use lineage tracing techniques to show that SKPs originate primarily from Col1a2-expressing dermal fibroblasts, and that basic fibroblast growth factor (bFGF) induces the reprogramming of mouse dermal fibroblasts cultured in monolayer to become Nestin+, Sox2+ SKPs. Furthermore, we show that a Col1a2-specific deletion of CCN2 impairs the ability of SKPs to differentiate into α-SMA-expressing myofibroblast-like cells, and that this impairment is associated with changes in cell surface receptor signaling, cell adhesion, and collagen degradation clusters of gene expression. Finally, we show that inhibition of focal adhesion kinase (FAK) and serum response factor (SRF) pathways prevent the induction of CCN2 and the differentiation of SKPs into myofibroblast-like cells. Taken together, these results suggest that collagen-producing fibroblasts possess a high degree of inherent plasticity, and that CCN2 is a downstream mediator of myofibroblast differentiation in progenitor cells.

Role of subcellular calcium elevation in polarization of osteoclasts toward the chemoattractant M-CSF

Benjamin D. Wheal, Ryan J. Beach, S. Jeffrey Dixon and Stephen M. Sims

Background and Rationale\\ A number of cytokines elicit directed migration of osteoclasts, including macrophage colony-stimulating factor (M-CSF), transforming growth factor beta and receptor activator of NF-kB ligand. Recruitment of osteoclasts and their mononuclear precursors to the bone is an essential first step in bone remodeling and is critical for the expansion of metastatic tumors in bone.\\ Objective\\ Our goal was to study subcellular changes of cytosolic Ca2+ in osteoclasts to understand their role in directing chemotaxis.\\ Methods\\ Osteoclasts were isolated from rat long bones and loaded with the Ca2+-sensitive dye fura-2. Dual wavelength fluorescence imaging was used to map cytosolic free Ca2+ concentration ([Ca2+]i) in motile cells. To study the functional importance of Ca2+ signaling, osteoclasts were loaded with the Ca2+ chelator, BAPTA, by incubating in BAPTA-AM for 30 min.\\ Results\\ Focal stimulation of osteoclasts with M-CSF elicited prompt lamellipodial ruffling, followed by lamellipod extension and migration toward the source of M-CSF. We found that M-CSF causes acute elevation of [Ca2+]i, apparent as a Ca2+ wave originating nearest the source of M-CSF and propagating distally through the cytosol. This elevation of [Ca2+]i was reduced when osteoclasts were treated with the phospholipase C inhibitor U73122, supporting a role for Ca2+-release from intracellular stores. The functional importance of Ca2+ signaling was examined by studying osteoclasts loaded with BAPTA to blunt changes of cytosolic Ca2+ concentration. Notably, osteoclasts continued to exhibit pronounced lamellipodial ruffling, however BAPTA impaired steering and directed migration in response to M-CSF.\\ Conclusions\\ In summary, we demonstrate for the first time that M-CSF causes acute rise of [Ca2+]i in osteoclasts and that rise of [Ca2+]i is required for polarization and directed migration of osteoclasts toward M-CSF.

Discoidin Domain Receptor 1 Mediates Collagen Remodeling By Compaction and Internalization

Nuno M. Coelho, Pamma D. Arora and Christopher A. McCulloch

The discoidin domain receptor 1 (DDR1) has been associated with collagen remodeling by matrix metalloproteinases but the contributions of DDR1 to collagen remodeling by traction and internalization are not defined. Using DDR1 siRNA knockdown or over-expression of DDR1, we found that DDR1 expression levels were weakly associated with matrix metalloproteinase 1 or 13 expression levels or with pericellular proteolysis of collagen in mouse fibroblasts and human breast cancer cells. In contrast, DDR1 expression levels were strongly associated with increased compaction of fibrillar collagen and enhanced collagen internalization, processes that involve non-muscle myosin IIA (NMIIA). By immunoprecipitation we found that NMIIA associates with the C-terminal kinase region of DDR1 and this region was essential for collagen compaction and internalization for intracellular degradation. The enhanced association of DDR1 with NMIIA was closely linked to collagen-induced DDR1 activation by tyrosine phosphorylation. Inhibition of DDR1 tyrosine phosphorylation with Nilotinib reduced DDR1 association with NMIIA by 60% and inhibited collagen compaction and internalization by 2-fold. These data indicate that DDR1 association with NMIIA enables mechanically-mediated collagen compaction and internalization.

Adseverin Mediates Alveolar Bone Loss Associated with Inflammation

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The cortical actin cytoskeleton is remodeled by the calcium-regulated actin-binding protein adseverin in chromaffin cells but how adseverin contributes to cortical actin remodeling, cell fusion and osteoclastogenesis in pre-osteoclasts has not been defined. We found that the fusion of pre-osteoclasts is dependent in part on the formation of cell extensions, which exhibited extensive actin remodeling at cell fusion sites in cells stably transfected with Lifeact-GFP. By using either microarray and Western blotting or microarray alone we and others found that adseverin, a member of the gelsolin family of actin capping and severing proteins, was strongly expressed in murine and human cells during osteoclastogenesis. We found that knockdown of adseverin in the osteoclast precursor cell line RAW264.7, or conditional knockout of adseverin in primary monocytes, decreased the ability of RANKL (RAW264.7 cells) or RANKL and M-CSF (primary cells) to induce cell extension formation and osteoclastogenesis. In luciferase assays, adseverin promoter activity was strongly increased by RANKL, which was further enhanced by IL-1beta. In an in vitro model of osteoclastogenesis, IL-1beta enhanced RANKL-induced cell fusion and increased tartrate-resistant acid phosphatase expression. In contrast, IL-1beta did not affect cell fusion in cells from adseverin knockout animals. We found that bone structure, morphology and turnover were normal in the long bone and jaws of mice with conditional deletion of adseverin. Experimental periodontitis initiated by silk ligatures caused marked alveolar bone loss in wild type mice within 4 weeks but bone loss was prevented in adseverin-conditional null mice. We conclude that adseverin is an important regulator of actin filament assembly and cell fusion in pre-osteoclasts at inflammatory sites. The discovery that adseverin mediates inflammation-mediated alveolar bone loss, suggests that adseverin may be a therapeutic target for prevention of alveolar bone loss in periodontitis.\\ Key words: Adseverin, osteoclastogenesis, inflammation, periodontitis.

Elucidating where and why the V-ATPase "a" subunit isoforms a1, a2, and a4 are glycosylated

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Background: Vacuolar H+-ATPases (V-ATPases) pump protons from the cytoplasm to luminal and extracellular compartments. Osteoclast V-ATPases are responsible for acidifying bone surfaces, essential for bone resorption, while plasma membrane V-ATPases on metastatic cells acidify extracellular spaces to facilitate cellular invasion. The "a" subunit is the largest of 14 different subunits making up V-ATPases. In mammals there are four isoforms, a1–a4. Evidence suggests that the isoforms are responsible for targeting V-ATPase to specific locations. Isoforms a1 and a2 are localized to intracellular compartments and a3 is localized to the osteoclast membrane while a4 is in the apical membrane of α-intercalated cells of distal nephrons. In cancer cells, plasma membrane expression of a3 and a4 are required for cellular invasion. Our lab has shown that the a3 subunit is glycosylated, however, the glycosylation status of a1, a2, and a4 subunits is unknown. Hypothesis: Glycosylation of "a" subunits is important for assembly, folding and/or targeting of V-ATPases. Objective and significance: To elucidate unique features of a subunit isoforms that govern structure, function and targeting of V-ATPases in order to facilitate development of specific therapeutics to prevent bone resorption or cell metastasis. Methods: Mammalian cells were transfected with the FLAG-tagged version of the V-ATPase a1, a2 and a4 subunits with or without mutations that eliminate putative N-glycosylation sites. Expression and localization were assessed by immunoblotting and immunofluorescence. Glycosylation was assessed by mutational analysis and treatment with endoglycosidases. Results: Immunoblots of extracts from cells transfected with FLAG-tagged a1, a2 or a4 revealed a diffuse 95-100 kDa band. When treated with Endo-H or PNGaseF, the bands decreased to 93-95 kDa suggesting that a1, a2 and a4 subunits are all glycosylated with high mannose oligosaccharide. Immunoblots of a1, a2, and a4 bearing a2-N484Q + N505Q, a1-N495Q and a4N489D/Q mutations, respectively, resulted in identical 93-95 kDa bands showing that N489, N484 +N505, and N495 are sites of N-glycosylation for a1, a,2 and a4, respectively. Cycloheximide pulse chase experiments suggested that unglycosylated a2 and a4 subunits degraded faster than N-glycosylated forms. Immunofluorescence showed unglycosylated a4 cannot exit the ER while ongoing co-immunoprecipitation studies suggest that it does not associate with the ATP-binding B subunit. Conclusions: All mammalian V-ATPase a subunits are glycoproteins. For a4, glycosylation is required for ER exit, protein stability, and complex assembly. Investigations are underway to determine whether glycosylation of all V-ATPase a isoforms are important for assembly, folding, and/or targeting of the V-ATPase complex.

Synergistic effects of cellular and plasma fibronectin in tissue organization in vivo

Heena Kumra, Laetitia Sabatier, Marian Chen, Amani Hassan, Takao Sakai, Pierre Chambon, Dieter P. Reinhardt

BACKGROUND: Fibronectin (FN) is an abundant and ubiquitously expressed protein present in plasma and in the extracellular matrix (ECM) of various connective tissues. Soluble plasma FN (pFN) is synthesized by liver hepatocytes, whereas cellular FN (cFN) is secreted and assembled by various mesenchymal cells including fibroblasts and smooth muscle cells. FN plays a crucial role in development, demonstrated by the early embryonic lethality (E8.5) of the FN null mouse. Evidence from cell culture systems exists demonstrating that FN is a “master organizer and stabilizer” for the assembly of various connective tissue proteins including fibrillin-1, collagen I and III and latent TGF-β binding protein-1 (LTBP-1). While the experimental in vitro evidence supports the concept of FN as a master organizer, it is not clear whether it has a similar role in tissue organization in vivo, and what the specific contributions are of the two FN isoforms. \\HYPOTHESIS: In this project we hypothesize that cellular and plasma FN play synergistic roles in tissue organization. \\RESULTS: To test this hypothesis, the Cre-lox recombination technology was used to generate a smooth muscle-specific and tamoxifen-inducible cFN knockout (KO) mouse model to target blood vessels and lungs. To study the role of pFN in these organs, a liver-specific FN KO mouse model, in which pFN becomes deleted postnatally at P3 was generated. In addition, we have also produced a double KO (dKO) mouse model of cFN and pFN. In preliminary analyses of aorta and lungs from the inducible cFN KO mouse, a reduced and disorganized smooth muscle layer was observed in the bronchioles of experimental mice as compared to the controls. The aortic wall of the experimental mice was also disorganized, featuring numerous breaks and forks in the elastic lamellae. In addition, there was reduced and loose deposition of collagen in both tissues. pFN infiltration was observed in these tissues indicating that pFN can partially replace the function of cFN. In preliminary analyses, the pFN KOs did not have any obvious histological phenotypes. However, the tamoxifen injected dKO mice die postnatally between P6 – P15, indicating a severe phenotype when both FN isoforms are absent. This study indicate a synergistic effect of cFN and pFN in tissue organization of blood vessels and lungs.

Sessions 3&4: Musculoskeletal system

Invited Lecture

Microenvironment influence on skeletal muscle progenitor cells

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Skeletal muscle possesses an incredible regenerative capacity, thanks to its resident progenitor cells. Unfortunately, this potential can be impaired following a trauma and could lead to the formation of mature bone, so-called heterotopic ossification (HO). Our previous work demonstrated that contrary to other bone morphogenetic proteins (BMPs), BMP-9 can induce muscle HO only in damaged muscle, underscoring the robust influence of the microenvironment on the onset of HO pathophysiology. In this presentation, we will describe ongoing experiments that demonstrate the multifaceted impact of BMP-9 on muscle resident progenitor cells, triggering imbalanced muscle regeneration. More specifically, we will show the effect of BMP9 on muscle progenitor cells, and propose a hypothesis explaining the shift of their myogenic potential towards an osteogenic one. On the other hand, we will demonstrate how BMP9 could potentiate the osteogenic differentiation of the muscle resident stromal cells within damaged muscle. In a clinical point of view, these results illustrate the complexity of the interrelations between the microenvironment and progenitor cells during tissue regeneration. In addition, the strong osteogenic activity of BMP9 combined with an important source of muscle resident progenitor cells could be advantageously used for developing novel strategies aiming to heal persistent fractures.

An ex vivo model for dynamic culture and tissue repair of human intervertebral disc degeneration

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Low back pain is directly related to intervertebral disc (IVDs) degeneration, a common ailment with tremendous healthcare and socioeconomic costs. Long-term organ culture of intact IVDs is necessary to develop ex vivo models of human IVD degeneration and repair, where the relationship between mechanobiology, disc matrix composition and metabolism can be better understood in the context of degenerative disease. To establish this ex vivo organ culture model, a bioreactor was developed that facilitates organ culture of intact human lumbar discs in a controlled, dynamically loaded environment. The bioreactor is used in combination with a previously reported harvesting method which maintains the integrity of the intervertebral discs by preserving the non-calcified part of the cartilage endplate with collagen fibers attached to it. Here, we determine IVD tissue integrity and cell viability under 3 different loading conditions (low: 0.1-0.3 MPa; medium: 0.1-0.6 MPa; high: 0.1-1.2 MPa), and investigate the suitability of this model towards cell supplementation for tissue repair. After 14 days in dynamic culture, cell viability was maintained at greater than 80% throughout the disc at low and medium loads, yet dropped to approximately 70% (NP) and 50% (AF) under high loads. Proteoglycan content remained stable in all loading schems (approximately 50 µg sGAG/mg tissue). No fragmentation of chondroadherin was observed with the low, medium or high loading regimes. Labeled human NP cells injected into discs within an HA-pNIPAM hydrogel showed strong localization to the NP region after 14 days of medium dynamic loading. This study indicates feasibility of culturing human IVDs for 14 days under various physiological (moderate to strenuous) loads, as well as in the presence of injected cell therapies. When combined with the capabilities of the bioreactor, this ex vivo approach can help to elucidate the role of load in both disc degeneration and tissue regeneration.

Intramedullary Wire Fixation of Bone Allograft for Reconstruction of Critically-Sized Radius Segmental Defect in a New Zealand White Rabbit Model

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PURPOSE: Pre-clinical in vivo testing in animal models plays a critical role in advancing new orthopaedic therapies/implants for potential use in human patients. The New Zealand White rabbit (NZWr) radius segmental model is an appropriate long bone defect model to test biocompatibility of graft materials because the NZWr bone exhibits Haversian remodeling similar to human bone, matching the tubular shape of the radius in implants is easier, the model's reproducibility allows for comparison of graft materials, and reportedly no fixation is required due to the support of the intact ulna. However, given the significant curvature of the radius, we would argue that fixation is required to achieve a stable anatomic reconstruction and avoid non-unions associated with biomechanical instability. The aim of this study was to establish and evaluate intramedullary (IM) wire as the ideal fixation method within the NZWr radius defect model.\\METHODS: Ten female retired breeder NZWr (7-9 months old) were used. Younger rabbits with open growth plates were excluded to avoid epiphyseal slipping. Ethics approval was obtained. Bone allografts were first harvested from both radii of five donor age-matched female retired breeder NZWr. Under general anesthesia, a 15-mm critically-sized defect was created in the radius (mid-diaphysis) of recipient NZWr using a micro-oscillating saw. The previously harvested donor allograft was then cut and sized to fit the radial defect, and a 0.8 mm Kirschner wire (K-wire) was used for graft fixation as an IM device. The reconstructions were monitored with bi-weekly radiographs until sacrifice at 12 weeks. A musculoskeletal radiologist quantified the bony healing based on the radiographic scoring system described by An and Friedman. Upon retrieval of the reconstructed radii at 12 weeks, the specimens were scanned with microCT and analyzed with static histomorphometry.\\RESULTS: Stable IM wire fixation was achieved in nine rabbits, with only one graft minimally displacing after slight K-wire migration at 2 weeks. Radiographic scoring analysis revealed no healing at 2 weeks, mild-moderate periosteal bridging callus localized to the osteotomy sites and 50% bridging union from 6-8 weeks. Cortical remodeling was noted at 10 weeks. By 12 weeks, there was complete proximal osteotomy site union, near full circumferential union at the distal osteotomy site, significant cortical remodeling, and periosteal callus formation over the entire reconstruction, while still maintaining the interosseous space between the radius and ulna. Union and cortical remodeling at 12 weeks were confirmed with microCT and histologic analysis. No infections or mortality were observed.\\CONCLUSION: This study demonstrates that IM wire fixation in the NZWr radius segmental model successfully achieves an anatomic and stable construct to accurately study the biology of large segmental defect reconstruction using bone allograft.

Fluid Flow-Induced Migration of Osteoclasts: Crawling Against the Tide

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Background: Cells display a wide range of directed movements in response to environmental cues. One of these is rheotaxis, the orientation and migration of a cell in response to extracellular fluid flow. Positive rheotaxis, the tendency of a cell to move against the flow, was first reported for spermatozoa. When bone experiences mechanical loading, fluid flows through its canalicular network. Osteoclasts are large multinuclear cells responsible for bone resorption. The ability of fluid shear stress (FSS) to affect osteoclast behaviour is poorly understood. Our goal was to investigate the effects of FSS on osteoclasts. We hypothesized that fluid flow directs osteoclast movement.\\ Materials and Methods: Osteoclasts were isolated from the long bones of neonatal Wistar rats. Fluid flow was applied to single osteoclasts by using glass micropipettes with tip diameters of 12-15 µm. The micropipettes, filled with the same medium bathing the cells, were placed about 200 mm from the cell, and fluid flow (2-4 mL/min) was directed towards the osteoclast. Osteoclast morphology and motility were monitored using time-lapse phase-contrast microscopy.\\ Results: Migrating osteoclasts were identified by 1) a polarized morphology, consisting of a lamellipod at the leading edge and a uropod at the rear (trailing edge), and 2) net displacement of the osteoclast's centroid of at least 50 mm toward the micropipette tip over a 40-minute observation period. In many cases, FSS induced prompt polarization, turning, and migration of osteoclasts toward the flow. As a control, micropipettes were positioned in a similar manner but without flow. To quantify the effect of FSS on osteoclast movement, the net displacement of the osteoclast's centroid from the point of origin was measured over a 40-minute period. Of the 38 osteoclasts exposed to FSS, 28 migrated at least 50 mm against the flow (toward the micropipette tip). In the absence of FSS, 10 of 10 control osteoclasts showed random movement and no significant orientation toward the micropipette tip. Chi-square analysis revealed significant positive rheotaxis in response to fluid shear ($p < 0.01$).\\ Conclusion: Our data reveal for the first time that osteoclasts exhibit positive rheotaxis in response to FSS. In vivo, osteoclasts are known to accumulate at sites of microcracks. Fluid flow from microcracks may serve to recruit osteoclasts and initiate bone remodeling at these sites. \\ This study is supported by the Canadian Institutes of Health Research.

Role of Matrix Gla Protein in Craniofacial Development

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Introduction: Craniofacial defects constitute a significant part of congenital deformities in humans. Both environmental and genetic factors may lead to abnormal growth of orofacial skeletal elements affecting the overall structure of the face. For example, fetal exposure to warfarin leads to Warfarin Embryopathy, a congenital disease characterized by severe midface hypoplasia and depressed nasal bridge. Warfarin impairs vitamin K-mediated post-translational gamma carboxylation of several Gla proteins. Likewise, mutation in a Gla protein called Matrix Gla Protein (MGP) leads to Keutel Syndrome, a rare genetic disease, hallmarked by midface hypoplasia and abnormal calcification of cartilaginous tissues. To date, it is not known how MGP affects midface development. Also, it is not known whether inactivation of Osteocalcin (Oc), another Gla protein, which shares ancestral origins and structural similarities with MGP, may also lead to midface hypoplasia. In the current study, we characterized a mouse model that lacks MGP and examined the possible mechanism underlying the facial abnormalities seen in this model. // Objectives: Characterize the craniofacial phenotype in the Mgp-/- mice and investigate the underlying cause of the craniofacial abnormalities in these mice. // Methods and Results: In the current study, we compared the craniofacial phenotype of both Mgp-/- and Oc-/- mice. Although there was a severely malformed skull in Mgp-/- mice, no such abnormalities were seen in Oc-/- mice. These findings suggest that Oc does not play any role in craniofacial development. Then, we analyzed the craniofacial phenotype of the Mgp-/- mice by cephalometric analyses of micro-CT images and found a severe midface hypoplasia and a class III malocclusion caused by impaired maxillary growth. Alizarin red staining of the cranial skeleton, von Kossa staining of cranial sections and micro-CT imaging showed ectopic calcification of the nasal septum of the Mgp-/- mice indicating that MGP deficiency in the cartilaginous tissues is the cause of the observed facial hypoplasia. Finally, we showed that transgenic restoration of Mgp expression in the chondrocytes alone is sufficient to prevent the craniofacial anomalies in Mgp-/- mice. // Conclusions: MGP deficiency in mice causes mid-face hypoplasia and skeletal malocclusion. The local expression of Mgp in the cartilaginous tissues prevents the calcification of the nasal septum cartilage and plays a major role in craniofacial development.

Epiphyseal cartilage vascular and matrix changes at sites predisposed to osteochondrosis detected by 3T MRI, second harmonic generation microscopy and histology

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INTRODUCTION : Osteochondrosis (OC) is an ischemic chondronecrosis of epiphyseal growth cartilage (EGC) and a focal failure of endochondral ossification leading to osteochondritis dissecans (OCD). It afflicts many juvenile animals and humans at specific sites in susceptible joints bilaterally. The upstream events leading to the focal ischemia remain to be elucidated. Hypothesis: Subregional variations in EGC matrix proteoglycan (PG) and collagen in neonatal foals predisposed to OC render it susceptible to focal trauma of cartilage vessels. Objectives: Investigate femoral trochlea EGC: 1. Matrix with quantitative 3T MRI (qMRI) and second harmonic generation (SHG) microscopy; 2. Vascularity with 3T MRI susceptibility-weighted imaging (SWI). MATERIAL & METHODS : MRI: Femoral trochleas of OC predisposed (n=5) and control (n=4) foals were imaged ex vivo with 3T MRI. Sequences were T1ρ for PG assessment; T2 for collagen structure/content and SWI for vascularity. Mean T1ρ and T2 relaxation times were measured at each ROI (n=8) for intra-site comparisons (OC and control groups) and inter-site comparisons. Histology & SHG: Site-matched sections were stained with safranin O fast green (SOFG) for PG content and immunochemically for type II collagen. SHG scans were made at selected sites for collagen ultrastructural assessment. RESULTS : T1ρ and T2 relaxation times were significantly increased (p

Comparison of Mechanical Measures Used to Assess Orthodontic Mini-Implant Stability

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\\Stability of mini-implants, used as skeletal anchorage devices during orthodontic treatment, is achieved by mechanical retention at the screw-bone interface. However, mini-implant loosening remains a complication associated with its use. As such, appropriate measures are required to assess mini-implant stability to better predict their clinical success. Within the literature, techniques for measurement of mini-implant mechanical stability have been discussed, but few studies have compared them to determine their level of association with one another. This study investigated various mechanical measures, to determine their agreement and reliability in assessing mini-implant stability. \\Ninety self-drilling orthodontic mini-implants from six different manufacturers were inserted into artificial bone blocks (Sawbones®) using a custom-made device. A load sensor (6 DOF, Advanced Mechanical Technology Inc.,) secured at the base of the bone block measured insertion torques during mini-implant placement. Immediately following mini-implant insertion, the mobility of the implanted systems was assessed using the Periotest® Stability Measurement System (Periotest®). Subsequently, the mini-implants were placed in a materials testing machine (Instronâ 8874) for pull-out testing. To compare the stability measurements (insertion torque, Periotest value, pull-out load, pull-out displacement) for the various mini-implants, coefficients of variation (% COVs) were used to test the reliability among the various measures, and multiple correlation analyses were used to test the strength of the agreement between the individual stability measurements. \\Overall, Periotest showed greatest variability in its results (% COV: 11–100%) compared to insertion torque (≤ 11%), pull-out load (≤ 4%) and displacement (≤ 19%), for all mini-implants. When testing agreement between the measures, the strongest correlations were found between insertion torques and Periotest values ($r = -0.68$; $p < 0.0001$), and insertion torques and pull-out load ($r = 0.66$; $p < 0.0001$). In comparison, mini-implant displacements showed weak (Periotest: $r = -0.42$; $p < 0.0001$) or no ($p > 0.05$) correlations with other stability measures. \\Stable mini-implants are characterized by high insertion torques, low Periotest values and high pull-out loads. Since Periotest showed the greatest variability compared to insertion torque and pull-out load, it may be less reliable in predicting mini-implant stability. Based on the correlations found between insertion torque, Periotest and pull-out load, these measures agree in their assessment of mini-implant stability. However, it is important to note that each of these measures assess uni-directional stability of mini-implants, and therefore, should be used in collaboration (i.e., not independently) to comprehensively predict the clinical success of mini-implants.

Toll-like Receptor 2 Regulates Nerve Growth Factor via NF-κB Signalling in Intervertebral Disc Cells

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Introduction: Intervertebral disc degeneration is a leading cause of chronic low back pain (LBP), but how degeneration contributes to LBP is poorly understood. Nerve growth factor (NGF) levels increase during disc degeneration and evidence suggests NGF promotes disc innervation, neuronal sensitization and low back pain, making NGF a possible therapeutic target. However, current anti-NGF therapeutics have limited efficacy. Mechanisms leading to increased NGF in the disc are poorly understood and the molecular signaling mechanisms regulating NGF in inflammatory mediator rich environments, such as a degenerating disc, are unknown. During degeneration, proteoglycans and extra-cellular matrix (ECM) proteins are degraded and fragmented. ECM fragments can act as endogenous danger signal ligands for toll-like receptors (TLR). TLR activation induces cytokine and chemokine expression, and could thus regulate expression of inflammatory mediators, such as IL-1β or TNFα, during disc degeneration. NGF is often increased in environments with elevated levels of inflammatory mediators.// Hypothesis: TLR activation induces NGF expression in intervertebral disc cells.// Methods: Non-degenerate human intervertebral discs from organ donors without a history of low back pain were collected for cell isolation. NP and AF tissues were separated and enzymatically digested. Cells were treated with IL-1β (control), peptidoglycan (PGN, TLR2 agonist) and lipopolysaccharide (LPS, TLR4 agonist). siRNA was used to knockdown TLR2 in cells prior to treatment with PGN. Activated signaling pathways were evaluated by western blot. Small molecule inhibitors blocking p38 MAPK (SB203580) and NF-κB (BMS-345541) activity were added to cell cultures in combination with IL-1β and PGN. NGF expression was evaluated by qRT-PCR after 6 hours, western blot and ELISA after 48 hours.// Results: TLR 2 activation significantly increased NGF gene expression in NP cells following 6, 12 and 24 hours of treatment and in AF cells after 24 hours of treatment, while TLR 4 had little effect. TLR 2 activation also significantly increased NGF protein after 48 hours in NP and AF cells while TLR 4 activation did not increase NGF levels. TLR2 knockdown showed that induced NGF expression is directly regulated by TLR 2 rather than by cytokines produced in response to TLR activation. TLR activation induced p38 MAPK and p65 (NF- κB pathway) in disc cells. Blocking the p38 pathway had little effect on NGF expression, while blocking NF-κB significantly decreased TLR 2 induced NGF expression.// Conclusion: These results suggest TLR2 activation directly increases NGF expression via NF-κB signaling. These results represent a novel regulatory mechanism NGF, which could lead to the development of new therapeutic targets to target NGF and treat LBP associated with disc degeneration.

Histological characteristics of new-born foals metacarpal growth cartilage: to a better understanding of the pathophysiology of osteochondrosis

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Osteochondrosis (OC) is a developmental orthopedic disease affecting both humans and horses. In the juvenile epiphyses avascular articular cartilage overlies a vascularized growth cartilage that is transformed to bone at the ossification front. The vessels are housed in cartilage canals. OC is characterized in the early stages of disease by a focal chondronecrosis of the growth cartilage, that does not undergo subsequent endochondral ossification. This necrotic zone fissures to form a and osteochondral fragments that cause joint pain. It is impossible to study the early stages of the disease in humans but studies of the naturally occurring disease in animals such as horses provides valuable insight into its etiology, which remains to be fully elucidated. Focal ischemia of the growth cartilage is believed to be one of the earliest events in the development of OC. Our objective was to investigate cartilage and cartilage canals in joints of OC predisposed and control newborn foals to detect structural differences that could predispose to OC. OC predisposed foals (OC: n=5) were conceived by embryo transfer from OC parents and non OC control foals (Control: n=3) were euthanized for non-orthopedic reasons at the veterinary teaching hospital. The distal 3rd metacarpal (MCP) bone was harvested and sections were obtained from multiple sites were OC arises and control sites. All slides were then digitalized for image processing. An analysis of the cartilage canals was performed to detect differences between groups and sites within groups. Parameters assessed included cartilage canal number, surface density, circularity, predominant cartilage canal angle relative to the section plan (ossification front), and cartilage thickness. Data was analyzed by general linear models with Bonferroni adjustments. Significant differences were detected between sites within the joints. The sagittal ridge of MCP, the most common site of clinical OC, had significantly more cartilage canals and a greater cartilage thickness compared to other areas within the joint. The dorsal part of the sagittal ridge where clinical OC arises also had a wider range of diameter of cartilage canals (small, medium and large) compared to the palmar part of the same zone where only small and medium canals were identified. The sites where OC arises in newborn equine MCP epiphyses had thicker cartilage and larger cartilage canals. Combined, this structure may predispose the vessels to focal trauma at these sites leading to focal ischemia. Future studies are planned to investigate the biomechanical properties of the cartilage at these sites

Ca2+ Regulates the Expression of Type II Collagen and Aggrecan in Intervertebral Disc Cells by Activating the Extracellular Calcium-Sensing Receptor

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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. The role of IVD calcification in the development DDD is unknown. Our preliminary data suggest that ionic calcium content and expression of the extracellular calcium-sensing receptor (CaSR) are increased in the nucleus pulpous (NP) and annulus fibrosis (AF) of degenerate discs, however, its role in DDD remains unclear. Material and Methods IVD Cells: Bovine and human NP and AF cells were incubated in culture media supplemented with various concentrations of calcium (1.0, 1.5, 2.5, 5.0 mM) a CaSR agonist [5 μM], or IL-1β [10 ng/ml] for 7 days. Lysates were extracted and the expression of aggrecan and type II collagen (Col II) were measured by Western blotting. IVD Cultures: Caudal IVDs from the tails of 20-24 month old steers were isolated and the vertebral bone was removed. IVDs were cultured for 4 weeks in culture medium supplemented with calcium (1.0, 2.5, or 5.0 mM), or a CaSR agonist [5 μM]. NP and AF tissue were subjected to guanidium extraction and Western blotting was performed to determine the expression of aggrecan and Col II. Histological sections were prepared to determine degree of mineralization by von Kossa staining and expression of alkaline phosphatase. Results - The expression of aggrecan and Col II decreased dose-dependently in both NP and AF cells following supplementation with calcium or the CaSR agonist. A similar phenomenon was observed for the expression of aggrecan and Col II in IVDs following calcium supplementation. In addition to decreases in Col II and aggrecan, increases in mineralization and expression of alkaline phosphatase, and the pain marker NGF, were observed in IVDs supplemented with calcium. Conclusion - Our results suggest that changes in the local concentrations of calcium are not benign, and that activation of the CaSR may be a contributing factor in IVD degeneration. Acknowledgements - This research is supported by the Canadian Institute of Health Research (CIHR) and AO Foundation.

Role of exosomes released by breast cancer and myelogenous cells in stimulation of osteoclast formation by cancer-derived factors

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Breast cancer commonly metastasizes to bone, resulting in painful osteolytic lesions. We have previously shown that breast-cancer derived factors can directly stimulate the formation of bone resorbing cells, osteoclasts. Breast cancer-derived factors were shown to induce osteoclast formation from late precursors in a RANKL-independent manner. Using mass spectrometry, we identified a number of proteins present in the extracellular fluid of breast cancer cells, and confirmed that removal of these proteins results in reduction of osteoclastogenic potential of breast cancer cells. Interestingly, these proteins are known for their cytosolic functions, but are not normally expected to be found in the extracellular milieu. Recently, exosomes, nanovesicles of 40-100 nm in diameter, were implicated in a number of processes, including information transfer by cancer cells. We hypothesize that such exosomes may be responsible for the transport of the osteoclastogenic proteins from tumor cells to osteoclast precursors. We isolated exosomes from the MDA-MB-231 human breast carcinoma cells and K562 human myelogenous leukemia cells through differential centrifugation. The morphology of these exosomes was examined using electron microscopy, their size was determined using nanoparticle tracking analysis, and the expression of exosomal markers CD9 and TSG101 for MDA-MB-231 and transferrin receptor 2 (TFR2) for K562 cells was confirmed by immunoblotting. Using immunoblotting, we confirmed that the purified exosomes contain the potential osteoclastogenic proteins. Thus, exosomes released by breast cancer cells can potentially participate in activation of osteoclast formation at the sites of bone metastasis, resulting in the formation of a permissive environment for metastatic growth of incoming tumor cells.

Role of Bone Sialoprotein in the Tendon-Bone Insertion

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Background The site at which tendons and ligaments insert into bone, the enthesis, is important for musculoskeletal stability and locomotion. Entheses transition from tendon through a fibrocartilaginous tissue into bone. Within the fibrocartilage a sharp boundary, the tidemark, separates mineralized tissue from soft tissue. A common injury is the separation of tendons and ligaments from bone. Current restorative surgical techniques are inadequate, as they exhibit high failure rates. Bone sialoprotein (BSP) is an intrinsically disordered phosphoprotein that has been shown to promote the mineralization of calcified tissues. We hypothesize that BSP is involved in mineralization of entheses, facilitating the attachment of ligaments and tendons to bone. \\ Methods Animals studied were male 129/CD1 mice, age 15 weeks. BSP was localized by immunohistochemistry. Haematoxylin and eosin staining was used to assess the gross morphology. Three parallel transverses were measured from tidemark to the bone interface in the entheses of five Bsp-/- and wild type (WT) littermates. Tidemark-to-bone transverses were also used to measure mineral content using microCT and Raman spectroscopy. Mineral content was determined from the ratio of the 960 cm-1 hydroxyapatite peak to the 2940 cm-1 collagen peak. Patella-patellar tendon-tibia complexes were isolated and prepared for mechanical testing as described (Miller et al., J Biomech Eng 134(3) 2012). \\ Results Immunohistochemical analyses show that BSP is present in the calcified fibrocartilage (CFC) of the quadriceps tendon enthesis (QCT) and supraspinatus tendon enthesis (SST). In the absence of BSP the CFC remains mineralized. The size of the Bsp-/- QCT enthesis is 229.1±17.5 µm compared to the WT 178.8 ±15.5 µm (n=5, p

CD109 INHIBITS TGF-BETA SIGNALING AND ECM PROTEIN PRODUCTION IN HUMAN CHONDROCYTES.

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Transforming growth factor-beta (TGF-β) is a multifunctional growth factor that maintains the integrity and function of cartilage by promoting the synthesis of extracellular matrix (ECM) proteins such as type II collagen. Dysregulation of TGF-β action is implicated in joint tissue diseases as osteoarthritis (OA). Therefore, determining the factors that regulate TGF-β action in cartilage may provide a novel strategy to improve cartilage function. TGF-β initiates its action by binding to the TGF-β type II receptor which activates the TGF-β type I receptor (Activin-receptor Like Kinase 5, ALK5), followed by phosphorylation of the intracellular proteins, Smad2/3. The activated Smad2/3 forms a heteromeric complex with Smad4 and translocates to the nucleus leading to regulation of gene transcription. Purpose: Our group has reported the discovery of CD109 as a novel TGF-β co-receptor and has shown that CD109 is a potent negative regulator of TGF-β signaling and action in skin. The objectives of the current study were: (i) to determine whether CD109 is expressed in human articular chondrocytes, and (ii) to examine whether CD109 regulates TGF-β signaling and ECM protein expression in human primary articular chondrocytes. Methods: Primary articular chondrocytes were isolated from normal cartilage harvested from adult patients with traumatic open joint injury who have no history of OA or from osteoarthritic cartilage from OA patients at hip replacement surgery; or harvested from facet joint cartilage of organ donors from Transplant Quebec. CD109 expression in chondrocytes was determined by Western blot. Furthermore, CD109 function was analyzed by blocking CD109 expression using CD109-specific siRNA. The TGF-β-induced transcriptional activity, the level of the receptor expression, Smad phosphorylation, and ECM protein production were analyzed. Results: Our results show that CD109 is expressed in human articular chondrocytes and that loss of CD109 expression markedly enhances TGF-β type I receptor (ALK5) expression, TGF-β-induced phosphorylation of Smad3, Smad3-driven transcriptional activity, and expression of type II collagen and plasminogen activator inhibitor-1 (PAI-1). Conclusion: Our findings suggest that CD109 negatively regulates TGF-β receptor expression and signaling, and TGF-β-induced ECM production in human articular chondrocytes, and thus may play an important role in maintaining cartilage function and integrity.

Sessions 5&6: Tissue engineering and biomaterials

Invited Lecture

Functional tissue engineering: Optimization of the ECM architecture through microfabrication and bioreactor technologies

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There is a clinical need for engineered tissues intended for regenerative medicine applications. It is possible, through various approaches, to produce living engineered tissues using synthetic materials or to use the extracellular matrix (ECM) produced by cells in culture as a scaffold to support three-dimensional growth various cell types. It has previously been shown that tissue-engineered 2D and 3D constructs can be produced using fibroblasts and smooth muscle cells (SMCs). By combining these approaches with microfabrication techniques and bioreactor technologies, it is possible to develop living tissues or decellularized ECM sheets presenting anisotropic properties that can recapitulate the physiologic architecture of native tissues. These anisotropic tissues can be either grown on microfabricated substrates presenting a specific topography allowing for cell alignment and directing ECM assembly, or stimulated in a dynamic environment also resulting in specific tissue structure and organization. This overlap between engineering, cell biology and biomedical sciences highlights the vast potential of collaborative and interdisciplinary work performed in the field of regenerative medicine.

Self-Assembled Tubular Heart Valves from Human Fibroblasts

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High hopes are placed on tissue engineered heart valves (TEHVs) to circumvent the restricted availability of allografts, the coagulation risks caused by mechanical valves and the limited durability of pericardial bioprostheses. In an effort to replicate the complex physiological geometry, cell culture has been done on a wide variety of three-dimensional supports, from decellularized xenogeneic valves to polymeric scaffolds. Although these methods have shown promising results, many difficulties have yet to be overcome such as rejection, partial recellularization and post-operative contraction. Instead of the native valve geometry, a simple tubular shape has recently been suggested, allowing easy fabrication, fast implantation, and minimal crimped footprint from a transcatheter delivery perspective. This minimalistic design is well-suited for the self-assembly method, with which the only support to the cells is the extracellular matrix they themselves produce, allowing the tissue to be completely free from exogenous materials during its entire fabrication cycle. The objective of this research is to adapt the self-assembly tissue engineering method to the design of tubular heart valves.\\ Tubular constructs were produced by rolling human fibroblastic self-assembled sheets on solid mandrels. After maturation, the tubes were installed at the root of an artificial aorta, fixed along their entire circumference on the ventricular side and with only three single point attached commissures (SPACs) on the aortic side. This allows the tube to collapse under back-pressure, adopting a shape similar to that of a tri-leaflet heart valve during diastole. A custom pulsed flow bioreactor was used to evaluate the valves performances. The tissue microstructure was analyzed at its different fabrication steps using Masson's trichrome staining.\\ Preliminary results show good functionality with excellent leaflet coaptation and opening area, confirming the effectiveness of the tubular concept, the fixation method and the choice of diameter. Aortic flow conditions have yet to be tested and durability has to be assessed. Histology confirmed abundant collagen content, good cell distribution and homogeneous tube fusion. This research shows that the self-assembly method, which has already proven its potential for small diameter vascular grafts, can be used to achieve functional tubular heart valves.

PLATELET RICH PLASMA ENHANCES THE INTEGRATION OF BIOENGINEERED ARTICULAR CARTILAGE WITH NATIVE TISSUE

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Background: Osteoarthritis (OA) is a debilitating disease characterized by degradation of articular cartilage and subchondral bone remodeling. Current therapies for early or midstage disease range from lifestyle modifications to arthroscopic procedures. These procedures do not regenerate articular cartilage, and therefore there is great interest in developing biological approaches to cartilage repair. Some of these approaches are limited by their inability to integrate with the native cartilage. We have shown previously that platelet rich plasma (PRP) can enhance cartilage tissue formation. PRP is obtained from a patient's own blood, and once activated, is an autologous source of many growth factors, cytokines and other molecules which may aid in healing. This raised the question as to whether PRP could enhance tissue integration.// Hypothesis: PRP will enhance integration of bioengineered cartilage with native cartilage.// Methods: Chondrocytes were isolated from bovine metacarpal-phalangeal joints, seeded on a porous bone substitute (calcium polyphosphate (CPP)) and grown in the presence of FBS to form an in vitro model of osteochondral-like tissue. After 7 days, the biphasic constructs were soaked in PRP for 30 minutes prior to implantation into the core of a ring-shaped biphasic explant of native bovine cartilage and bone. Controls were not soaked in PRP. The resulting implant-explant construct was cultured in a stirring bioreactor in serum free conditions for 2 weeks. The integration zone was visualized histologically. A push-out test was performed to assess the strength of integration. Matrix accumulation at the zone of integration was assessed biochemically. Significance (p

A Reconstructed Urethra by Tissue Engineering: In Situ Grafting in Rabbits for Proof of Concept

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Introduction//Urethral pathologies often need reconstruction but procedure complications and the limited availability of urologic tissues raised our interest for the development of a tissue-engineered urethral substitute. We propose a substitute produced by the self-assembly method, free of exogenous materials. The aim was to create a rabbit model for autologous urethral replacement in vivo.//Materials and Methods// Enzymatic cell extraction was accomplished from bladder and skin biopsies. Harvested dermal (DF) and vesical (VF) fibroblasts produced and assembled their own extracellular matrix and sheets were rolled on a mandrel. VFs were used in order to produce a more organ specific tissue. The impact of the use of DF, group A or DF and VF mixed together, group B, on mechanical proprieties and graft results was evaluated. Ring tensile tests were performed to evaluate load failure and strain as well as estimated burst pressure. Suture retention tests were also done. To demonstrate the proof of concept, reconstructed urethras (1,5cm long, 6.2mm diameter) were grafted in 4 rabbits. Allogeneic circumferential replacement surgeries were performed. Characterization was performed by histology (Masson's Trichrome) and immunofluorescence.//Results and discussion//Just as porcine and human VF, when cultured in two dimensions, rabbit fibroblasts from bladder clearly organized their extracellular matrix differently to the DF. After 3 weeks of maturation, 3D constructs presented suture retention strength of 23,64±3,99 gram-forces (gf) for group A and 36,75±5,74 gf for B. The estimated burst pressure averaged 458,40±101.62mmHg for group A and 559,18±73,60mmHg for B, whereas a native porcine urethra reached a maximum of 418±66mmHg. Exploratory surgeries confirmed the feasibility of the technique used for the reconstruction and provided information about future improvement. The size and mechanical resistance of tissues were appropriate for suturing to the native urethra. Four weeks after the surgery, vascularisation was abundant and urothelial cells completely covered the urethral lumen. At this point, our results have showed no difference between the 2 groups in transplant results, but there are some variability in the quality in the fusion of the tissue.//Conclusion//This study showed our capacity to produce an autologous rabbit urethra that can resist to physio-mechanical constraints and is ready for urothelial cell seeding before long-term in-vivo study in rabbits.

Nucleus pulposus and chondrocyte cell growth and tissue deposition on 3D-printed scaffolds

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Painful degeneration of soft tissues such as articular cartilage and intervertebral discs (IVDs) accounts for enormous social and economic concerns. Tissue engineering and regenerative medicine aim to provide suitable biomimetics recapitulating native tissues. Biocompatible, implantable thermoplastics for desktop 3D-printing are available for design of high resolution structures resembling extracellular matrix structure and mechanical function. This study investigated cell growth and matrix production within large-pore 3D-printed acrylonitrile butadiene styrene (ABS) and polylactic acid (PLA) scaffolds for cartilage and intervertebral disc tissue regeneration applications. Primary bovine articular chondrocytes and nucleus pulposus (NP) cells from IVDs were cultured on ABS and PLA scaffolds for three weeks. Both cell types adhered and proliferated, with NP cells almost completely filling in the pores on both ABS and PLA scaffolds. Chondrocytes and NP cells both showed high cell viability, and both produced ample proteoglycan and collagen type II on scaffold surfaces indicating that large-pore scaffolds drive massive extracellular matrix production. Immunofluorescence and western blot data revealed that both cell types evenly distribute collagen type II and aggrecan protein on both ABS and PLA scaffolds. Mechanical testing revealed stability of scaffolds after 3 weeks in culture medium. This study confirms that large-pore ABS and PLA scaffolds printed with simple and affordable desktop 3D-printers provide mechanical stability and suitable space for cell ingrowth and matrix deposition towards cartilage and IVD tissue engineering. Future scaffold designs better mimicking native tissue structure combined with more mechanically flexible and resorbable materials may provide implantable constructs with proper structure, function and cellularity necessary for potential cartilage and disc tissue repair in vivo.

Early detection of extracellular matrix disorganization and cytoplasmic accumulation of TDP-43 in tissue-engineered skins derived from ALS patients: an optimized approach to recover secreted proteins for biomarker discovery

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Amyotrophic lateral sclerosis (ALS) is a fatal adult-onset disease characterized by the selective degeneration of motor neurons (MNs) in the central nervous system (CNS). The identification and development of disease-modifying therapies is difficult and novel strategies for early diagnosis of ALS, to monitor disease progression and to assess response to existing and future treatments, are urgently needed. Due to the common embryonic origin of both skin and neural tissues, many neurological disorders, including ALS, are accompanied by skin changes that often precede the apparition of neurological symptoms. We have developed a unique tissue-engineered skin model (TES) derived from symptomatic, sporadic and familial ALS patients as well as pre-symptomatic FALS patients carrying a known pathological DNA mutation. TES were generated from isolated keratinocytes and fibroblasts and analyzed using different biochemical, immunohistological and molecular methods. Our ALS-TES presents a number of striking structural and molecular features, uniquely seen in patients-derived TES, including extracellular matrix (ECM) disorganization and cytoplasmic TDP-43 inclusions, a pathological signature found in the majority of ALS cases in the affected regions of the CNS. To facilitate ongoing research involving secreted proteins, we revisited cell culture protocols and whole secreted protein enrichment. The proteins secreted by a particular type of cell, the secretome, play important roles in the regulation of many physiological processes via paracrine/autocrine mechanisms and they are of increasing interest to help understanding rare diseases and to identify potential biomarkers and therapeutic targets. Consequently, our ALS-TES could represent a renewable source of human tissue to better understand the physiopathological mechanisms underlying ALS, accelerate the identification of disease biomarkers for early diagnosis and disease monitoring, as well as provide a unique tool for the development of drug screening assays. Given the importance of secreted proteins as a source to elucidate the pathogenesis of rare diseases, especially neurological disorders, this approach may help to discover novel candidate biomarkers with potential clinical significance.

Articular Cartilage Tissue Engineering with Redifferentiated Human Chondrocytes

Vanessa Bianchi and Rita Kandel

Articular cartilage (AC) tissue lines the surfaces of bones of the synovial joints to facilitate low friction articulation and transmission of loads to the underlying subchondral bone. Healthy intact AC tissue is essential for proper joint articulation however it is susceptible to irreparable damage from injury or diseases such as osteoarthritis (OA), which leave affected individuals suffering from joint pain and limited mobility. AC tissue has a limited ability for self-repair and currently used therapies are insufficient, prompting research into new biologically based therapies using autologous chondrocytes (the cells of AC). As only a limited number of chondrocytes can be isolated from autologous donor sites they need to be grown in monolayer culture to increase cell numbers before use. This causes the cells to lose their phenotype in a process referred to as dedifferentiation and prevents AC tissue formation. Therefore in order for biological replacement therapies to be successful the chondrocyte phenotype needs to be re-established. We hypothesize that human passaged chondrocytes can regain a chondrogenic phenotype when cultured in a defined serum-free media supplemented with an appropriate factor(s). We show that through the use of 3D culture and a defined media supplemented with transforming growth factor beta 3 (TGFβ3), human passaged (P2) chondrocytes can regain an articular chondrocyte-like phenotype and produce articular cartilage tissue in vitro. With TGFβ3, human passaged chondrocytes produce AC tissue with significantly more proteoglycan and collagen than untreated cells. Immunohistochemistry shows that TGFβ3 supplemented cultures produce tissues rich in collagen type II and aggrecan (the two major ECM components of AC, and markers of the articular chondrocyte phenotype), and no collagen type 1. Additionally, the rounded chondrocyte morphology that is lost with dedifferentiation is recovered. With re-establishment of the chondrocyte phenotype, passaged chondrocytes from autologous donors can be used to bioengineered AC tissue constructs for use in treating cartilage damage and OA.

Tri-Culture of Vascular Cells from Cellularised Collagen-based Scaffolds Promotes Vascular Tissue Remodeling

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INTRODUCTION Collagen gel is a commonly used scaffold in vascular tissue engineering due to its biological properties including a high potential for supporting and guiding vascular cells in the regeneration process. The approach we privileged consists in first reproducing the media, which provide the high elastic properties of the vessel wall, thus making it an essential and effective component for blood and nutrients transportation. Starting from an original method, previously reported and aimed to process collagen and smooth muscle cells (SMCs), the overall goal of this project was to design and develop an endothelialised two-layers collagen cell-based scaffold in a disc shape. The underlying layer is composed of fibroblasts (FBs) seeded within collagen. The upper layer is composed of SMCs seeded within collagen and endothelial cells (ECs) are seeded on this construct. This construct is finally expected to provide vascular tissue remodeling due to cells/cells and cells/matrix interactions and to produce an engineered tissue with properties close to that of blood vessel walls. It is also expected to provide a valid in vitro model for further studies of vascular patho-physiology. \\ RESULTS AND DISCUSSION After 24 hrs of growth, the non-circular morphology of endothelilals cells suggests that the cells are growing and proliferating to form a monolayer on top of the collagen gel layers. The presence of SMCs in the background, inside the gel, was confirmed by 3D confocal images. Transversal section of collagen gels shows the two layers of collagen, one containing SMCs and the others FBs, nested one within the other. After one week of culture, cells density in the gels increased because of gels compaction due to SMCs activity. ECs layer was shown to remain intact. The alignement of cells due to the specific mold anchoring retaining the compaction in one direction was also observed. The interaction between cells enhanced the matrix remodeling and the properties of the arterial construct. Finally, hemo-compatibility tests were performed on this cellularised scaffold. \\ CONCLUSION Tri-culture of vascular cells were achieved on collagen scaffold without losing endothelial cells. Furthermore, immunofluorescence staining suggested that cells established focal attachments. This experiment showed that tri-culture of vascular cells from cellularised collagen-based scaffold is a promising strategy for the regeneration of the vascular tissue. This talk will present the overall strategy behind this project and the characterization of cells localization in the collagen matrix will be discussed.

The culture of Nucleus Pulposus Cells on a continuously expanding culture surface enhances their phenotype for tissue engineering purposes

Julien T. Gravel, Derek H. Rosenzweig, Lisbet A. Haglund

Introduction: Degenerative Disc Disease (DDD) is characterized by tissue decellularization and phenotypic alteration of cells of the nucleus pulposus (NP), leading to tissue degradation and back pain. Standard therapeutic procedures do not foster regeneration. A potential avenue to drive regeneration is autologous cell implantation. A caveat of this therapy is that multiple passages lead to dedifferentiation during cell expansion. Continuous expansion culture surfaces (CE) have previously been shown to inhibit articular chondrocyte dedifferentiation. CE culture can yield NP cells with improved phenotypes. Furthermore, these cells will, when transferred to a hydrogel, produce a neo-matrix closer to healthy NP tissue.//Methods: Polystyrene dishes were used as is or were covered with silicone rubber. Silicone coated and High Extension Silicone Rubber dishes were functionalized with collagen type I. NP cells were isolated from steer IVDs. The tissue was cut and digested overnight using collagenase type II. 2.5x105 cells were plated in each dish. Static cultures were passaged on increasingly large dishes. Between each passage, 5 days were allocated to correspond to the size of CE cultures. Once this protocol was completed, RNA was extracted. cDNA was synthesized and qPCR was performed using primers for collagen type II, Aggrecan, CHAD, Sox9, collagen type I and GAPDH. One million static or CE cultured cells were seeded in Hyaluronic acid/Chitosan hydrogel and cultured 21 days. Proteins were extracted and Collagen type II was analyzed by western blotting.//Results: Static culture and passaging of NP cells on polystyrene dishes resulted in decreased collagen type II, aggrecan and CHAD gene expression compared to freshly isolated cells. Numbers of cell doublings were the same on both culture surfaces. Gene levels of collagen type II and aggrecan were increased with CE. Once seeded in a hydrogel, Cells multiplied by CE produced 2 fold more collagen type II protein.//Discussion: Continuous expansion culture of NP has the potential to produce clinically relevant cell populations, with high collagen type II and aggrecan expression. Our data suggest that this culture method preserves the phenotype of NP cells to a higher extent than standard culture techniques. We further demonstrate that chitosan/HA hydrogels are suitable for NP-like tissue formation. Collagen type II is a crucial structural element of NP ECM and its higher synthesis level confirms that cells multiplied by continuous expansion maintain a superior phenotype. //Significance: The present study suggests a new culture method for improved NP cell phenotype for tissue engineering purposes.

Study of the impact of glycation in wound healing using a tissue-engineered skin

Kiefer Thouin, Sébastien Cadau, François Berthod

The wound healing process is essential to restore the skin barrier function to prevent fluid lost and risk of infection. The peripheral nervous system plays an important role in this process by inducing neurogenic inflammation. Diabetes induces neuropathy that aggravates impairment of wound healing leading to ulcer development and high risks of lower limb amputation. This process is mainly due to diabetes-induced hyperglycemia causing tissues glycation and advanced glycated endproducts (AGE) formation such as N-carboxymethyl-lysine.//Our hypothesis is that AGE's deleterious effects in wound healing observed in diabetes could be inhibited by a topic treatment with anti-glycation and AGE-breaker molecules.//The project's aim is to develop a glycated tissue-engineered reconstructed skin with a wound in vitro to evaluate the influence of AGEs on the reepithelialisation process and to develop a topic treatment targeting AGEs.//Method: We developed an in vitro wound-healing model by using a reconstructed skin in which a 8mm diameter wound is realized. The treatment of this model with glyoxal induced expression of AGEs and subsequent inhibition of wound closure. We have evaluated the effect of a treatment with aminoguanidin, an anti-glycation molecule, and alagebrium, an AGE-breaker molecule, to establish if these molecules could improve different parameters in glycated wound healing.//Results and conclusion: Results suggest that the action of aminoguanidin and alagebrium inhibits partially AGEs production and improve the reepithelialisation of the wound in glycated models. Thus, their topic application on ulcers could be a valuable approach to improve wound healing with minimal systemic side effects.

Session 7: Connective tissue remodeling III

Invited Lecture

New Biological Roles for MMPs Revealed by Proteomics of the ECM in vivo

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Proteases have both detrimental and beneficial roles in innate immunity and pathology. In contrast to the traditional dim view of matrix metalloproteinases (MMPs) being dowdy matrix degraders, we show MMPs are protective in inflammation. We explored the roles of macrophage MMP12 by quantifying global proteome and protein N-termini (the N-terminome) in inflammation and viral infection. In exploring the role of macrophage MMP12, we found that *Mmp12*-/- mice were protected from viral endocarditis and display earlier and dramatic severe arthritis vs. wild-type mice characterized by massive neutrophil infiltrations. Moonlighting nuclear transcription roles for MMP12 are revealed for the first time (Marchant et al Nature Medicine 20, 493-502). Using knockout mouse models we found that the secreted macrophage metalloproteinase, MMP12, translocates to the nucleus of virus-infected cells *in trans* and is essential for secretion of IFNα. We rescued IκBα expression and IFNα secretion in *Mmp12*$^{-/-}$ cells by transfection or addition of recombinant MMP12. A number of protease-regulated genes during viral infection were identified by ChIP-SEQ. By combining ChIP-SEQ with Terminal Amino Isotopic Labelling of Substrates (TAILS, *Kleifeld et al Nature Biotech 28, 281-288; Prudova et al 2010 Mol Cell Proteomics)*, a targeted N-terminomics procedure for identifying protease substrates by enrichment of the cleaved neo N-terminal peptides, we developed a proteogenomic approach to identify substrates whose expression is repressed at both the gene and protein levels by the protease to rapidly deplete the protein from the system, that in viral infection is essential for the IFNα response and survival. Thus, MMP12 is a key host protective protease essential in dampening inflammation and for survival in viral infections by cleaving substrates other than ECM molecules.

DEVELOPMENT OF A BIOMARKER FOR OSTEOARTHRITIS: CATHEPSIN K DEGRADATION OF CARTILAGE

Beatriz Noé, Francis Beaudry, Kathleen Théroux, Hélène Richard, John S. Mort, A. Robin Poole, Sheila Laverty

Introduction: Osteoarthritis (OA) is a degenerative joint disease characterized by the progressive destruction of articular cartilage. Cartilage matrix triple helical type II collagen molecules are key structural components highly resistant to degradation. The matrix metalloproteinase (MMP) collagenases can cleave intact type II collagen at primary cleavage sites, a key event in its subsequent degradation. Biomarkers are now available to measure the MMP cleavage fragments in body fluids. We have recently identified the cysteine proteinase cathepsin K (catK) as an active collagenase in equine OA cartilage specimens. We hypothesize that cat K also has an important role in the degradation of the collagen network in OA and that biomarker development will help elucidate its role and will also be potentially diagnostic. The aim of our investigation is to develop a novel competitive inhibition-assay against a neo-epitope generated by catK cleavage of equine type II collagen. \\Methods: Equine type II collagen was digested with cat K and the cleavage products characterized by mass spectrometry. Anti-neoepitope (C2K77) polyclonal antibodies were raised in rabbits against the most N-terminal cleavage products. The antibodies were employed to investigate cat K collagen cleavage in OA cartilage samples employing immunohistochemistry. C2K77 was used to establish a novel equine specific competitive inhibition (CI) assay. Briefly, the C2K77 neoepitope was bound to 96 well immunoassay plates at a concentration that permits detection of subsequent variable rabbit anti-neoepitope antibody binding following pre-exposure to standard concentrations of the C2K77 peptide or to samples containing C2K77. The assay is employed to measure cat K degradation fragments in the supernatants of stimulated cartilage explants and in banked body fluids from normal and OA horses.\\Results: Six cat K cleavage sites distributed throughout the triple helical region were identified in equine type II collagen. The most N-terminal site was within three residues of the previously reported site in bovine type II collagen. Western blotting using anti-neoepitope antibodies revealed that the initial cleavages occurred near the N-terminus. Immunohistochemistry of cartilage sections of OA samples revealed stained lesion areas but none in unaffected sites. The C2K77 competitive inhibition assay is being validated and preliminary results will be presented. \\Conclusion: Cat K cleaves triple helical collagen by erosion from the N-terminal region with subsequent progressive cleavages. The C2K77 immunoassay holds promise to detect specific cat K activity in vitro and in vivo and is a potential biomarker of OA.

LPS-Induced Inflammation Inhibits Bone Repair

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Background & Rationale: Bone repair in the adult skeleton starts with a transient inflammatory response in which cells involved in the repair process, including mast cells, migrate to the site of injury. In recent work we showed that repair of a femoral bone defect in mast cell deficient mice was impaired compared with control mice (Behrends et al 2014 eCM v28 p209). Lipopolysaccharide (LPS) on the surface of gram negative bacteria can induce sustained systemic inflammation, which has been associated with impaired bone healing in the clinical setting. The goal of this study was to characterize the repair of femoral bone defects in mice with LPS-induced inflammation, particularly with respect to mast cell involvement.// Materials & Methods: Bilateral 1mm x 2mm cortical defects were drilled in the femoral diaphysis of adult wild type C57BL/6 mice. The treatment group received daily IP injections of 100µL of 0.25mg/ml LPS from post-operative Day 1 to Day 7, while the control group received 100µL of PBS. Cohorts of mice were euthanized on Day 7 or Day 14 for tissue harvest including serum for TNFα, skin and lung for mast cell counts and bone for micro CT, histochemical and immunochemical analysis of repair.// Results Chronic, systemic inflammation on Day 7 post-operative in LPS treated mice was confirmed by elevated circulating levels of the pro-inflammatory cytokine TNFα and accumulation of mature mast cells in the classic target tissues of lung and skin. In contrast, mast cell numbers were decreased in the bone callus of LPS treated mice on Day 7, but equal to control at Day 14. The early decrease in mast cell numbers in the bone callus was reflected in impaired bone healing and non-union at Day 14, similar to that seen in the mast cell deficient mice. Markers of angiogenesis, bone formation and resorption were all reduced in the LPS treated compared with PBS treated mice.// Conclusion: LPS induced systemic inflammation resulted in an increase in mast cells in soft tissues but an anomalous reduction of mast cells in healing bone. The results confirm previous data supporting a pivotal role for mast cells in early bone repair and suggest differences in the mast cell phenotype in bone compared with classic target tissues like skin and lung.

Calcium suppresses parathyroid hormone-related peptide-induced synthesis of aggrecan and type II collagen in osteoarthritic chondrocytes

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INTRODUCTION: Osteoarthritis (OA) is a chronic degenerative joint disorder that affects millions of Canadians. It is characterized by the destruction of articular cartilage due to an imbalance in the anabolic and catabolic activities of chondrocytes. Parathyroid hormone (PTH) is synthesized by the parathyroid gland and functions to maintain serum calcium (Ca2+) levels by acting on calcium-handling tissues (bone and kidney). The synthesis and secretion of PTH is negatively regulated by Ca2+ acting through the extracellular calcium-sensing receptor. PTH-related peptide (-rP) is expressed in a variety of tissue that are not involved in calcium metabolism. PTHrP plays an important role in endochondral ossification by regulating chondrocyte proliferation and chondrogenesis, due to its ability to mitigate chondrocyte hypertrophy. PTH and PTHrP activate a common receptor, the type 1 PTH/PTHrP receptor (PTH1R). Recent studies have demonstrated reduced cartilage degeneration following administration of PTH in animal models of OA. Our preliminary data demonstrate significantly elevated levels of Ca2+ (2.1 ±0.3 mM) in the synovial fluid of OA patients, and that Ca2+ negatively regulates the expression of aggrecan (Agg) and type II collagen (Col II) in human articular cartilage. The purpose of this study is to determine the negative impact of Ca2+ on PTHrP-induced matrix protein synthesis in human OA articular chondrocytes. METHODS: OA cartilage was obtained from donors undergoing total knee arthroplasty (range 50-65 years) with informed consent. OA chondrocytes were prepared from each donor and stabilized for 7 days under standard culture conditions. Normal chondrocytes (PromoCell) were expanded under the same conditions and used as control. After stabilization, the cells were exposed to increasing doses (0.1-1000 pM) of PTHrP [1-86] (Sigma-Aldrich) for 7 days. The effect of Ca2+ on PTHrP was determined by incubating chondrocytes with PTHrP supplemented with increasing concentrations of Ca2+ [0.5-5 mM]. The expression of Col II, Agg, ADAMTS-4 and -5, MMP-13, and TIMP3 was evaluated by Western blotting. RESULTS: PTHrP dose-dependently increased the synthesis of Agg and Col II in chondrocytes. The expression of ADAMTS-5 was decreased and TIMP3, an inhibitor of MMP-13, was increased following PTHrP treatment. The effects of PTHrP on Agg and Col II expression were abrogated by Ca2+, particularly at higher concentrations of 2.5 and 5.0 mM. The effects on Ca2+on PTHrP-induced Agg and Col II synthesis will be evaluated in human OA cartilage explants. CONCLUSIONS: PTHrP has therapeutic potential in the treatment of OA, however, its effects may be mitigated by elevated levels of Ca2+ in synovial fluid.

Degeneration of Articular Cartilage is mediated by Calcium and the Extracellular Calcium-Sensing Receptor in Osteoarthritis.

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Introduction: Osteoarthritis (OA) is a multifactorial disease that affects millions of individuals, and, although, the mechanism(s) of OA onset are complex, the biological outcome is cartilage degradation and cortical bone loss. The degradation of cartilage is typified by the progressive loss of extracellular matrix components, such as the proteoglycan aggrecan, and type II collagen, resulting from the upregulation of catabolic enzymes aggrecanases (ADAMTS-) 4 and 5 as well as matrix metalloproteinases (MMPs) and type X collagen. We recently discovered elevated levels of free calcium in the synovial fluid of OA patients and raised a question on its relevance in affecting cartilage degeneration. The extracellular calcium-sensing receptor (CaSR) is a G protein-coupled receptor (GPCR), and principle regulator of parathyroid hormone (PTH) synthesis and secretion. It functions to maintain calcium homeostasis, and is the only known mechanism in sensing both local and systemic calcium fluctuations. Recently, tissue-specific and inducible chondrocyte knockouts of CaSR have provided evidence for an important role in chondrogenesis, however, its role in human chondrocytes, and more specifically, its contribution to the pathology of OA remains unclear. In this study we aimed to determine the role of CaSR in the pathological development of OA. Materials and Methods: Articular cartilage was isolated from 5 donors undergoing total hip replacement. Cells were recovered from the cartilage of each knee by sequential digestion with Pronase followed by Collagenase, and expanded in DMEM supplemented with 10% heat-inactivated FBS. OA and normal articular chondrocytes (PromoCell, Heidelberg, Germany) were transferred to 6-well plates in culture medium containing various concentrations of calcium (0.5, 1.0, 2.5, and 5.0 mM CaCl2), allosteric agonist (cincalcet, 1 uM) and CaSR antagonist (antagonist, 1 uM), and replenished every third day for a duration of 7 days. Cartilage explants were prepared from the same donors, and included cartilage with the cortical bone approximately 1 cm2 in dimension; bovine articular cartilage (12 months) was used as a control. Explants were cultured in the above mentioned media for 21 days. Immunohistochemistry was performed on cartilage explants to measure CaSR expression and markers of OA (ColX, MMP-13, IL-1R). The sulfated glycosaminoglycan (GAG, predominantly aggrecan) content of cartilage was analyzed using the 1,9-dimethylmethylene blue (DMMB) dye-binding assay, and aggregan fragmentation was determined by Western blotting. Western blotting was also performed to measure CaSR expression, MAPK, MMP-3 and -13, ADAMTS-4 and -5, Collagen II and Aggrecan. Results: CaSR expression was markedly increased in superficial cartilage of OA donors when compared to bovine articular cartilage. Proteoglycan content of the cartilage explants decreased as a function of calcium, as determined by the DMMB assay and Western blotting of aggrecan. The expression of CaSR was also higher in OA versus normal human articular chondrocytes, a property that was reflected by the degree of MAPK activity following stimulation with calcium. When compared to normal cells, OA chondrocytes demonstrated a reduced capacity to synthesize proteoglycan. This property was reversed when OA cells were incubated with the CaSR antagonist. Conclusion: We provide evidence that prolonged activation of CaSR in human chondrocytes decreases proteoglycan and collagen II synthesis. Significance: Inhibition of CaSR may support a role in cartilage regeneration.

Enhancing Nucleus Pulposus Tissue Formation In-Vitro: A Novel Approach

Rahul Gawri, Robert Pilliar, Rita Kandel.

Purpose: Disc degeneration can be associated with low back pain, affecting 70% of the individuals over the age of 50. Currently there is no optimal therapy so there is a great need to identify new treatment approaches. Inorganic polyphosphates (iPP) are linear polymers of orthophosphate units varying in chain length and widely present in cells. As iPP has anabolic effects on chondrocytes, we hypothesized that iPP treatment will enhance matrix accumulation, making it a suitable to use to induce biological repair of degenerated IVDs.// Methods: NP cells isolated from bovine caudal discs were grown in 3D on porous ceramic substrates and grown in the presence of various amounts and size of polyphosphate. Selected cultures were also grown under hypoxic conditions (2% O2). Gene expression was determined using RT-PCR using gene specific primers. Matrix accumulation was quantified biochemically by measuring proteoglycan and collagen contents. DAPI fluorescence shift was used to determine presence of iPP in native bovine IVD tissue. Alamar blue assay was also performed to evaluate the metabolic state of iPP treated NP cells.// Results: DAPI staining showed iPP in native IVD tissue which was present diffusely throughout the NP. iPP enhanced matrix accumulation in a chain length dependant manner. iPP was also effective under hypoxic culture conditions. NP cells grown in the presence of iPP-22 showed increase in gene expression of aggrecan, Collagen II and Sox 9, which was maintained up to 14 days. ADAMTS-4 expression levels were decreased as early as day 7. MMP-3 gene expression was decreased by day 14, whereas MMP-13 gene expression was increased. iPP had no effect on NP cell metabolism when compared to controls as determined by Alamar blue assay.// Conclusions: Polyphosphate enhanced NP tissue formation in vitro by modulating expression of chondrocyte matrix genes and downregulation of proteases leading to increased proteoglycan and collagen accumulation. Elevated levels of MMP-13 expression may reflect tissue remodelling. As iPP is effective even under hypoxic conditions, this suggests that iPP may be a novel treatment for disc degeneration.

Session 8: Cell-ECM interactions

Invited Lecture

Urinary bladder smooth muscle-matrix interactions: molecular and epigenetic insights

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While urinary bladder function is generally unappreciated by its owner, it is one of the few organs subject to lasting pathology, dysfunction, and altered contractility induced by improper use. The bulk of work in this area has focused on pressure studies and on the principal cholinergic and contractile pathways mediating bladder smooth muscle contraction. Often due to outlet obstruction generating excessive wall stretch and pressure, bladder smooth muscle – extracellular matrix interaction becomes imbalanced leading to a fibroproliferative response characterized by excess smooth muscle cell overgrowth and hypertrophy, and extracellular matrix accumulation. Interestingly, these responses are often irreversible even after obstruction is relieved. Moreover, we also noted that matrix can impart a powerful growth stimulus to smooth muscle. Since these are not mutationally driven responses, we are currently exploring the epigenetic mechanisms that may in part mediate this pathobiology, with an emphasis on the DNA methylation machinery.

Development of a three-dimensional (3D) cell culture model that mimics female genital mucosae

Ingrid Saba, Alexandre Rousseau, Hazem O. Orabi and Stéphane Bolduc

Background: Vaginal mucosae are often exposed to chemicals, irritants, microorganisms and viruses that induce inflammation and enhance women' susceptibility to infections. Using a self-assembly technique, we report here the production of a unique 3D fully autologous vaginal equivalent model that is very specific for studying female mucosae responses. Objective: Our main objective is to validate that our human 3D model mimics female genital mucosae. Methodology: Vaginal stromal and epithelial cells were isolated from vaginal biopsies of the same HIV-1-negative female donor. Stromal cells were cultured and allowed to form their own extracellular matrix, and vaginal epithelium was seeded on top of the stromal layer and differentiated in vitro. Results: Our data indicate that stromal and epithelial vaginal cells have different estrogen requirements. Using H&E and Masson Trichrome staining, we are able to assess the general vaginal tissue architecture, cellular organization and morphology of our equivalents. Mucus secretion and the presence of glycogen are validated using PAS staining. Immunofluorescence using antibodies against cytokeratin, Lamin and collagen identified the 3 key structures of the equivalent (epithelium, basement membrane and stroma component). Conclusion: Our experimental model indicates that the 3D vaginal equivalent engineered in our laboratory is similar to native vaginal tissue, and can be used to study intravaginal delivery of microbicides, contraceptives and/or anti-HIV agents.

Regulation of osteoclast formation by collagen type I degradation products

Boraschi-Diaz, Iris; Komarova, Svetlana V.

Introduction: Bone destruction by specialized multinucleated cells, osteoclasts, is important for physiological processes of bone remodeling and tooth eruption. Osteoclasts are also critical contributors to numerous diseases such as rheumatoid arthritis, periodontitis and cancer metastasis to bone. Bone matrix is composed of hydroxyapatite and organic matrix, major component of which is collagen type I. Osteoclasts destroy bone by lowering the extracellular pH to dissolve hydroxyapatite and releasing proteolytic enzymes, such as cathepsin K to digest the organic matrix.// Objective: We hypothesized that while osteoclasts mediate collagen destruction, the resulting degradation products may in turn regulate osteoclast formation and function.// Methods: The effect of collagen type I degradation products on formation of osteoclasts from mouse bone marrow was examined.// Results: We characterized the degradation of mouse and human collagen type I by active recombinant cathepsin K. We have found that addition of 0.2 µM of active cathepsin K to collagen type I in an acidic solution (pH 5.5) containing 50 mM sodium acetate , 2.5 mM dithiothreitol, 2 mM EDTA, 0.15% (w/v) C4-S chondroitin sulphate for a period of 30-60 min at 32ºC resulted in formation of reproducible fragmentation pattern. The collagen fragments as well as control solutions without cathepsin K or collagen type I were dialyzed against tris buffered saline. FVB mouse bone marrow cells were treated with MCSF (50 ng/ml) and RANKL (50 ng/ml) for 6 days to induce osteoclast formation. The dialyzed collagen type I degradation fragments (5-30 µg/ml) or control solutions containing undigested collagen type I (5-30 µg/ml), or all the digestion components without the collagen were added to the osteoclast differentiation media for the duration of an experiment. No significant change in the numbers of osteoclasts formed was observed in the presence of the control solutions. In contrast, addition of collagen type I degradation fragments significantly and dose-dependently inhibited osteoclast formation. When the fragments were separated according to their weight using chromatography, the strongest inhibition was produced with fragments of approximately 20 kDa in size.// Conclusions: This study demonstrates that collagen degradation fragments inhibit osteoclast formation, providing a novel mechanism for the physiological control of osteoclastogenesis.//

Pro-proliferative α1 integrin subunit over-expression is regulated by the c-MYC oncogenic factor in colorectal cancer cells.

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INTRODUCTION: In the intestine, the α1β1 collagen receptor is specifically expressed in proliferating crypt cells supporting a reported role for α1, via caveolin-1 and Shc, in the downstream activation of the Ras/ERK proliferative pathway. In mouse models, the α1β1 integrin supports breast cancer cell motility and, together with the Kras oncogenic factor, potentiates tumour growth. As we have recently shown that integrin α1β1 is present in 65% of colon adenocarcinomas and from the early stages of colorectal cancer (CRC), we postulated that integrin α1β1 has a pro-tumoral contribution in CRC related to α1 sub-unit (SU) expression and regulation. METHODS: α1β1 function was studied in T84, HT29 and SW480 CRC cell lines using shRNA silencing α1 (shα1) compared to an sh-control (shC). Cell proliferation was assessed and migration was tested by the scratch test assay in medium supplemented with 0.5% serum. In silico analysis of the promoter of the α1 gene (ITGA1) revealed 2 responsive elements for the oncogenic c-MYC transcription factor which we named EBOX1 and EBOX2. mRNA and protein levels of α1 were quantified in the three cell lines infected with shRNAs knocking down c-MYC or treated with 50 µM of 10058-F4, a pharmacological inhibitor of c-MYC. The α1 promoter controlled by c-MYC was verified by luciferase assay in 293T cells including co-transfections with the dominant negative inhibitor, Mad-MYC, and selective mutagenesis. The in vivo association of c-MYC and RNA Pol-II with the α1 promoter was tested by the chromatin immunoprecipitation (ChIP) assay. Immunohistochemistry (IHC) analysis of c-MYC expression was performed on a TMA containing 65 adenocarcinomas and their matched margins. Results: T84, HT29 and SW480 shα1 cells showed reduced cell proliferation and delayed wound healing compared to controls. c-MYC knockdown in the three cell lines or treatment with the c-MYC inhibitor showed a drastic reduction in α1 mRNA and protein levels. Transient over-expression of c-MYC led to a significant increase in α1 promoter activity which was abolished with Mad-MYC co-transfection or EBOX1/2 disruption. ChIP confirmed the association of c-MYC and RNA pol-II with the α1 promoter. IHC showed that MYC and α1 expression are correlated in 72.3% of the studied tumours. CONCLUSION: Our results show that α1β1 is involved in cell proliferation and migration of CRC cells. Our data suggest that the expression of the pro-proliferative integrin α1β1 could be regulated by the oncogenic factor c-MYC in CRC cells. (Supported by the CIHR)

Fibrillin-1 interaction with cells regulates microRNAs

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Background and Aim: Fibrillins are the major components of microfibrils in the extracellular matrix of elastic and non-elastic tissues. They are multi-domain proteins, containing mainly calcium-binding epidermal growth factor-like domains and 8-cysteine/TB domains. Fibrillin-1 contains one evolutionarily conserved Arg-Gly-Asp (RGD) sequence in its fourth TB domain which is required to mediate cell-matrix interactions by binding to cell-surface integrins. Mutations in this domain lead to heritable disorders, including Marfan syndrome and stiff skin syndrome. This study aims to determine the cell signaling function of the fibrillin-1 RGD sequence. Experimental Procedures: Two recombinant fibrillin-1 fragments were produced, one wild-type RGD-containing fragment and one fragment containing a mutant RGA sequence. The different characteristics of interactions between human skin fibroblasts (HSFs) and the two fragments were analyzed by light and fluorescence microscopy. To determine the differential regulation of signaling pathways, microarray analysis of microRNA (miRNA) as well as mRNA expression was conducted. MiRNAs play a key role in the regulation of gene expression by targeting mRNAs. The expression levels were compared after 24 hours of interaction between HSFs and the two fragments. Real-time quantitative PCR was used to validate the microarray results for a selection of miRNAs and mRNAs, as well as to perform a time course analysis of the differential expression of the selected miRNAs. Interactions between miRNAs and mRNAs were predicted by bioinformatics analysis and predicted interactions were validated in vitro using the mirTrap system. Cell culture studies using miRNA mimics or inhibitors were applied to study the functional effects of miRNA overexpression or inhibition in vitro. Results: After 24 hours, HSFs attached differently to plates coated with the wild-type fragment in comparison to the RGA-containing fragment. The interaction of HSFs with the RGD sequence of fibrillin 1 showed proliferative potential in immunofluorescence assays. Surprisingly, the microarray displayed differential expression of many miRNAs and mRNAs after 24 h of interaction between HSFs and the two fragments. Differential miRNA expression occurred after only 2 hours of interaction. Pathway analysis indicated that the differentially expressed miRNAs act together in regulating cell adhesion, migration and growth factors, all of which are relevant for extracellular matrix biology and pathology. Overexpression of certain miRNAs in human skin fibroblasts has significant effects on the actin cytoskeleton as well as the activity of focal adhesion kinase, a downstream mediator of integrin signaling. miR-612, miR-1208 and miRNA-3185 are three of the most interesting candidates because they are significantly downregulated upon integrin ligation and regulate not only known growth factors controlled by fibrillin-1 (TGF-β2/BMP2), but also Wnt, fibroblast growth factor and Notch pathways, all not previously described as important players in extracellular matrix biology and pathology. A variety of those growth factors were shown to be targets of miR-612, miR-1208 and miR-3185 in vitro.

Comparison between osteoarthritic and osteonecrotic bone marrow-derived mesenchymal stem cells: Differences in the expression of the extracellular calcium-sensing receptor and osteogenesis

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Insert your abstract without any formatting mark such as bold (Plain text only). Line breaks must be marked by two backslashes. IMPORTANT !!! Please keep it under 400 words !!! INTRODUCTION: Osteonecrosis of the femoral head (ON) is a painful disorder that often leads to the collapse of the femoral head and subsequent total hip replacement. Risk factors include corticosteroid therapy, alcohol abuse and sickle-cell anaemia. Core decompression of the hip is often performed to treat early-stage ON, however, in later stages of the disease it does not prevent the inevitable, arthroplasty. The use of bone marrow-derived mesenchymal stem cells (MSCs) to treat ON has been explored in small trials. The procedure involves decompressing the femoral head followed by injection of autologous bone marrow obtained from the patient's iliac crest. Although improvement was demonstrated, the procedure appeared to temporarily delay the need for surgery in a number of patients during follow-up. Evidence suggest that ON MSCs are deficient in the ability to differentiate into osteoblasts, secrete growth factors, and maintain survival. In addition, the environment of the ON hip may not be suitable for MSC survival and differentiation. Therefore, priming ON MSCs toward an osteoblastic lineage may improve survival in the affected joint. The extracellular calcium-sensing receptor (CaSR) has recently emerged as a target in the osteogenic differentiation of MSCs, as demonstrated in several studies. In this study we plan to investigate the role of CaSR in the differentiation of ON and OA MSCs. METHODS: Osteoarthritic (OA) and ON MSCs were isolated from the bone marrow of osteonecrosis patients undergoing total hip replacement and expanded in culture in regular growth medium. OA and ON MSCs were cultured in osteogenic differentiation medium without or with 5 µM CaSR agonist (Cincalcet). Media was changed every 3 days up to a period of 21 days. CaSR expression was evaluated by Western blotting from ON and OA MSCs collected from several donors. Osteogenic differentiation was monitored by von Kossa for mineralization, and Western blotting for the expression of calcification markers: type X collagen, alkaline phosphatase, and osteopontin. RESULTS: The expression of CaSR was downregulated in ON MSCs. ON MSCs had a reduced capacity in becoming osteogenic when compared to OA MSCs, as determined by mineralization potential and expression of osteogenic markers. CONCLUSION: CaSR activation may provide a simple means of enhancing osteogenic differentiation in ON MSCs and improve transplantation survival in ON patients. ACKNOWLEDGEMENTS: We thank the Canadian Institute of Health Research (CIHR).

Posters (In numerical order corresponding to display)

Session I: Even posters: evaluation on Friday 29th, 12:30 pm to 2:00 pm

Session II: Odd posters: evaluation on Saturday 30th, 12:30 am to 2:00 pm

P01 Integrin-linked kinase expression supports RhoA/ROCK-mediated cell contractility through its role in fibronectin assembly

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Fibronectin matrix assembly is a crucial step in the cell's response to extracellular matrix (ECM) stiffness leading to RhoA-dependant cell contractility. In the small intestine, fibronectin is predominantly expressed in the interstitial matrix and basal lamina of crypts of the crypt-villus axis. We have shown previously that integrin-linked kinase (ILK) siRNA-depleted human intestinal epithelial crypt (HIEC) cells exhibit a significant reduction in ECM fibronectin deposition that is responsible for the inhibition of their proliferation, spreading and migration. In the present study, we show that ILK siRNA-depleted HIEC cells loose stress fibers, mature focal and fibrillar adhesions, concomitant to a decrease in the activation of the RhoA/ROCK pathway. The defective contractile phenotype of ILK-depleted HIEC cells is rescued by seeding the cells onto an exogenous fibronectin substrate that also restores fibronectin fibrillogenesis as well as RhoA/ROCK pathway activation levels. Conversely, exogenous activation of the RhoA/ROCK pathway in starved ILK-depleted HIEC cells reveals a marked decrease in the isometric tension-support capacity of the ECM interface when these cells are grown on uncoated surfaces. Finally, the detection of tensin, the α5β1 integrin and fibronectin in fluorescence microscopy suggests that ILK-depleted HIEC cells specifically block fibrillogenesis of the soluble form of fibronectin at the sites of fibrillar adhesion complex formation. Altogether, our data suggest that ILK expression in proliferative intestinal epithelial cells is important for proper fibronectin/ECM assembly, which in turn is responsible for the mechano-dependent promotion of the RhoA/ROCK pathway and ECM-integrin-actin axis isometric tension development.

P02 Nanoparticle ultrastructure of avian eggshell

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The calcitic avian eggshell protects the chick embryo from physical trauma and microorganisms, and provides calcium to its growing skeleton. The eggshell contains abundant proteins including osteopontin (OPN), a mineral-binding phosphoprotein also found in calcitic human inner ear otoconia. To investigate protein-mineral relationships in eggshell at the ultrastructural level, atomic force microscopy (AFM) and transmission electron microscopy (TEM) were used to reveal calcitic nanoparticles having an average size of 19 nm in the outermost vertical crystal layer (VCL), 29 nm in the middle palisades layer (PL), and 55 nm in the innermost mammillary layer (ML). Immunoblotting of demineralized eggshell protein extracts showed prominent OPN bands. Immunohistochemical localization of OPN in sections of demineralized eggshell revealed the highest amount of OPN in the VCL, followed by the PL, and then the ML. Determination of the mechanical properties of these three shell layers by nanoindentation demonstrated that higher hardness values correlated with smaller nanoparticle size, thus showing decreasing hardness across the shell thickness from the outside to the inside (VCL>PL>ML). To examine whether OPN might regulate nanoparticle size, we grew calcite crystals in the presence of OPN. SEM revealed that OPN altered external crystal morphology, and Raman spectroscopy (crystals washed with NaOH to remove surface-bound protein) revealed a broad peak between 2850 and 3000 cm-1 indicating OPN occlusion within the crystals. Protein recovery from the same crystals, evaluated by immunoblotting, confirmed characteristic bands for crystal-occluded OPN. AFM from the internal structure of the grown crystals revealed smaller nanoparticles (avg. 16 nm) at high OPN concentration (5.9 μM) relative to those found at low 0.9 μM OPN concentration (avg. 38 nm). Nanoparticles were absent from control calcite crystals (no added OPN). In conclusion, these observations provide details on protein-mineral relationships related to hardness in avian eggshell. Funded by CIHR.

P03 Toward Understanding The Pathogenesis Of Keratoconus: Insight From A Tissue-Engineered Stromal Model

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Keratoconus (KC) is a corneal pathology that causes progressive thinning and weakening of the corneal stroma. The pathogenesis of KC is unclear, however, gene and cellular changes have been reported. The goal of this study was to investigate the pathogenesis of KC using tissue-engineered (TE) stromal models. Corneal stromal cells were isolated from human KC (n=2) and healthy eye bank (n=2) corneas. Cells, cultured in presence of ascorbic acid and fetal calf serum, were analysed on day 7 (cells) and day 30 (TE models). At both time points, a 24h conditioned-medium was collected to analyse the secreted proteases (Proteome profiler human proteases array kit, R&D Systems) and RNA was extracted in order to perform transcriptome analysis by DNA chip (Agilent SurePrint G3 Human Gene expression microarrays). Analysis of the proteases revealed that day 7 KC cells secreted a higher amount of MMP8 (3.4), MMP9 (4.5), MMP12 (3.0), MMP13 (2.8), ADAM8 (3.2), ADAMTS1 (2.7), ADAMTS13 (3.5), Cathepsin V (30.4), Kalikrein-6 (6.0), Kalikrein-7 (3.6), Kalikrein-10 (3.4), Neprilysin (2.6), Proprotein convertase 9 (8.0) and Proteinase 3 (4.6) as compared to healthy cells (fold-changes). Transcriptome analysis also showed an upregulation for ADAMTS1 (2.3), MMP8 (1.8), MMP12 (1.9), MMP13 (2.8) and Neprilysin (3.3) genes (fold-changes), at day 7 in KC cells. This study demonstrated the possibility of to produce an in vitro model of KC that allows investigation of this complex pathology using omics approaches. Characterization of this model will be of great interest to develop new therapies for this eye disease.

P04 Short Link N as a Therapeutic Agent to Treat Early Intervertebral Disc Degeneration

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Purpose: Induced repair of disc tissue may be possible by intradiscal injection of growth factors. We previously demonstrated that Link N could act as such factor in the disc. We have recently discovered that AF cells can process it to a fragment spanning amino acid residues 1-8 (US Patent # 61870394) – short Link N (sLink N). Our in vitro data indicates that the biologically active sequence is preserved within this fragment, which could represent a potential stable growth factor for disc repair. Purpose was to evaluate the effect of sLink N and compare its efficacy to Link N. Method: Caudal IVDs from the tails of 20-24 month old steers were isolated with adjacent vertebral bone. After 7 days of preconditioning, degeneration was induced with NP injection of 100-µg of trypsin. Seven days after, the trypsin-treated discs were injected with sLink N or Link N (100 µg/disc, n=6 discs/group). Four trypsin-treated degenerate discs were injected with PBS while four discs served as non-degeneration controls. At 2, 4 and 8 weeks post treatment, two discs from each treatment and control groups were processed for biochemical analyses. Proteoglycan (predominantly aggrecan) synthesis in the NP was monitored as sulfated glycosaminoglycan using the 1,9-dimethylmethylene blue dye-binding assay and Western blotting used to determine the expression of aggrecan and type II collagen. Results: GAG content in the degenerate discs decreased approximately 50% compared to controls. When degenerate discs were treated with sLink N or Link N, significant increases in GAG content was observed. However, sLink N was more potent at inducing proteoglycan and type II collagen in degenerate discs compared to Link N. Conclusion: Our results reveal that sLink N or Link N have the ability to restore tissue content and sLink N may be more potent than Link N in treating early disc degeneration.

P05 3D TISSUE ENGINEERING BLADDER MODEL FOR CANCER INVASION STUDY

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INTRODUCTION: Bladder cancer is the ninth most common cancer worldwide and was estimated to be the 13th leading cause of cancer death1. 70% of diagnosed patients have tumor recurrence and 15% of such recurrent cases will see their non muscle-invasive cancer progress to invasive disease2-3. Since the invasion of the basal membrane by tumor cells is the first step towards metastasis, the current lack of 3D in vitro models for bladder carcinoma (BCa) impedes the study of biological mechanisms regulating the NMIBT to MIBT transition. Hence, we propose to establish a unique 3D tissue engineering in vitro human BCa model to study mechanisms involved in tumor invasion. METHODOLOGY: To develop and characterize BCa 3D model, human fibroblast and urothelial cells were cultivated in order to reproduce an bladder equivalent which reproduces the histological properties of human bladders. RT4 (low-grade) and T24 (high-grade) BCa cell lines were be stably transduced with lentivirus expressing dsRed fluorescent protein. Spheroids from these cells were seeded on bladder equivalent urothelium. Invasiveness was assessed at 1, 2 and 3 weeks by immunofluorescence microscopy. RESULTS: The two BCa cell lines developed as tumors on the bladder equivalent. T24 cells were able to migrate beyond the basement membrane in the submucosa while the RT4 did not. Invasive cells covered 3,2% of the submucosa surface. CONCLUSION: We have a unique 3D cancer model to identify mechanisms involved in the development of invasive tumors. 1. Siegel R, Naishadham D, & Jemal A. Cancer statistics, 2013. CA: a Cancer Journal for Clinicians, 63(1), 11–30, 2013. 2. Lerner SP, Schoenberg MP, Sternberg CN. Textbook of Bladder Cancer. Oxon UK: Taylor and Francis. 2006. 3. Pashos CL, Botteman MF, Laskin BL, & Redaelli A. Bladder Cancer ; Epidemiology, Diagnosis, and Management. Cancer Practice, 10(6), 1–12, 2002.

P06 SURGEON'S PERSPECTIVE ON RELIABILITY OF FINE NEEDLE ASPIRATION CYTOLOGY IN SALIVARY NEOPLASMS

Kabir Hussain

Fine needle aspiration (FNA) is a type of biopsy procedure. In fine needle aspiration, a thin needle is inserted into an area of abnormal-appearing tissue or body fluid. As with other types of biopsies, the sample collected during fine needle aspiration can help make a diagnosis or rule out conditions such as cancer. The surgical intervention is planned depending on cytology. Not infrequently, this springs some surprises when the histopathology reports a malignancy. The aim of this study is to evaluate the efficacy of FNA and cytology on salivary neoplasms and its influence on treatment outcome. A retrospective study was conducted in 120 patients who underwent surgery for FNA-proven salivary neoplasms at our institute. FNA was found to have a sensitivity, specificity and diagnostic accuracy of 66%, 97.1% and 70% respectively. FNA has a reliable sensitivity and specificity. However, limitations were encountered with specific lesions especially some cystic and malignant lesions which resulted in modification of treatment.

P07 α2-Macroglobulin (A2M) Mediates Glucocorticoid-induced Hypofibrinolysis in Primary Human Endothelial Cells

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Background: High-dose glucocorticoid therapy is associated with the development of Osteonecrosis of the Femoral Head (ONFH), an irreversible pathology thought to be initiated by a local decrease in blood flow. This decrease is believed to be caused by an abnormal activation of endothelial cells, leading to an imbalance in the coagulation/fibrinolysis state that favors coagulation and thrombosis.\\ A tissue microarray of affected hips in a rat model of the disease revealed a 3.5 fold up-regulation of A2M. In a subsequent study, our laboratory showed an early, sustained elevation of A2M circulating levels only in affected rats.\\ A2M is a broad-spectrum protease inhibitor expressed predominantly in the plasma but also by macrophages, Schwann cells, and fibroblasts. Once A2M binds a protease, it becomes activated and is then rapidly engulfed by its ubiquitously expressed receptor, Low-weight Lipoprotein-receptor Related Protein 1 (LRP1), activating various signaling cascades that modulate gene expression of a variety of effectors including prostate specific antigen (PSA), matrix metalloproteinases (MMPs), and platelet activating factor (PAF).\\ Methods: Nearly confluent Human Umbilical Vein Endothelial Cells (HUVEC) were treated with 1mM Dexamethasone for 48 hours. 10ng/ml of TNFα and 1.5 unit/ml thrombin were added 4 hours and 8 hours, respectively, before cell harvest to stimulate the cells. To study A2M signaling 100nM methyl-amine activated A2M were used to treat HUVEC. Total RNA was extracted via Trizol; expression levels of A2M, tissue plasminogen activator (tPA), plasminogen activator inhibitor 1 (PAI-1) and thrombomodulin (TM) were assayed via qPCR.\\ Results: 1mM dexamethasone treatment caused a 3.5-fold up-regulation of A2M gene expression in HUVEC, concomitant with a 2.8 fold up-regulation of PAI-1, with no significant effect on tPA levels. A2M expression was further up-regulated by thrombin (4.1-fold) but not TNFα. The inflammatory mediators TNFα and thrombin further enhanced the glucocorticoid-mediated PAI-1 up-regulation (4.7 fold and 4.4 fold respectively). The same effect was reproduced by treatment with exogenous A2M. Activated A2M treatment lead to a 3-fold up-regulation of PAI-1 expression and a 2-fold suppression of TM expression, with no effect on tPA expression.\\ Conclusions: α2M mediates glucocorticoid-induced hypofibrinolysis by up-regulating PAI-1 gene expression while down-regulating TM expression in primary human endothelial cells. This is the first study demonstrating the expression of α2M in endothelial cells and these results suggest a plausible endothelial mechanism for the vascular consequences of glucocorticoid treatment. Western blots and functional studies are under way to correlate findings at the protein and function levels.

P08 Effects of phosphoamino acids on calcite dissolution

Valentin Nelea1,3, Jeanne Paquette2 and Marc D. McKee1,3

Biomineralization commonly involves an extracellular matrix and specific proteins that are phosphorylated particularly on serine residues (threonine and tyrosine residues can also be phosphorylated). Osteopontin is one such mineral-binding phosphoprotein which plays a role in the development of calcitic (calcium carbonate) structures (e.g. inner ear otoconia and avian eggshell), and also influences calcite dissolution (e.g. that occurs during eggshell thinning and chick hatching). To assess the effect of phosphorylation on calcite dissolution, the (1 0 4) cleavage surface of calcite was dissolved in the presence of aqueous solutions of the phosphoamino acids serine (P-Ser), threonine (P-Thr) and tyrosine (P-Tyr). The resulting etch pits shapes were imaged by atomic force microscopy and compared with those produced in the presence of inorganic phosphates and phosphoryl-ethanolamine (PEA). In the presence of inorganic phosphates and PEA, similar teardrop-shaped pits were observed, with the calcite glide-plane symmetry retained. At short incubation times (

P09 The collagen VII correction by gene therapy and tissue engineering to develop a treatment for recessive dystrophic epidermolysis bullosa

Angela Dakiw Piaceski, Francis Bisson, Karim Ghani, Manuel Caruso, Lucie Germain

Recessive dystrophic epidermolysis bullosa (RDEB) is a rare genetic disease characterized by intense blistering of the epidermis caused by a lack of adhesion at the dermal-epidermal junction. The severity of this disease varies among subtypes with mild blistering to severe bulla formation, erosions, scarring, and mutilation that can lead to a lethal outcome. There is no cure for RDEB and only palliative treatments that are restricted to individual wounds are offered to the patients. Mutations in the COL7A1 gene are responsible for RDEB. This gene encodes collagen VII, normally secreted into the extracellular space by dermal fibroblasts and epidermal keratinocytes. This protein is a major structural component of anchoring fibrils that ensure the cohesion between the epidermis and the dermis. Restoring a functional gene into skin cells to correct the collagen VII production is the basis of the gene therapy, and focus has been given to improve the safety and efficacy of this approach. Therefore, the objective of this project is to develop a gene therapy strategy for RDEB, using autologous tissue-engineered skin substitutes optimized to express collagen VII in keratinocytes and fibroblasts. The integration of the COL7A1 gene into human cells with a safe retrovirus vector and its expression in cultured cells or in tissue-engineered skin (TES) were assessed by immunofluorescence and western blot. Our results show that keratinocytes and fibroblasts in culture express collagen VII after transduction. Transduction efficiency reached about 20% in keratinocytes and about 30% in fibroblasts. In TES, foreign collagen VII transduced in fibroblasts is localized at the dermal-epidermal junction, like native collagen VII and histological appearance was similar to control TES. Work is in progress to validated new safer vectors constructed with different pseudotypes of viral particles and coding for GFP for their ability to transduce human fibroblasts and keratinocytes. These results indicate that RDEB is potentially treatable by gene therapy in an autologous TES optimized for their efficiency and safety. Acknowledgments (Fondation GO, Réseau ThéCell du FRQS)

P10 Immune tolerance of a skin substitute produced with allogeneic or xenogeneic dermis and a syngeneic epidermis grafted on mice

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1 Centre de recherche en organogénèse expérimentale de l'Université Laval / LOEX et département de chirurgie, Université Laval, Québec, Qc, Canada, et centre de recherche du CHU de Québec, axe médecine régénératrice, Québec, Qc, Canada. Skin substitutes made by the self-assembly approach of tissue engineering allow for permanent wound coverage. However, the production delay is quite long. The aim of this study was to reduce the production time by using an allogeneic dermis associated with an autologous epidermis without rejection. Allogeneic epidermis is known to be rejected. Thus, various tissue-engineered skin substitutes (allogeneic, xenogeneic, chimeric and autologous) were tested according to the self-assembly method. Fibroblasts were extracted from adult skin of BALB/c and C3H/HeN mice, and normal human skin whereas keratinocytes were extracted from newborn BLAB/c and C3H/HeN and adult human skin. To reconstruct the dermis, dermal fibroblasts were cultivated in the presence of ascorbic acid, which promotes extracellular matrix assembly and allows the formation of thick sheets of collagenous tissue. Keratinocytes were then added in order to reconstruct the epidermis. Following epidermal proliferation and differentiation, these substitutes were transplanted on a syngenic mouse C3H/HeN model in triplicate to estimate their functionalities. Allogeneic, autologous, xenogeneic and chimeric skin substitutes were grafted and compared according to rejection criteria 19, 35 and 56 days after grafting. Histological and immunohistochemical analysis of specialize immune cells (lymphocyte T CD4, CD8 and lymphocyte B) suggested the establishment of a tolerance of skin substitutes composed of an allogeneic or a xenogeneic dermis associated with a syngeneic epidermis. In contrast, we observed a systematic rejection of all skin substitutes produced with allogeneic or xenogeneic epidermis as soon as 19 days after grafting. In conclusion, this work is a new approach that could decrease the waiting time for transplantation of tissue-engineered skin substitutes.

P11 The Tissue-Engineered Human Cornea as a Model to Study Matrix Metalloproteinases Expression during Wound Healing

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Purpose: The reepithelialization of the corneal surface, occurring mainly after an injury to the eye, requires an important extracellular matrix (ECM) remodeling which is suspected to be regulated by the matrix metalloproteinases (MMPs) -2 and -9. The goal of this study was to analyze the gene expression and enzymatic activity of MMP-2 and MMP-9 during wound healing using tissue-engineered human corneas as a model.// Methods: The self-assembly approach was used to produce human tissue–engineered corneas (hTECs) as previously described (Germain et al., Pathobiol. 1999). hTECs (N=11) were wounded with a 8-mm diameter biopsy punch and deposited on another reconstructed human corneal stroma to allow wound closure on a natural ECM. Undamaged hTECs (N=3) were used as controls. After 4 to 6 days of culture, hTECs were separated in 3 sections: the central (designed as the wound), the internal and the external rings, using trepans of 8-mm, 13-mm and 19-mm diameters, respectively. Total RNA was extracted from the cells isolated from each of the three sections to perform microarrays and qPCR analysis. Culture media was collected each day during wound healing to monitor MMP-2 and MMP-9 activities by gel zymography.// Results: Expression of both the MMP-2 and MMP-9 genes was found to increase in the central ring compared with their level in the external ring, as revealed by microarray and qPCR analyses. Consistent with these results, the MMP- 2 and MMP-9 enzymatic activities increased from day 0 to day 4 during the wounding process. Most of all, microarray analyses identified other deregulated target genes whose respective protein products may contribute to the efficiency of the wound healing process.// Conclusions: This study will improve our understanding of the cellular and molecular mechanisms that modulate human corneal wound healing by exploiting a new, innovative 3D reconstructed tissue much closer to the native cornea.

P12 Deep tissue imaging methods for the characterization of tissue-engineered substitutes

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INTRODUCTION: Tissue engineering strategies are now enabling the reconstruction of substitutes featuring increasing complexity. In vitro multistep procedures are often used to produce thick human substitutes containing multiple cell types. For example, endothelial cells can be incorporated during tissue production and form capillary-like network structures to promote proper vascularization of the substitutes upon grafting. Optimal imaging methods are therefore needed in order to perform timely detailed characterization of those three-dimensional constructs of appreciable size. Confocal microscopy has demonstrated its ability to produce images of great resolution from tissue sections but also to modelize tridimensional tissue structures. However, this technology is limited to rather thin histological specimens due to the scattering of light when penetrating deeper into the tissue. This is a great limitation because reconstructed tissues should be observed in their entirety to analyze their structural organization.\\OBJECTIVE: This study describes a combination of methods involving optical clearing and multiple labellings for spectral confocal microscopy to characterize whole samples of skin and adipose tissues (native and tissue-engineered). In particular, features such as the vascular networks as well as structural components and the cellular function (apoptosis) have been evaluated, including the benefits of performing quantitative analysis using an appropriate 3D image analysis software.\\RESULTS: Optical clearing techniques significantly reduced the scattering of light and allowed the acquisition of high quality images to a significantly greater depth. Within the native and tissue-engineered constructs, optical clearing enabled a comprehensive analysis of the spatial distribution of structures of interest such as the presence of a vascular network and adipocytes. These techniques also enabled the detection of apoptotic cells in fixed tissues allowing quantification and spatial representation of cell death in engineered substitutes.\\CONCLUSION: The optical clearing techniques represent sample treatment processes which, when used in combination with different imaging modalities, contribute to exceptional image quality. They represent a considerable advantage in the field of tissue engineering by providing both a quantification and a morphological 3D representation of these structures. Supported by the CIHR.

P13 The impact of IL-1a/TGF-B1 ratio on the production of matrix metalloproteinases in human dermis reconstructed by the self-assembly method

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Fibrosis is a pathological condition compromising function of the affected organ and is characterized by an excessive deposition of extracellular matrix as well as by a decrease in the activity of matrix metalloproteinases (MMP). Various biomolecules including cytokines are highly involved in the fibrotic process. Among cytokines, TGF-B1 shows a well-known pro-fibrotic action but the effects of IL-1a remain controversial, pro- or anti-fibrotic, according to results. The aim of this study is to evaluate the effects of IL1a alone or in presence of TGF-B1 on fibrosis using dermal sheets constructed by self-assembly methodology. Dermis were constructed using normal fibroblast populations cultured in DME medium containing 10% FCS in 6-well plates with anchoring paper until confluence. After confluence, the cells were treated with DME medium containing 10% FCS + ascorbic acid (50 ug/mL) supplemented with IL-1a, TGF-B1 and different ratios of both cytokines during 3 weeks. Then, 48h-supernatants and 8 mm-circular punches of reconstructed dermis were collected for assessment of total MMP activity (by fluorimetry) and hydroxyproline (by colorimetry), respectively. . The assay of total MMP activity showed that addition of IL-1a alone, even at low doses (from 6 x 10e-5 nM) was able to strongly stimulate the MMP production while TGF-B1 addition weakly inhibited MMP production. When, the two cytokines were put together at various ratios, it appears that the TGF-B1 was able to counteract the effects of IL-1a on the production of MMP, regardless of the concentration used and even if the concentration of TGF-B1 alone is too low to inhibit MMP production. The assay of quantity of Hydroxyproline present into dermis samples demonstrated that both TGFß1 and IL-1a were able to increase the final amount of deposited collagen but the cell sensitivity to these cytokines were lower for IL1a than TGFß1 . All dermal sheets cultured in presence of both cytokines exhibited a further increase in the amount of Hydroxyproline compared to each cytokine alone. These results demonstrate that TGF-B1 and IL-1a have strong profibrotic action but that their action is different according to the cytokine used.

P14 Molecular characteristics and binding properties of the latent transforming growth factor beta binding protein 4 (LTBP-4)

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The latent transforming growth factor beta (TGF-β) binding proteins (LTBPs) are extracellular matrix proteins involved in the regulation of tissue development and TGF-β growth factor activity. Human mutations in LTBP-4 lead to autosomal recessive cutis laxa type 1C, characterized by severe craniofacial anomalies, lax skin and severe abnormalities in the lung. Elastogenesis is severely deficient. Mice knockout models of LTBP-4 generally replicate these clinical symptoms. Here we characterize physical and interaction properties of human- (h) and mouse- (m) LTBP-4. These proteins were recombinantly produced by HEK 293 cells and purified to homogeneity. Surface plasmon resonance spectroscopy was used to assess binding of LTBP-4 to heparin, tropoelastin, short fibulins (fibulin-3, -4 and -5) and fibronectin. Particle sizes and molecular conformation of LTBP-4 in solution were determined by dynamic light scattering. Atomic force microscopy (AFM) was applied to visualize LTBP-4 molecules as adsorbed on functionalized mica surfaces with a negative or positive charge-carrying reagent. Both h- and mLTBP-4 bound to fibulin-4, fibulin-5, and to fibronectin with high affinity (KD=2-13 nM). Both forms of LTBP-4 did not bind to fibulin-3 or to tropoelastin. hLTBP-4 interacted with heparin with high affinity (KD=3-4 nM), while similar experiments with mLTBL4 did not detect binding. Particle sizes (hydrodynamic radius) in physiological solution (TBS, 2 mM CaCl2) were ~10 nm and ~12 nm for h- and mLTBP-4, respectively. These values correlate with an extended LTBP-4 conformation. In higher ionic strength solutions (500 mM NaCl) particle sizes were smaller (~7 nm and ~9 nm, respectively), suggesting a more closed molecular conformation. AFM experiments revealed that LTBP-4 condensate on negatively-charged surfaces forming compact, and relatively homogenous aggregates with rounded and elongated shapes of 50-150 nm dimension. Interestingly, on positively-charged surfaces, filaments and fibers developed, very likely by a surface charge-driven self-assembling process. These filamentous structures were few hundreds of nm up to few micrometers in length with widths of 7-20 nm. On uncharged mica, LTBP-4 was observed as a mixture of round (20-30 nm in diameter) and elongated (61 nm average length, 7-10 nm width) molecules. Our data document the interaction repertoire of LTBP-4 and indicate that LTBP-4 is able to self-assemble, a property that might be crucial for its role in elastogenesis.

P15 An accessible method for real-time imaging of live cells during dynamic fluid shear

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\\Introduction: Mechanotransduction is the process by which cells sense – and respond to – the local mechanical environment. This ability to react to external loads and forces is a critical component of mammalian physiology and is essential for normal functioning of our bones, lungs, and blood vessels; yet, the underlying mechanisms are still poorly understood. A form of mechanical stimulation that is commonly implicated in mechanotransduction is shear stress due to fluid flow. Our goal is to observe the immediate responses of cells to pulsatile fluid shear. Here, we describe the development of a microfluidic chamber for live cell cultures, which is compatible with real-time optical microscopy. \\Methods: A microfluidic chamber was designed and fabricated from polydimethylsiloxane (PDMS) using a replica molding technique. Thin PDMS membranes were applied to a cell-culture dish with a thin glass-bottom window. Chambers were then cast and sealed to this base membrane, creating a microfluidic chamber with channels from about 1500-µm wide by 100-µm tall to 500-µm wide by 100-µm tall. A saline solution containing 6-µm diameter beads was pumped through the chambers. Imaging of the channels under flow was performed using an inverted microscope and high-speed digital camera (1200 FPS). Flow parameters were calculated by micro-particle imaging velocimetry, using the polystyrene beads as markers. Chambers were disinfected, sterilized, and subsequently pretreated to enhance cell attachment. MC3T3-E1 osteoblast-like cells were then seeded into the chamber and imaged. In addition, cells were treated with fluorescent calcium dye fura-2 and exposed to shear stress during photometry. \\Results: Several prototype microfluidic flow chambers have been successfully fabricated in a reproducible manner. Steady flow rates up to 30 µL/min have been introduced into the chambers, generating a range of shear stresses from 1 to 3 Pa. In addition, MC3T3-E1 cells adhered and survived within the microfluidic chamber for at least 24 hours. Calcium photometry shows an increase in intracellular Calcium levels in response to fluid shear stimulus. \\Conclusions: We have developed, fabricated and tested a microfluidic system capable of delivering physiologically relevant fluid shear stresses, under steady flow conditions. Such stresses can be applied to a chamber capable of hosting live cells, which can be imaged while subjecting cells to controlled flow-induced shear stress. Further development of the platform will enable application of high-frequency oscillatory

P16 Proposed Research for the Development of a Validated Computational Model of the Ligament to Bone Insertion Using Second-Harmonic Generation Microscopy

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Introduction: Ligament insertions into bone (entheses) represent a natural adaptation to severe material mismatch. Load is transferred from relatively flexible connective tissue to relatively inflexible bone over typically less than a millimeter [1]. Adequate load transfer at an enthesis is necessary for normal joint function while preventing injury [2]. A few models have been used to assess different aspects of insertional mechanics, but all suffer from limitations. Primarily, there has been an inability to observe enthesis behaviour under applied load. Accurate quantification of enthesis mechanical properties and model validation has thus been lacking.//Objective and Hypothesis: The goal of the proposed research is to use second-harmonic generation (SHG) microscopy to evaluate a finite element (FE) representation of an enthesis. The intent is to analyze the load transfer mechanics at the ligament to bone interface more accurately. The hypothesis is that dynamic changes in collagen fibre organization, relative movement between fibres and fibrocartilage and pressurization of non-fibrous extracellular matrix relieve interfacial stress concentrations.//Methods: The MCL of rabbits will be studied as it is representative of several other insertion sites [3]. Dissected samples of entheses will be affixed to a custom-built loading device and mounted in the SHG microscope's viewing plane. Images will be captured at multiple tissue depths at different applied loads. Custom-written software will quantify microscopic strain, relative movement and collagen fibre reorganization. Entheses will be modeled as two-phase fibre-reinforced composite materials. Fibres and their surrounding matrix will have varying material properties along the length of entheses. High-resolution MRI will be used to extract detailed gross 3D geometry. Refining material properties, including the effects of fibre reorganization and relative movement will be performed such that modeled enthesis behaviour closely approximates observed behaviour. The mechanisms of alleviating stress concentrations will then be analyzed.//Expected Outcomes: Visualization of enthesis behaviour under load by SHG microscopy is expected to provide a means of FE model validation. The roles of changing collagen fibre orientation, sliding between fibres and pressurization of non-fibrous matrix in eliminating stress concentrations are expected to be revealed.//Significance: The combination of SHG imaging and FE modeling will provide insight into enthesis load transfer, contributing to tissue, biomaterial and general engineering as well as to improved surgical attachment procedures.//References: [1] Liu, Y et al., J Eng Mater Technol, 133:011006, 2011. [2] Thomopoulos, S et al., J Orthop Res, 21:413-419, 2003. [3] Matyas, J et al., J Biomech, 28:147-157, 1995.

P17 Characterization of the cellular response of annulus fibrosus cells to cyclic tensile strain

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Introduction: Mechanical loading is known to play a vital role in intervertebral disc (IVD) homeostasis. Similar to other musculoskeletal tissues, physiological loading contributes to the maintenance of IVD tissue homeostasis while over-loading contributes to tissue degeneration. Importantly, the differences between the effects of mechanical loading on distinct cell types of IVD and the cell type-specific effectors of mechanotransduction are poorly understood. Previous studies investigating changes in IVD cells in response to mechanical loading have largely focused on nucleus pulposus cells. The current study aims to quantify the effects of cyclic tensile strain (CTS) on annulus fibrosus (AF) cells to determine the importance of loading frequency and duration of the applied load on the cellular response using a mouse model. We aim to compare the response of AF cells to protocols of cyclic strain that model either physiological or super-physiological loading.\\ Methods & Results: AF cells were isolated from the IVDs of NotoCre; ROSAmTmG fluorescent reporter mice at 8 weeks of age, enabling the distinction of NP (GFP expressing) and AF cells (Tomato expressing) as a result of Cre expression in notochord-derived cells. Primary AF cells were cultured in high-density monolayer and exposed to multi-directional cyclical strain (10% strain; 0.1, 0.5, 1.0 or 2.0 Hz; 30 minutes) using the Cell Scale Mechanoculture device. Cells were harvested and total RNA was extracted at 2, 6, 12, or 24 hours following mechanical loading. Real-time polymerase chain reaction was used to quantify the expression of extracellular matrix (ECM) genes and ECM degrading enzymes.\\ Characterization of our in vitro model system demonstrates that primary cells maintain an AF-like phenotype when maintained in high-density monolayer culture, with expression of type I collagen, type II collagen and Gdf10 at levels similar to those detected in the intact murine IVD. No expression of markers of nucleus pulposus cells was detected. Following acute exposure to cyclic tensile strain (10% strain at 1.0 Hz), the expression of AF-associated matrix genes (including type I collagen, aggrecan, decorin & biglycan) was not significantly changed; however, a significant induction of both type X collagen and Adamts-4 expression was detected.\\ Significance: Our preliminary findings suggest that the cellular response of AF cells to cyclic tensile load is time- and frequency-dependent. Further studies will focus on identifying the mechanosensitive pathways activated by cyclic loading in IVD cells in order to better characterize the response of this complex tissue to its mechanical environment.\\

P18 Injectable Hydrogels for Cell Delivery and Repair of Intervertebral discs

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Intervertebral disc (IVD) degeneration is the leading cause of chronic low back pain. It is an aging process with cell-mediated responses to progressive structural deterioration. Disc degeneration is currently irreversible and is thought to develop through gradual or acute overloading of disc tissue. Conservative treatments include routine physiotherapy and pain relief medications for early stage diagnosis. Invasive surgical procedures such as spinal fusion, disc arthroplasty and posterior dynamic stabilization are performed for severe cases, and these do not prevent degeneration of adjacent discs. In recent investigations, thermo-responsive hydrogels are introduced as novel treatment options due to their injectable nature and gelling properties. This indicates that hydrogels can potentially be used for tissue repair in degenerate IVDs, filling in fissures and facilitating tissue regeneration. Thus, the purpose of this study was to evaluate nucleus pulposus cell viability in two types of thermo-responsive hydrogels: hyaluronan grafted poly(N-isopropylacrylamide) (HA-pNIPAM) and chitosan-hyaluronic acid composite (CHT/HA), under in vitro conditions in a unique agarose culturing construct which protects the hydrogel/cell suspensions. The agarose culturing constructs were made with 1.5% agarose in DMEM culture media. One million cells/mL of nucleus pulposus (NP) cells and human mesenchymal stem cells were seeded within each hydrogel separately. These mixtures (~400 µL) were loaded into agarose constructs and cultured for up to 21 days. Assessment of cell viability was performed with live/dead assay. After 7, 14 and 21 days, cells maintained above 80% viability within both hydrogels types. To test whether these cell-seeded hydrogels are suitable for disc implantation, fluorescently labeled NP cell-seeded HA-pNIPAM and CHT/HA were injected into intact human IVDs and bovine caudal discs, respectively, and cells remained viable and localized to the center of the discs after 14 days in the culture. In conclusion, HA-pNIPAM and CHT/HA hydrogels are viable compositions to be used as an injectable medium for delivery of cells into degenerate discs. Further investigations can be performed to study the extracellular matrix generation potential within these hydrogels for the development of possible tissue regeneration treatments for IVD degeneration.

P19 Engineering of an entirely human autologous 3D-bone tissue produced by the self-assembly approach

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Regenerative medicine is an increasingly important field in medical research. Many approaches, in orthopedic and maxillofacial reconstruction, are possible to achieve bone repair using prosthetics, biomaterials or autografts. Tissue-engineering strategies targeting the production of graftable bone tissues using autologous patient cells are promising. In particular, adipose tissue is an abundant source of highly multipotent cells that can be harvested with a minimally-invasive lipoaspiration procedure. In this context, our goal was to produce an entirely human 3D-bone tissue that will be graftable to restore mandibular continuity defects. First, we hypothesized that an Autologous Bone Tissue (ABT) could be produced using a self-assembly method which relies on the osteogenic capacity of human Adipose-derived Stromal/Stem Cells (ASCs) to become osteoblast cells capable of forming their own endogenous calcified matrix. Then, we evaluated the probability of ABT's integration and efficacy to heal bone defects in an immunocompromised rat model. //In vitro studies contained groups with ASCs and dermal fibroblasts cultured in osteogenic induction and control media both supplemented with freshly prepared ascorbic acid at 50 µg/ml to stimulate matrix production. After 5 weeks of in vitro culture, histology, alizarin red, hydroxyapatite and quantitative calcium assays were performed, and showed major differences between osteogenically induced ASC samples compared to non-induced ASCs and both induced/non-induced fibroblast controls. A significant calcification (p

P20 Targeted deletion of Smpd3 in osterix expressing cells causes skeletal dysplasia and early lethality

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Sphingomyelin phosphodiesterase 3 (SMPD3), a lipid metabolizing enzyme present in the membranes of the endoplasmic reticulum and the inner leaflet of the cell membrane, has been identified as a key regulator of skeletal development. SMPD3 cleaves sphingomyelin and generates ceramides, a class of lipid second messengers and phosphocholine, an important metabolite for a number of metabolic pathways. Currently, there are two reported mouse models that lack functional SMPD3, which have been used to extensively study the physiological roles of this enzyme. The first model was generated by a conventional gene targeting method (Smpd3-/-), while the other model, known as fragilitas ossium (fro), was generated by chemically inducing the deletion of 1,758 bp of the Smpd3 gene resulting in the loss of part of intron 8 and exon 9. SMPD3-deficient fro/fro mice, show severe congenital skeletal defects hallmarked by poor mineralization of the developing growth plate cartilage and bone. In the current work, we report the development of Smpd3flox/flox mice for the conditional ablation of Smpd3 using the Cre-LoxP system. In order to test the functionality of this model, we mated these mice with Osx1-GFP::Cre mice, which express Cre recombinase under the control of the murine osterix promoter. This promoter has been reported to be active in the differentiating chondrocytes and in the cells of the osteoblast lineage. In agreement with this expression pattern, we find that the skeletal phenotype in Smpd3flox/flox;Osx1-GFP::Cre mice closely mimics that of the fro/fro mice. This work confirms that SMPD3 has a local role in the skeletal tissues instead of a systemic role during development. Smpd3flox/flox mice will be a useful model to investigate the tissue-specific roles of SMPD3 and its metabolites in future studies.

P21 Porcine animal model of scaphoid fractures complicated by avascular necrosis or non-union

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Background: The scaphoid is the second most common fractured bone of the upper extremity and is frequently complicated by avascular necrosis and non-union. Despite significant efforts, surgery remains challenging due to the bone's retrograde blood supply, small size and curved anatomy. Inadequate management can have lifetime consequences for the patient because of the permanent wrist deformation that ensues. Hence, the development of a reliable treatment is necessary and the efficacy of a new surgical procedure must be tested in vivo. The minipig is an ideal model as its radiocarpal characteristics closely resemble the human scaphoid.\\Purpose of the study: Characterization of the porcine radiocarpal vasculature and establishment of a model of scaphoid fractures complicated by AVN or non-union.\\Methods: To visualize the vasculature of the radiocarpal bone, the brachial artery of a piglet was perfused with radiopaque barium sulfate. To create a model of non-union, a 3mm osteotomy was created in the bone and a dense collagen spacer inserted in the gap on the experimental leg while the bone fragments were fixed on the control side. In the AVN group, the volar fragments were excised completely, devitalized in liquid nitrogen, reapposed to the dorsal fragment and fixed. No nitrogen was used in the control group. Subsequently, the radiocarpal bones were scanned with a micro CT scanner for quantification of bone healing. Calcein labelling and histological staining were used to assess the viability of the radiocarpal fragments.\\Results: The 3D reconstruction model showed that the anatomy and blood supply of the radiocarpal bone were similar to the scaphoid. For the non-union model, the control side presented complete bridging of the gap on micro CT analysis which was confirmed with alizarin red staining. The fragments on the experimental side failed to unite. Bone quantification in two regions of interest of 0.5 mm thick rings around the screw showed statistically significant better healing in the control group (58.2±2.9mm3 and 71.2±3.0mm3) than in the experimental group (43.1±7.7mm3 and 44.6±0.6mm3). For the AVN model, micro CT analysis and histology showed that regardless of the use of liquid nitrogen, the volar fragment had been resorbed and replaced by fibrous tissue.\\Conclusion: The vasculature of the minipig was shown to have a similar retrograde blood flow as the human scaphoid. While the non-union model yielded significant results and was proven to be suitable for development of surgical treatment, further research is required for an AVN model.

P22 Leukocyte integrins αLβ2, αMβ2 and αXβ2 as collagen receptors - Receptor activation and recognition of GFOGER motif

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Integrins αLβ2, αMβ2 and αXβ2 are expressed on leukocytes. Their primary ligands are counter transmembrane receptors or plasma proteins, such as intercellular cell adhesion molecule-1 (ICAM-1) or components of complement system (iC3b, iC4b), respectively. Function blocking antibodies for these integrins may also reduce cell adhesion to collagens. To make the first systematical comparison of human αLβ2, αMβ2 and αXβ2 as collagen receptors, we produced the corresponding integrin αI domains both in wild-type and activated form and measured their binding to collagens I-VI. In the "closed" (wild-type) conformation, the αLI and αMI domains bound with low avidity to their primary ligands, and the interaction with collagens was also very weak. Gain-of-function mutations αL I306G, αL K287C/K294C and αM I316G are considered to mimic "open", activated αI domains. The binding of these activated αI domains to the primary ligands was clearly stronger and they also recognized collagens with moderate avidity (Kd

P23 Investigating the link between adenosine transport and ectopic mineralization of spinal tissues

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The equilibrative nucleoside transporter 1 (ENT1) transfers adenosine across plasma membranes. Adenosine regulates many cell functions and has been linked to connective tissue mineralization, including the induction of alkaline phosphatase (ALP). ALP functions by breaking down inorganic pyrophosphate (PPi) into inorganic phosphate (Pi), thus supplying a source of phosphate for mineral formation. We reported previously that ENT1-/- mice exhibit ectopic mineralization of spinal tissues including the annulus fibrosus (AF) of the intervertebral disc (IVD). Consistent with loss of ENT1, plasma levels of adenosine are greater in ENT1-/- mice than in wild-type (WT) mice. Our objective was to investigate the mechanism underlying aberrant mineralization in ENT1-/- mice.\\ IVDs were isolated from ENT1-/- and WT mice by microdissection and either processed for histochemical staining of alkaline phosphatase activity or subjected to enzymatic dissociation for cell isolation and culture. AF cells were maintained in high-density micromass cultures in the presence of ascorbic acid and β-glycerophosphate to permit synthesis and mineralization of extracellular matrix. AF cell cultures were supplemented with adenosine (6 μM) and a selective inhibitor of ENT1 6-S-[(4-nitrophenyl)methyl]-6-thioinosine (NBMPR 50 nM), either alone or in combination. Following 1 or 3 weeks in culture, alkaline phosphatase activity was quantified in fixed cells. Histochemical staining of IVD sections demonstrated alkaline phosphatase-positive cells at the AF-vertebral body interface, consistent with previous reports. Interestingly, alkaline phosphatase activity was also detected in cells of the inner AF (to a greater extent in ENT1-/- versus WT mice). Consistent with the pattern observed in vivo, micromass cultures of ENT1-/- AF cells demonstrated significantly higher alkaline phosphatase activity after 1 and 3 weeks of culture, compared to cultures of cells from WT mice (n=5, p

P24 Chi3L1 and Chi3L2 are Stress Response Proteins in Intervertebral Disc Degeneration

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Introduction: Intervertebral disc degeneration is a leading cause of chronic low back pain. During degeneration the extracellular matrix (ECM) of the inner nucleus pulposus (NP) and outer annulus fibrosus (AF) is enzymatically degraded. IL-1β, TNFα and more recently toll-like receptors (TLRs) have been suggested to regulate matrix degrading protease production. The Chitinase-like proteins Chi3L1 (YKL-40) and Chi3L2 (YKL-39) are glycoproteins that increase during cellular stress. In cartilage, Chi3L1 may have a protective role by inhibiting protease production, while Chi3L2's biological role is unknown. Little is known about Chi3L1 or Chi3L2 expression, regulation and function in intervertebral discs. This study hypothesizes that degenerating discs secrete increased levels of Chi3L1 and Chi3L2 and that cytokines and TLR signaling regulate their expression.// Methods: Degenerating discs from low back pain patients were surgically removed and non-degenerating discs were harvested from human organ donors. Discs were then cultured for 48 hours and the conditioned media was collected. Other non-degenerate organ donor discs were used to separately isolate NP and AF cells. Cells were then treated with IL-1β, TNFα, PGN (TLR2 agonist) or LPS (TLR4 agonist). RNA was collected after 6 hours and conditioned media was collected following 48 hours. Western blot was used to analyze Chi3L1 and Chi3L2 protein secretion by degenerating and healthy discs, as well as NP and AF cells treated with different agonists. Chi3L1 and Chi3L2 gene expression induced by the different agonists in NP and AF cells was analyzed by qRT-PCR.// Results: Degenerating discs from low back pain patients secrete significantly greater amounts of Chi3L1 and Chi3L2 compared to healthy discs from pain free donors. IL-1β, TNFα, PGN or LPS did not significantly increase Chi3L1 gene expression or protein levels in NP or AF cells. In contrast, Chi3L2 gene expression was induced by IL-1β, TNFα, PGN and LPS, while only IL-1β and PGN increased Chi3L2 protein levels in NP cells. In AF cells IL-1β, TNFα, and LPS increased Chi3L2 gene expression while all treatments significantly increased Chi3L2 protein levels.// Conclusion: CHI3L1 and CHI3L2 expression is increased in many pathological conditions and can be used as molecular markers of cellular stress and inflammation. Here, we show that both are increased during disc degeneration. Interestingly, Chi3L2 is more stringently regulated than Chi3L1 with the factors we used in this study. Future studies will aim to elicit the biological function of Chi3L2 on disc cells.

P25 TG activity is required for osteoclastogenesis

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Osteoclasts are multinucleated macrophage lineage cells capable of resorbing mineralized bone. Increased osteoclast activity causes bone loss, i.e., osteopenia. Protein crosslinking enzymes, transglutaminase 2 (TG2) and Factor XIII-A (FXIII-A) are both expressed in monocyte/macrophage lineage cells; however, their expression and potential role in osteoclasts or during osteoclastogenesis have not so far been explored. To address the role of TGs in osteoclasts, we used mouse bone marrow-derived macrophages (BMMs) which were differentiated into osteoclasts with M-CSF and RANKL. We report that mRNA of both TG2 and FXIII-A are indeed expressed in BMMs and mature osteoclasts but not increased during osteoclastogenesis. Immunofluoresence microscopy analysis of TG2 and FXIII-A in osteoclasts showed the presence of proteins and colocalization of the two enzymes. To examine the role of TG2 and FXIII-A in osteoclastogenesis and bone resorption activity, BMMs were treated during the osteoclastogenesis with NC9 - an irreversible TG inhibitor. A low dose NC9 inhibited osteoclast differentiation and fusion and high concentration of NC9 blocked differentiation completely without effecting cell viability. Consistent with this, osteoclasts after NC9 treatment showed decreased bone resorption activity as analyzed by resorption pit assay. Since the NC9 can only inhibit the TG activity without affecting other non-enzymatic functions of TG2 and FXIII-A, our data suggests that TG activity is required for osteoclastogenesis. Our study identifies TG2 and FXIII-A as novel enzymatic factors that promote osteoclastogenesis. Funded by CIHR.

P26 Cytokines supplementation analysis of a 3D psoriatic human skin model by gene expression profiling

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The pathogenesis of plaque psoriasis involves genetic, immunological and environnemental factors. Despite conspicuous progress in terms of genetic data, we are still far from a comprehensive understanding of the immune regulation of the skin in psoriasis. To overcome the temporary grunt adition of immune cells, we aim to characterize a novel in vitro psoriatic human skin model produced by tissue-engineering and supplemented with a cocktail of four cytokines (TNF-α, IL-1α, IL-6, IL-17). DNA microarrays analyses were performed to determine the level of mRNA expression in psoriatic skin substitutes supplemented or not with cytokines. Cyanine 3-CTP labeled cRNA targets were prepared and incubated on a G4851A SurePrint G3 Human GE 8 × 60 K array slide. Slides were then hybridized, washed, and scanned on Agilent SureScan Scanner. The results showed that cytokines supplementation acts on the structure and the organization of epidermis, which was thicker and more irregular. A large number of genes was disregulated in psoriatic substitutes supplemented with cytokines : upregulated genes (DEFB4A, S100A12, KYUN, IL8, CX3CL1) and downregulated genes (CCL27, ACSBG1, SERPINA12). The set of upregulated genes is mainly involved in immune response pathways or cell chemotaxis, whereas downregulated genes seem to play a crucial role in skin differentiation. These data suggest that the supplementation with cytokines could enhance the psoriatic phenotype in cultured tissues leading to a closer mimicking of the pathology. This model could be used as a new relevant tool in dermopharmaceutical research.

P27 Expression of nuclear factor of activated T cells (NFAT) and the downstream targets myoferlin and myomaker in ground squirrel skeletal muscle during hibernation

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PURPOSE: 13-lined ground squirrels undergo remarkable adaptive changes during hibernation where the animals enter deep torpor and allow body temperature (Tb) to fall as low as 0-5°C. While hibernating, there is little to no skeletal muscle loss despite prolonged periods of inactivity over the months of hibernation. Thus, it has been proposed that there is a unique mechanism of skeletal muscle remodelling taking place during hibernation. We were interested in studying the role of the nuclear factor of activated T-cell (NFAT) family of transcription factors and their recently discovered sarcolemmal targets; myoferlin and myomaker, with the adaptations taking place in skeletal muscle during hibernation.\\ METHODS: Squirrels were wild-captured and experiments were conducted in an environmental chamber at 4-5°C to enable natural transitions into torpor-arousal cycles during the hibernation season. Whole heart samples were collected at 7 different time points over the torpor-arousal cycle including active at room temperature (ART), euthermic control (EC), entrance into torpor (EN), early torpor (ET), late torpor (LT), early arousal (EA), and late arousal (LA) back to euthermia; each stage differs in squirrel Tb and respiration rates. Total protein extracts were prepared and immunoblotting was performed using antibodies specific for mammalian NFATc1, c2, c3, c4, myoferlin, and myomaker. Bioinformatic analysis was conducted on the 1500 bp region upstream of the myomaker transcription start site to identify novel NFAT transcription factor binding sites.\\ RESULTS: In skeletal muscle, NFATc2, c3 protein levels increased during torpor, while NFATc1 decreased during torpor, and NFATc4 increased throughout the torpor-arousal cycle. Myoferlin showed large increases during torpor (4.75 fold increase in comparison with control), whereas myomaker levels remained stable throughout hibernation. Bioinformatics analysis and cross-referencing using the NFAT consensus binding sequence resulted in the identification of a putative novel NFAT binding site 1095 bp upstream of the myomaker transcription start site.\\ CONCLUSIONS: This study is the first to identify a possible NFAT-binding region within the myomaker promoter and suggest that this recently identified protein, which is required for skeletal muscle hypertrophy and repair, could be a target of NFAT. Elevations in NFATc2-4 and myoferlin at various points throughout hibernation appear to be part of a molecular mechanism underlying the skeletal muscle remodelling taking place to prevent disuse-induced muscle loss in the hibernating squirrel. This mechanism may be harnessed to develop more effective therapeutics for muscle wasting diseases and to reduce muscle loss resulting from inactivity.\\

P28 Physiological Models of Intervertebral Disc Degeneration for Evaluation of Novel Regenerative Therapies

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Introduction Degenerative disc disease (DDD) is characterized by decreased cell density, loss in proteoglycans and increased inflammatory factors. These factors will ultimately contribute to the deterioration of load bearing function. Models representing the various characteristics of DDD will be required for assessment of novel therapies. Since discs are under constant load in vivo, bioreactors applying load are required. The load environment and culture medium can be modified in various ways to mimic degeneration and test feasibility of regeneration.//Objective We aimed to develop models representing different individual characteristics of DDD. Trypsin was used to deplete proteoglycan content, low nutrient supply to affect cell viability, and lipopolysaccharride (LPS) to induce inflammatory factors, in bovine caudal discs.//Methods Degeneration was induced by injecting trypsin into the center of the discs. The discs were then maintained in a bioreactor, and cultured in DMEM with varying amounts of glucose (2g/L) with medium changes twice weekly. Unloaded discs were cultured in low glucose (1g/L) for 48, 72 or 96 hours. Glycosaminoglycan (GAG) content was measured in both sets of discs using DMMB method and cell viability was assessed by live/dead analysis. Isolated bovine nucleus pulposus (NP) cells were cultured with LPS. TLR2, TLR4, IL1β, TNFα, NGF, MMP3, MMP13, ADAMTS4 and ADAMTS5 gene expression was assessed using q RTPCR.//Results Cell viability was decreased by 50% in discs cultured unloaded for 96 hours in medium containing low glucose (fig. 1), mimicking early degeneration. Proteoglycan content was depleted but viability was retained in trypsin treated discs loaded in medium containing 2g/L glucose; these discs had noticeable holes, simulating a more severe degeneration. LPS treatment of isolated NP cells created an inflammatory state marked by an increase in inflammatory gene expression without effects on cell viability.//Conclusion We have established 3 potential models representing different aspects of DDD. These models may be used to assess novel treatments such as cell and biological therapies for disc degeneration. Future studies will assess the regenerative capacities of mesenchymal stem cells.

P29 Cancelled

P30 DIFFERENTIAL ROLES OF PI3-K ISOFORM COMPLEXES IN THE REGULATION OF HUMAN IEC CELL SURVIVAL AND ANOIKIS

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BACKGROUND: In human IECs, the PI3-K/Akt-1 pathway is crucial for the β1/Fak/Src-mediated promotion of cell survival and suppression of anoikis. PI3-K consists of a complex formed by a catalytic (C) and regulatory (R) subunit. Three R (p85α, β, and p55γ) and four C (p110α, β, δ and γ) isoforms are known. It is established that: i) PI3-K isoform complexes can be selectively expressed and/or activated depending on the cell type studied; and ii) these isoforms can perform distinct roles, even within the same given cell type.// AIMS: The main goal here was to analyze the expression of PI3-K isoforms and determine their roles in the regulation of human IECs survival and β1/Fak/Src-mediated suppression of anoikis, according to the state of enterocytic differentiation.// METHODS: Two established human intestinal epithelial cell models (cell lines HIECs and Caco-2/15) were used in order analyze undifferentiated vs. differentiated IECs in vitro. Predominant PI3-K isoform complexes were determined by immunoprecipitation (IP) of R subunits and verification of association (co-IP) of C subunits by Western blot (WB). Fak (Y397 phosphorylation) and Src (Y418 phosphorylation) activation, as well as functional Fak/Src interactions (phosphorylation of Y576/577 of Fak, by Src), were monitored by IP and WB. Additionally, cells were exposed to the following specific inhibitors: PF573228 (Fak), PP2 (Src), LY294002 (all p110s), PIK75 (p110α), or TGX221 (p110β). Expression silencing was carried out by siRNA transfection (for undifferentiated IECs) and overexpression by lentiviral infection. Anoikis was induced by maintaining cells in suspension. Apoptosis/anoikis was evaluated by fluorometric CASP-3 activity assays, and Akt-1 activation (S473 phosphorylation) was assessed by WB.// RESULTS: 1) distinct profiles of predominant PI3-K R/C isoform complexes are displayed by human IECs, according to the differentiation state; 2) these PI3-K isoform complexes are distinctly involved in human IEC survival as well as in Akt-1 activation, also according to the differentiation state; 3) distinct PI3-K isoform complexes are engaged by β1/Fak/Src signaling according to the state of differentiation; and 4) the distinct PI3-K isoform complexes engaged by the β1/Fak axis promote cell survival and suppress anoikis, whereas the ones engaged by the β1/Fak/Src axis only promote cell survival of human IECs, according to their state of differentiation.// CONCLUSION: These data demonstrate that PI3-K isoform complexes are selectively expressed, as well as distinctively engaged by β1/Fak/Src signaling and, consequently, perform selective roles in the survival and suppression of anoikis in human IECs according to their state of differentiation.

P31 Measuring macrophage-to-myofibroblast adhesion in vitro

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Background: Fibrosis is characterized by the co-existence of macrophages (Mϕ), which produce pro-fibrotic cytokines and myofibroblasts (MF), which excessively secrete and contract extracellular matrix. Both cells types have been reported to upregulate expression of the homotypic and calcium-dependent adherens junction protein cadherin-11 (cad-11) under pro-fibrotic conditions in vivo and in vitro. We hypothesize that strong adhesion of Mϕ to MFs through cad-11 is important to establish in close proximity which promotes persistent fibrotic activation of both cell types through paracrine signalling. It is our objective to develop Mϕ-to-MF adhesion assays suitable to quantify cad-11-mediated Mϕ-to-MF adhesion.// Methods: Fibroblasts explanted from mouse lungs were treated with basic fibroblast growth factor (FGF-2) to obtain fibroblast populations and with TGF-β factor to activate MFs. Primary Mϕ were obtained by flushing mouse bone marrow and cultured in the presence of Mϕ-colony stimulating factor, followed by 2-day treatment with interleukin-4/-13 to induce polarization into pro-fibrotic Mϕ2. To test cell-cell adhesion strength, Fibroblasts and MFs were cultured in the channels of parallel plate flow chambers to form confluent monolayers. Mϕ2 were then seeded onto the monolayers and allowed to adhere for 15 min before the flow chamber was mounted on a microscope stage and attached to a syringe pump. After removing non-attached Mϕ with gentle fluid shear stress (16 dyn/cm2), the shear stress was gradually increased to 79 dyn/cm2 and movies were recorded at 6 frames/min. The number of Mϕ remaining attached at the end of each flow rate step was quantified using ImageJ. We further established aggregation assays to assess the binding affinities of Mϕ-to-MF based on their surface characteristics. Suspended Mϕ were mixed with suspended fibroblasts or MFs in an Eppendorf tube and rotated in an incubator to facilitate cell-cell interaction. After 2 h, cell aggregates were plated onto a glass coverslip for 3 h. The percentage of Mϕ aggregates was quantified after immunostaining.// Results: In aggregation assays, Mϕ2a formed aggregates with MFs 2.5-times more frequently than with fibroblasts. Mϕ attachment to MF monolayers was 4-fold higher compared to fibroblasts at the highest shear force generated in a flow chamber. Preliminary results using cad-11-blocking agents and removing extracellular calcium indicate that cad-11 supports specific surface recognition and mediates strong adhesion between Mϕ and MFs.// Conclusion: We have established adhesion assays that are able to quantify differences in Mϕ adhesion to fibroblasts and MFs.

P32 The effect of exercise and high fat diet on glucose metabolism and circulating plasma fibronectin and osteocalcin levels

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Exercise is known to have positive effects on general health, however, the mechanisms of how those effects are mediated are not fully understood. In this study we have examined the effects of exercise on two circulating proteins linked to health profile; plasma fibronectin and uncarboxylated osteocalcin. Circulating plasma fibronectin, a glycoprotein, which forms networks of extracellular matrices throughout the body, plays an essential role in cell adhesion differentiation, proliferation, and survival of many cells. The soluble form of fibronectin is produced by hepatocytes in the liver and it is a major component in blood plasma, averaging 300 µg/ml in humans and 600 µg/ml in mice. Studies have shown that a number of cell types and many tissues uptake plasma fibronectin to use it as a tissue component. In addition, it has been shown that increased levels of circulating plasma fibronectin levels are linked to adverse health consequences including cardiovascular complications, cancer, arthritis, and chronic inflammation. Osteocalcin, is a hormone released from bone to serum during bone resorption. Circulating uncarboxylated osteocalcin has beneficial effects on glucose metabolism. In the study male mice were randomized into four groups: 1) Normal diet, sedentary; 2) Normal diet, exercising; 3) High fat diet, sedentary and; 4) High fat diet, exercising and subjected to a 24-week intervention period. Measures of glucose and insulin tolerance were taken and serum from mice was collected. Our data show that a high fat diet, as expected, increases bodyweight and fat mass of the whole body and impairs glucose tolerance, which is reversed with exercise. Plasma fibronectin levels, measured in serum samples of all mouse groups, showed dramatic alterations. Plasma fibronectin levels were significantly decreased in exercising groups on normal diet and high fat diet groups. Osteocalcin analysis shows a clear trend which suggests that uncarboxylated osteocalcin levels are elevated with exercise and reduced with high fat diet. In conclusion, these data suggest that exercise lowers plasma fibronectin levels and increases serum osteocalcin levels, linking both with a healthier metabolic profile. Funded by CIHR.

P33 Proliferation and differentiation of rat BMSCs and ADSCs seeded on a 3D printed PEEK scaffold: an in vitro study

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Introduction// When combined with tissue engineering, additive manufacturing offers the capability to print on demand three-dimensional complex structures that are accurately reproducible and highly customizable. Polyether ether ketone (PEEK) is a polymeric material with similar mechanical properties to bone that exhibits biocompatibility and capability to enhance osseointegration. Currently, biomaterials seeded with mesenchymal stem cells (MSCs) represent a particularly attractive approach for tissue engineering applications, since SCs can be expanded in vitro and can differentiate to diverse cell types. Purpose// This in vitro study investigates the potential of using 3D printed PEEK scaffolds seeded with mesenchymal stems derived from bone marrow (BMSCs) or adipose derived (ADSCs) for bone tissue regeneration. Materials and Methods// PEEK scaffolds were printed using selective laser sintering and their porosity was assessed using microcomputed tomography (µCT). BMSCs and ADSCs were isolated from male Sprague Dawley rats and expanded according to standard protocols. After seeding the scaffolds with either BMSCs or ADSCs, the viability, proliferation and morphology of these cells were assessed. Osseodifferentation was induced by osteogenic differentiation medium and was assessed by measuring the alkaline phosphatase (ALP) activity. Results// Alamar blue assays showed that the PEEK scaffolds maintained the viability of both MSCs and supported their proliferation. Specifically, cell viability increased 4.36% and 2.72% at 5 days relative to the positive control in ADSCs and BMSCs groups, respectively. Scanning electron microscopy (SEM) images revealed a flat fibroblast-like phenotype for both MSCs before inducing osseodifferentation. ALP assays revealed that ADSCs and BMSCs seeded on the scaffold have the potential to osseodifferentiate; however, the results were more reproducible in the ADSC group Conclusion// These results indicate that 3D-printed PEEK scaffolds are biocompatible with MSCs and sustain their proliferation. These scaffolds can induce osseodifferentation in ADSCs and may offer a viable alternative to current bone grafting methods.

P34 In vitro functionality and long-term stability of tissue-engineered human adipose tissues

Meryem Safoine, Kim Aubin, Marie-Alice Audet-Casgrain, and Julie Fradette

Human adipose tissue is an endocrine organ possessing a wide secretome impacting on several physiopathological processes. Studying the biology of this important organ requires adequate models able to recreate the tridimensional structure and key functions of human adipose tissue. Tissue engineering is a promising approach for the production of human reconstructed adipose tissues (hrAT) that can be used in metabolic research. We hypothesized that: 1) hrAT will be functional and stable over an extended culture period and 2) hrAT will display similarities with native human adipose tissue regarding the secretion profiles of five molecules implicated in angiogenic and adipogenic processes (leptin, PAI-1, Ang-1, VEGF and HGF).\\ METHODS: hrAT were produced using adipose-derived stromal cells under ascorbic acid stimulation, enabling extracellular matrix production and organization into easily manipulatable cell sheets (self-assembly method). These cell sheets can then be superposed to generate thicker hrAT substitutes. hrAT were maintained in culture for up to 11 weeks of adipogenic differentiation. Cell sheets conditioned media (48h) were harvested weekly during 7 weeks of culture for leptin and Ang-1 quantification. Human fat explants and reconstructed tissues were incubated for 48h before collecting culture supernatants. The molecules of interest were then quantified using ELISA assays. Total DNA content of the tissues was used to normalize the data.\\ RESULTS: hrAT maintained their stability and functionality over an extended culture period. This was assessed by their histological appearance after 4, 7 and 11 weeks of adipogenic differentiation and the increase of the mean adipocyte's surface area due to lipid accumulation during that period. In addition, the continuous increases in leptin and Ang-1 secretion during 7 weeks confirmed the metabolic activity of the adipocytes. The comparison of hrAT and fat explants revealed similar secretion levels for leptin and PAI-1 while Ang-1 and VEGF levels were higher for hrAT (7 and 46-fold, respectively), and HGF levels were higher for fat explants (10-fold).\\ CONCLUSION: Human adipose tissue secretome analysis is a promising avenue to further our understanding of the role of bioactive molecules in the disorders affecting adipose tissue depots. Our hrAT model possesses interesting features such as tridimensional structure, long-term stability and cell functionality in an in vitro context and can be used as a relevant model for the study of the adipose tissue under physiological or pathological conditions. Supported by the CIHR.

P35 Reconstruction of human skin substitutes using adipose-derived stromal cells.

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INTRODUCTION: Human fat is an accessible and abundant source of stem/stromal cells (ASCs), which offers the potential for new applications in skin reconstruction by tissue engineering. Using a self-assembly strategy, ASCs can be used to produce a connective tissue similar to dermis. Moreover, inducing ASCs toward adipogenic differentiation, we can recreate a functional human adipose tissue, which is representative of the skin's deepest layer: the hypodermis. ASCs and adipocytes are also an important source of secreted bioactive molecules with pro-angiogenic and pro-healing properties. Our hypothesis is that ASCs can be advantageous for skin tissue engineering, in order to produce bilayered and trilayered skin substitutes. \\ METHODS: Bilayered skin substitutes were engineered by seeding keratinocytes directly onto either a connective tissue layer made of non-differentiated ASCs, on an adipose tissue layer containing adipocytes (differentiated ASCs), or a dermis made of fibroblasts. Trilayered skin constructs were also produced by adding an adipose layer under a bilayered skin containing dermal fibroblasts. Each type of reconstructed skin was cultured at the air-liquid interface for 14 days to induce terminal epidermal differentiation. Histological aspects of the different types of reconstructed skin were evaluated after Masson's trichrome staining. Specific immunohistochemical labelings were performed to detect epidermal differentiation markers and dermo-epidermal junction molecules. Furthermore, bilayered skin substitutes containing non-differentiated ASCs were grafted on athymic mice. \\RESULTS: Bilayered and trilayered skin constructs presented an histology similar to normal human skin. An abundant collagen presence was observed in the connective compartments. For skin featuring an adipose layer, lipid-filled adipocytes were embedded in the extracellular matrix. The epidermal thickness was similar (ANOVA) between the different bilayered skin constructs. These also revealed an appropriate pattern of epithelial differentiation, with expression of K19 and K14 in the basal layer, K10 in the suprabasal layers and transglutaminase in the granular layer. The presence of a continuous dermo-epidermal junction (laminin 5, collagens IV and VII), which is important for the skin's mechanical properties, was detected by immunolabelings. The presence of hemidesmosomes was also confirmed by transmission electron microscopy. Finally, reconstructed skin containing ASCs were successfully grafted and integrated to host tissues, with basal keratinocyte's proliferative potential being preserved for at least 21 days after grafting. \\ CONCLUSIONS: ASCs can be used as a complementary or alternative cell source for skin reconstruction by tissue engineering. These human skin represent new autologous substitutes for patients suffering from chronic ulcers or extensive burns.

P36 A ROSE IS A ROSE, OR IS IT? HOW TO JUDGE SUCCESS IN STEM CELL DIFFERENTIATION

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In natural differentiation stem cells acquire the properties that are characteristic of tissues, and enable tissue function. In stem cell differentiation in the laboratory much the same is attempted by cultivating of cells under conditions similar to those in natural differentiation, but may not be quite the same. Thereby it becomes an issue whether the cells resulting from the latter type of differentiation are fully identical to the cells that naturally occur in the target tissue. \\This study identifies and tries out several different criteria to judge whether they are. These range from alterations in the expression level of mRNAs that are held to be characteristic of the tissue, to estimates of the functional performance of the cells. The issue is confounded by the fact that cells of most tissue types are themselves heterogeneous, in terms of gene expression if not also in terms of function. \\We show that cells according to the usual criteria are fully differentiated into the tissue one aimed for, but are far from the desired state according to other criteria. We propose that for perfect in vitro differentiation all criteria should be satisfied, or the ones most closely related to function.

P37 Fatty Acid Supplementation of Culture Conditions During the Production of Psoriatic Skin Substitutes

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Background: Tissue-engineered psoriatic skin substitutes produced following a self-assembly method correctly represent the psoriatic phenotype. However, an increased permeability of their skin barrier, which may be caused by an essential fatty acid deficiency, was observed compared to human skin. Objective: The aim of this study was to improve the barrier function of the substitutes by restoring their lipid profile. To that end, essential fatty acid supplementation of the culture medium was performed and their implication in the pathology validated. Methods: Healthy and psoriatic skin substitutes were reconstructed using culture medium supplemented with different concentrations of linoleic acid, α-linolenic acid and palmitic acid and compared with their respective counterparts, which were free of supplements. Macroscopic, histologic, immunochemistry, gas chromatography, infrared spectroscopy and percutaneous absorption analyses were performed to investigate whether the medium supplementation could have positive impacts on the barrier function of the substitutes or not. Results: The macroscopic, histologic and immunochemistry assays have demonstrated that fatty acids have positive impacts on skin substitute appearance. Moreover, the characterisation by gas chromatography of the different fatty acids present in the phospholipid fractions of the epidermis showed that the lipid profile was restored with a concentration of 5 µM fatty acid supplementation. Confirmed by infrared spectroscopy and percutaneous absorption, the lipid organisation was also modified when the medium was enriched with lipids. Conclusion: These results show that implementing with fatty acids during reconstruction of psoriatic skin substitutes could restore their lipid profile in order to improve the mimicking of normal human skin.

P38 Comprehensive review of a relationship between Selective Serotonin Reuptake Inhibitors (SSRIs) and bone health

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Depression is one of the leading debilitating conditions today and it is linked to imbalanced activity and function of serotonin in brain. Serotonin is a monoamine neurotransmitter and a contributor to the feeling of wellbeing. In depression serotonin levels decrease in synaptic cleft due to increased uptake into the cells. Drugs that modulate and restore serotonin levels outside the cells are widely used to treat depression, among which are Selective Serotonin Reuptake Inhibitors (SSRIs) are most commonly prescribed antidepressants. SSRIs function via increasing synaptic serotonin levels; however, SSRIs are not specific to brain and are also known to increase peripheral, circulating serotonin levels. In addition to brain functions, serotonin regulates a wide range of physiological and biological processes. Several animal studies have demonstrated the negative role of peripheral serotonin in maintenance of bone mass. The serotonin-mediated bone loss arises from direct effects of serotonin on osteoblasts and osteoclasts via serotonin receptors in these cells. This literature review aims to assess current evidence on the relationship between the use of SSRIs and bone loss and fracture rates in both animals and humans. Following a comprehensive search strategy, relevant studies were identified from the Pubmed, Medline, Embase using a combination of keywords and mesh terms including serotonin, serotonin reuptake inhibitors, depression, antidepressants, bone, fractures, and bone mineral density. Our review concludes that most animal studies used mice between the ages 8–14 weeks, the duration of SSRI treatment (fluoxetine being most commonly used) varied ranging 4-6 weeks, with dosage ranging 5 - 20 mg/kg/day. The collected data from animal studies demonstrated that SSRI use reduces bone volume and trabecular thickness and decreases bone mineral density. The data collected from most longitudinal, cross-sectional and population-based cohort clinical studies demonstrated an overall association between the use of SSRIs and low bone mineral density with increased risk of fractures. Therefore, based on the current data, we conclude that SSRIs influence the skeletal system negatively. Further research is essential to better understand the all potential mechanism behind the deleterious effects of serotonin on skeleton. Understanding full array of these mechanisms will allow the design of efficient strategies to counter bone loss and fracture risks in those patients using SSRIs. Funded by CIHR.

P39 Mineralization-inhibiting effects of transglutaminase-crosslinked polymer osteopontin in vitro

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Osteopontin (OPN) belongs to the SIBLING (Small, Integrin-Binding LIgand N-linked Glycoprotein) family of mineral-binding matrix proteins found in bones and teeth. OPN is a well-known inhibitor of matrix mineralization, and we and others have shown that enzymatic modification of OPN can affect this inhibitory function. In bone, OPN exists both as a monomer and as a high-molecular-weight polymer – the latter is formed by tissue transglutaminase-mediated crosslinking of glutamine and lysine residues in OPN to create homo- or heterotypic protein assemblies. Polymeric OPN has increased binding to collagen and promotes osteoblast adhesion, but despite these initial observations, the role of polymer OPN in mineralization is not clear. Here, we investigate the effect of polymer OPN in two in vitro systems of mineralization. Using bovine OPN crosslinked by guinea pig tissue transglutaminase, we examined the effect of polymer OPN in a hydroxyapatite crystal growth assay and in 12-day mineralizing MC3T3-E1 osteoblast cultures. Polymer OPN was able to inhibit the growth of hydroxyapatite crystals in solution at concentrations similar to monomer OPN, but not in osteoblast culture mineralization when it was added at later time points after an extracellular matrix had been established. However, when polymer OPN was added to osteoblast culture for the entire duration of culture (during matrix formation and mineralization stages), it was able to inhibit mineralization, with no effect on osteoblast cell proliferation and alkaline phosphatase activity. Atomic force microscopy and dynamic light scattering analysis of the polymers revealed that OPN polymers were as large as 500-1000 µm in diameter, while OPN monomers ranged from 10-20 nm in size. Our results suggest that the increased size of polymer OPN could prevent it from accessing mineralization sites within the extracellular matrix, thus making it a less potent inhibitor than monomer OPN in osteoblast cultures. Supported by CIHR and FRQ-S.

P40 Glycosylation of fibulin-4 regulates tropoelastin interaction and assembly

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The fibulin family is a group of eight extracellular glycoproteins, characterized by a variable number of calcium-binding epidermal growth factor (cbEGF) domains followed by a fibulin-type module at the C-terminus. Fibulin-3, -4, and -5 are referred to as the short fibulins, and consist of six cbEGF domains followed by the C-terminal domain. The short fibulins are expressed in various elastic tissues, including blood vessels and skin, and are known to play important roles in elastogenesis. Fibulin-4 is of great interest as mutations lead to cardiovascular and skin disease in humans. Its absence results in perinatal death in the corresponding knockout mouse model. Human fibulin-4 contains two N-linked glycans of unknown function, one in the third cbEGF domain at position 592, and second one in the C-terminal domain at position 1180. We have recombinantly expressed histidine-tagged human fibulin-4 in the mammalian HEK293 expression system and purified the protein by chelating chromatography. After fibulin-4 was enzymatically deglycosylated, it exhibited enhanced binding to tropoelastin, the soluble form of elastin, as compared to the fully glycosylated fibulin-4. Based on these results, we hypothesized that fibulin-4 glycosylation plays a critical role in elastic fiber formation. To test this hypothesis, we generated mutants of fibulin-4 lacking the N-glycosylation site i) at position 592, ii) at position 1180, and iii) at both sites. The mutant constructs were transfected into HEK293 cells to ensure proper post-translational modifications. All glycosylation mutants were secreted in significantly lower amounts compared to the wild-type protein, and showed enhanced activation of the unfolded protein response pathway, indicating that folding in endoplasmic reticulum was stalled. This demonstrates that N-linked glycosylation of fibulin-4 is important for proper secretion of the protein into the extracellular matrix. To identify a suitable cell culture model for elastogenesis, various cell lines were surveyed by immunofluorescent staining of relevant elastogenic proteins. Pulmonary aortic smooth muscle cells (PAC1) showed expression of fibronectin, fibrillin-1, and tropoelastin. The fibulin-4 wild-type and the glycosylation mutants were transfected in PAC1 cells to study their consequences on elastogenesis. We found that overexpression of fibulin-4 enhances tropoelastin secretion from these cells. All glycosylation mutants enhanced the assembly of tropoelastin into extracellular fibers significantly more than the wild-type fibulin-4. The data suggest that the fibulin-4 interaction with tropoelastin is regulated by the presence of N-linked glycans, possibly within the secretory pathway as a chaperone function. The data further indicate that the N-linked fibulin-4 glycans inhibit the extracellular elastogenesis.

P41 Subtracting the matrix out of the equation: matrix metalloproteinases in diseases Subtracting the matrix out of the equation: matrix metalloproteinases in diseases

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Extracellular matrices (ECM) and connective tissues are constantly being synthesized and then dynamically remodelled to maintain proper tissue homeostasis. These biological events are accompanied by or accomplished by proteolytic activity, respectively, and are tightly regulated at many levels. Among several enzymes have been described to orchestrate these events the matrix metalloproteinases (MMPs) have been studied for decades in context of the ECM in numerous diseases. As their name suggests, MMPs were discovered as ECM protein degraders but we now know that MMPs are involved in a plethora of biological processes other than ECM remodeling, and many more interesting processes where they exert higher order control of cellular responses. Several ECM products can also affect cell migration and signalling therefore contributing to inflammation. Using a proteomics approach, TAILS (Terminal amine isotopic labeling of substrates), several ECM and non-ECM MMP substrates were identified and validated in cell and animal models. In arthritis, it is shown that MMPs contribute to matrix degradation but also play a dominant role in the control of inflammation. Other proteases such as the cathepsins appear to have more dominant role in matrix degradation than MMPs. MMPs have evolved to be even more fascinating and essential regulators of cell function than before in the drug development heyday. After more than 50 years of investigation, it appears that just like tadpole metamorphosis to frogs, and the more dramatic metamorphosis of a frog to a handsome prince upon a princess’s kiss, MMPs too have morphed from dowdy matrix remodellers to princes of the cell signaling realm.

P42 In vitro modelization of the impact of inflammation on human reconstructed adipose tissuesIn vitro modelization of the impact of inflammation on human reconstructed adipose tissues

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INTRODUCTION: Inflammation is a normal phase of the wound healing process likely to occur following tissue transplantation. Human reconstructed adipose tissues (hrAT) produced by tissue engineering represent a promising alternative to autologous fat grafts in reconstructive surgery. It is therefore important to determine the impact of inflammation on hrAT. We hypothesized that an in vitro inflammatory context will induce a modulation of the gene expression and secretion profile of hrAT and induce a degradation of their capillary network.\\ METHODS: hrAT containing or not a capillary network were produced by tissue engineering using adipose-derived stem/stromal cells and microvascular endothelial cells. They were incubated with 10 or 100 ng/ml tumor necrosis factor α (TNFα) and 10 ng/ml interleukin-1β (IL-1β) for up to 6 days in vitro. Tissue samples and culture supernatants were collected and analyzed by quantitative real-time PCR, ELISA assays and confocal microscopy following an immunostaining for the endothelial cell marker CD31.\\ RESULTS: In hrAT devoid of endothelial network, quantitative PCR established the gene expression of TNFα receptors TNFRSF1A and TNFRSF1B. Gene expression of CCL2 coding for the monocyte chemoattractant protein-1 (MCP-1), which has an important role for immune cell recruitment at the site of inflammation, was increased up to 10 fold after 6h and 24h of stimulation with TNFα. A decrease in expression of adipocyte's lipid metabolism related genes SLC2A4, FASN and LIPE was also observed. A robust increase in gene expression of 3 targets of the NF-κB pathway activation after TNFα exposure (TNFAIP3, PTGS2, TRAF1) was observed as well. Culture supernatants from hrAT treated for 24h with 10 and 100 ng/ml TNFα revealed an increase in MCP-1 (3.3 and 5.0 fold, respectively). NGF (1.6 and 2.3 fold) and HGF (1.6 and 1.5 fold) were also increased while no differences were observed for VEGF or leptin secretion. For hrAT containing a preformed network of capillaries in vitro, a chronic 6-day incubation with IL-1β alone or in combination with TNFα revealed a smaller (53% and 64%, respectively) and less ramified network (2.3 and 4.0 fold).\\ CONCLUSION: The results establish that stromal cells, adipocytes and endothelial cells contained in hrAT respond to an inflammatory context. Using hrAT as a model will facilitate the study of the impact of inflammation on human adipose tissue such as after reconstructed tissue implantation or in the context of obesity, a chronic low-grade inflammation state. Supported by the CIHR.

P43 Pirfenidone as an Inhibitor of Skeletal Muscle FibrosisPirfenidone as an Inhibitor of Skeletal Muscle Fibrosis

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We identified a population within the human skeletal muscle (CD90+) capable of differentiating into myofibroblasts, the most important cells involved in the development of fibrosis, after stimulation with transforming growth factor beta (TGFβ1). These progenitors were characterized and the potential of Pirfenidone, a drug currently used for the treatment of idiopathic pulmonary fibrosis, to inhibit their myofibroblastic differentiation was evaluated by measuring the expression of α-smooth muscle actin (αSMA) and collagen type I at gene and protein levels as well as contractility of the cells. Pirfenidone inhibited the myofibroblastic differentiation by decreasing αSMA and collagen expression, lowering contractility and inhibiting expression of pro-fibrotic genes. Pirfenidone hindered the formation of myofibroblasts by diminishing phosphorylation of ERK1/2. Reduction of Smad2/3 phosphorylation also seems to be involved in the mechanism through which Pirfenidone affects myofibroblastic differentiation. We also showed reduction of αSMA and pro-fibrotic genes expression as well as a significant decrease in the collagen content of the muscles of Duchenne's muscular dystrophy mouse model (MDX) after 6 months treatment with Pirfenidone. Identifying myofibroblast progenitors in human skeletal muscle and showing the anti-fibrotic effects of Pirfenidone are steps forward to better understand and treat muscle fibrosis observed in several muscle regenerative disorders.

P44 Organ-Specific ECM Biomimetics By Self-Assembly For the Study of Stromal Induction of Epithelial MaturationOrgan-Specific ECM Biomimetics By Self-Assembly For the Study of Stromal Induction of Epithelial Maturation

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Introduction and objectives: Tissue stromas influence the different epithelia, starting in utero and extending into adulthood. Synthetic extracellular matrices, including hydrogels scaffolds and nanofabricated collagen fibers, have many shortcomings and do not replicate normal extracellular matrix(ECM) biology. The self-assembly method for ECM formation using stromal cells, closely mimics natural ECM. In this study, we studied the impact of organ-specific stromal cells on the maturation of genitourinary epithelium and compared it to non-specific stromal cells. Material and methods: Tissue biopsies from human skin, urinary bladder and vagina were collected. Epithelial and stromal cells were individually isolated from each biopsy. Stromal cells were cultured under the influence of ascorbic acid to form collagen sheets. Stromal sheets were superimposed to form a single construct, followed by seeding of epithelial cells on the stromal matrix. Four types of equivalents were formed (dermal stroma/urothelium, bladder stroma/urothelium, dermal stroma/vaginal epithelium, vaginal stroma/vaginal epithelium). All four equivalents were cultured at the air-liquid interface for 3 weeks to allow maturation and further evaluation. The analyses of all equivalents included histological and functional assessments for epithelial maturation and differentiation. Different types of stromas were evaluated for ECM proteins and relevant receptor expression. Results: Different stromal sheets were formed and could be handled easily. Regarding urothelium, a well-stratified epithelium was formed on both stromas; however, it was more differentiated when placed on bladder stromal cells than on dermal fibroblasts as shown by evident expression of CK20 and lack of expression of CK14. Permeability test was similar among the 2 urothelial equivalents. As for vaginal epithelium, it was better stratified and differentiated when it was grown on vaginal stroma than dermal stroma. Mucin and glycogen production were seen only with vaginal stroma. Conclusions: Organ-specific stromal cells and ECM induces more efficiently their corresponding epithelium maturation, and it was even more evident with vaginal epithelium. Self-assembled Organ-specific ECM biomimetics of urogenital tract are novel models for studying mesenchymal-epithelial interactions involved in tissue development, repair and regeneration and epithelial differentiation.

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