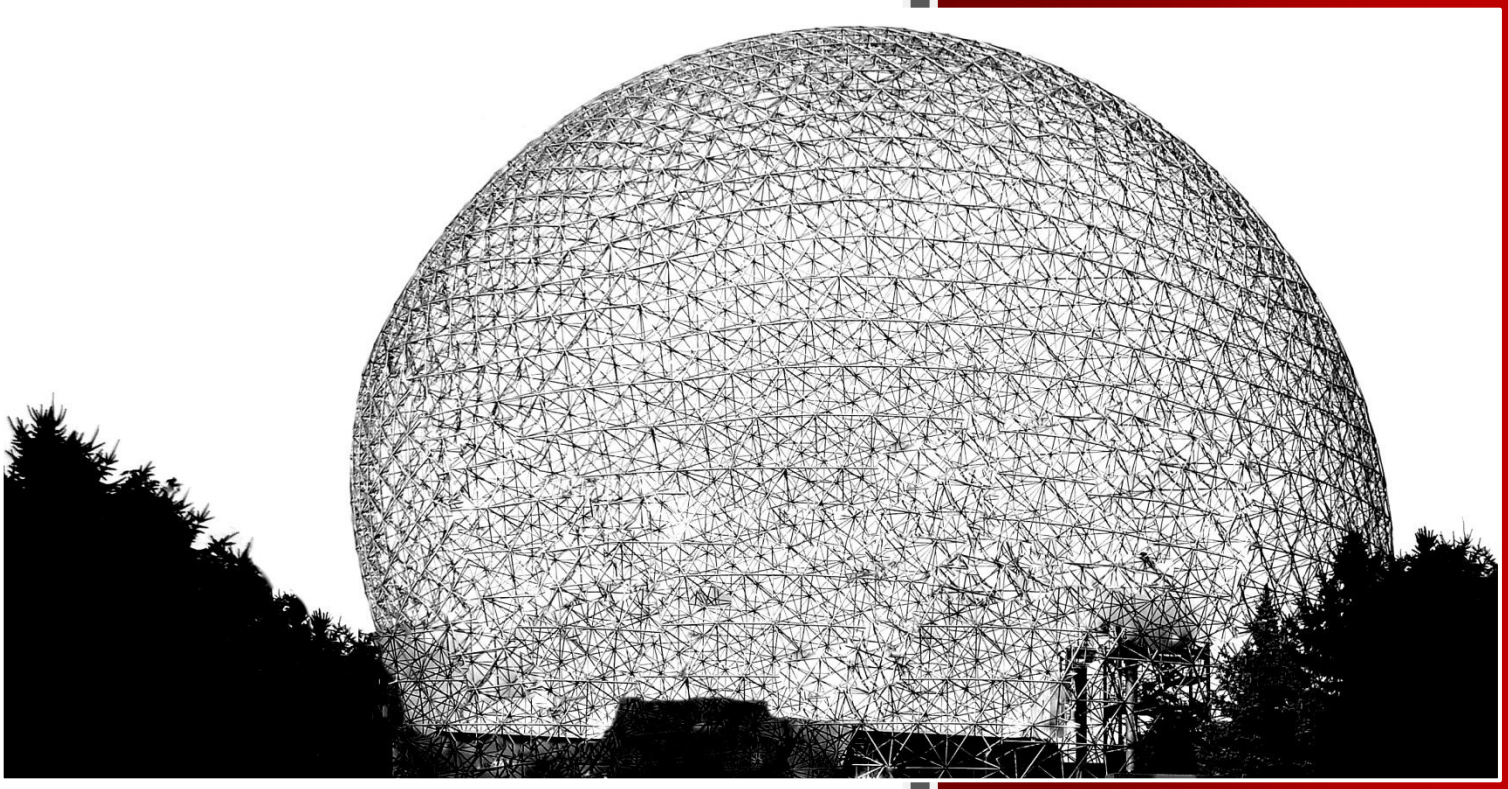
May 29-June 1
2013

19TH ANNUAL CANADIAN CONNECTIVE TISSUE CONFERENCE



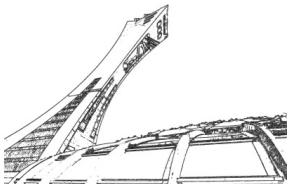
PROGRAMME BOOK

McGill University

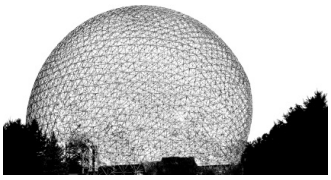
Montreal, Canada

CCTC 2013

http://connective-tissue-canada.com/cctc-2013/



CCTC Montreal **2013**



Dear Colleagues and Friends,

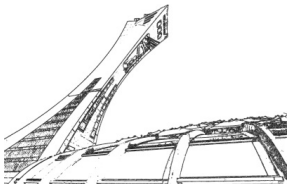
Welcome to Montreal and the 19th Canadian Connective Tissue Conference held at McGill University from May 29th- June 1st 2013.

CCTC 2013 will build on the historical CCTC model with the overall goals of providing a platform allowing the exchange of scientific knowledge, facilitate the generation of a network of collaborations and novel research teams. The mission of the CCTC is to help foster the next generation young Canadian scientists and thus trainees at the conference are given ample opportunity to chair the scientific sessions, to present their research and interact with leading Canadian and international researchers.

The program features 9 oral presentation sessions and 3 poster presentation sessions, starting Thursday morning and running throughout Friday and Saturday. A banquet will be held on Friday evening. New for this year are two guided poster sessions. The posters will be divided into several groups based on their area of research. The designated judges for a group of posters will visit each of the posters together with all the presenters in that group. Each presenter will get the opportunity to present his/her poster to the group.

This year's research themes are:

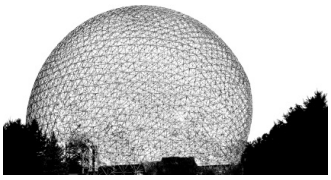
- **Connective tissue in disease - Clinical and fundamental aspects**
- **Stem cells in tissue regeneration and tissue engineering**
- **Genetic basis of connective tissue disease**
- **Bone, cartilage and tooth development**
- **Biomechanics, mechanobiology, and biomaterials**
- **The extracellular matrix in connective tissues**
- **Connective tissue remodeling**



CCTC

Montreal

2013

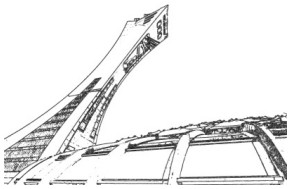


The Canadian Connective Tissue Society was formed in 2012 and will hold its second board meeting in Montreal. The aim of the CCTS is to fill the gaps in current scientific and clinical understanding of connective tissues in both health and disease through communication of health research evidence between Canadian basic scientists, clinicians, and small medium enterprises. Please visit http://connective-tissue-canada.com/ for more information.

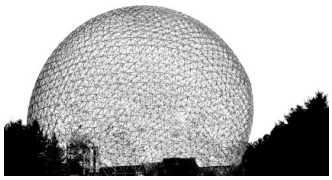
We look forward to seeing you and wish that you will enjoy the program and social activities of the 19th Canadian Connective Tissue Conference in Montreal.

Lisbet Haglund, Ph.D.
Assistant Professor, Surgery
Orthopaedic Research Laboratory
Montreal General Hospital, Room C9.173
1650 Cedar Ave, Montreal, H3G1A4

Monzur Murshed
Assistant Professor
Department of Medicine and
Faculty of Dentistry
1529 Cedar Avenue, Montreal, H3G1A6



CCTC Montreal 2013



WELCOME TO THE ANNUAL MEETING IN MONTREAL AT MCGILL UNIVERSITY

Dear Colleagues and Friends,

Since its founding in 1994, the Canadian Connective Tissue Conference (CCTC) has been held annually to bridge the gaps in our current scientific and clinical understanding of connective tissues, in both health and disease. The meeting gives us a wonderful opportunity to network, develop new collaborations, and learn the latest in the field.

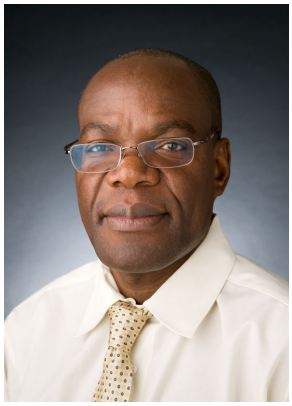
Over the years, the conference has helped foster the next generation of young Canadian scientists by giving them the opportunity to present their research, as well as enabling them to interact with leading Canadian and international researchers. In 2012, this network was formalized during the Toronto meeting by inaugurating the Canadian Connective Tissue Society (CCTS). A distinctive feature of the CCTS is our commitment to giving a platform to trainees. This unique approach will strengthen the future of connective tissue research in Canada.

On behalf of the members of the founding board, the co-chairs Dr. Haglund and Dr. Murshed and the local organizing committee it is my pleasure to invite you to attend the 2013 CCTS Annual Meeting in Montreal, from May 29 – June 1st.

We have put together an outstanding program that promises once again to encompass basic research, translation and clinical application. It will interest both trainees and experienced researchers.

The meeting will take place in a vibrant city and guarantees a great opportunity for many summer festivals. Montreal has a rich historic heritage and was recently named the UNESCO city of design. We look forward to hosting you in Montreal.

Best regards,



Fackson Mwale

President, Canadian Connective Tissue Society (CCTS)

Project Director and Associate Professor
Department of Surgery, McGill University
Orthopaedics Research LAB
Lady Davis Institute for Medical Research
3755 Chemin Cote Ste Catherine Rm 602
Montreal QC H3T 1E2 Canada

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Canadian Connective Tissue Conference 2013

Programme

Wednesday, 29.05.2013

5:30 pm-8:00 pm	Registration opens
6:00 pm-8:00 pm	Poster mounting (all posters on display all days)

Thursday, 30.05.2013

8:00 am	Registration opens (until 12:00)
8:00 am	Mounting of late posters
8:30 - 9:00 am	**Welcome Remarks by Lisbet Haglund and Monzur Murshed**. (Conference chairs)

9:00 – 10:15 am	**Session 1** **Genetics of Connective Tissue** *Co-chairs: J-F Lavoie and Nilesh Talele*
10:15 - 10:30 am	**Coffee Break.**

10:30 –11:45 pm	**Session 2** **Bone, Cartilage and tooth development** *Co-chairs: Michael P Grant and Chen Li*
11:45- 2:15 pm	**Lunch, Poster viewing (Poster Session I)**

11:45-2:15 pm		**Poster Session I (viewing of first four groups of posters)**

2:15 – 3:30 pm	**Session 3** **Connective Tissue in Disease - Clinical and Fundamental Aspects** *Co-chairs:* Corey Maggiano *and Rémi Parenteau-Bareil*
3:30 - 3:45 pm	**Coffee Break**

3:45 – 5:10 pm	**Session 4** **Stem Cells in Tissue Regeneration and Tissue Engineering** *Co-chairs: Derek Rosenzweig and Elizabeth Cambridge*

6:00 – 8:00 pm	 **Cocktail hour and Poster session II (all posters on display)**

Friday, 31.05.2013

8:30 – 09:35 am	**Session 5** **Biomechanics, Mechanobiology, and Biomaterials** *Co-chairs: Oladunni Babasola and Bashar Alkhatib*
9:35 - 10:00 am	**Coffee Break.**

10:00 - 11:15 am	**Session 6** **The Extracellular Matrix in Connective Tissues 1** *Co-chairs: Elie Abed and Rahul Gawri*
11:15- 1:30 pm	**Lunch, Poster viewing (Poster Session III)**

11:15 -1:30 pm	 **Poster Session III (viewing of last four groups of posters)**

1:30 – 2:50 pm	**Session 7** **The Extracellular Matrix in Connective Tissues 2** *Co-chairs: William W Du and Laurence Tessier*
2:50 - 3:15 pm	**Coffee Break**

3:15 – 4:20 pm	**Session 8** **Connective tissue remodeling 1** *Co-chairs: Irvens Fanélus and Justin Parreno*

7:00 pm: Dinner Banquet

Saturday, 01.06.2013

9:00 – 10:15 am	**Session 9** **Connective tissue remodeling 2** *Co-chairs: Elena Zimina and Charles-Hubert Lafantaisie-Favreau*
10:15-10:30 am	**Coffee Break**

10:30-12:00 pm	**Closing remarks and Award ceremony**

12:00 pm	**CCTS Business meeting**
1:00 pm	**End of Conference**

General Information

Conference Venue:

The SSMU Building
3480, rue McTavish
Montréal, Québec, Canada
H3A 0E7

On-site Registration:

Cash and cheque will be accepted for onsite registration up to the start of the conference. 135 CAD for trainees/assistants and 185 CAD for researchers

Wireless Network:

Please enquire at the registration desk about username and password.

Location of Sessions:

SSMU Ballroom, third floor of the SSMU building.

Location of Poster Sessions:

SSMU Ballroom, third floor of the SSMU building.

Wine and Cheese:

Thursday, May 30th from 6-8pm

Venue: SSMU Ballroom, third floor of the SSMU building.

Location of the Banquet:

Directions to Thomson House from the Confernce Venue:

3650 McTavish, Montreal, Quebec, H3A 1Y2

- Walk uphill on Rue McTavish, walk up the stairs and cross Ave du Dr. Penfield, continue walking up Rue McTavish and Thompson House is located on your left

Thomson House is an elegant mansion located in Montreal's Golden Square Mile district, which was the richest neighbourhood in Canada in the early 20th century. Rich in history Thomson House was purchased from the Gravel family in 1968 by McGill University and renamed the Thomson House after former Dean and Vice-Principal David L. Thomson in 1971. Since 1969 Thomson House has served as the home of McGill's Post Graduate Student Society and is filled with several bars, lounges, study areas, conference rooms and a banquet hall

Tickets: Please inquire at the registration desk

Directions to Conference Venue

Metro stop: Peel (on the green line). Follow the signs for Rue Metcalfe to exit the metro station. Walk up hill on Rue Melcalfe, cross Rue Sherbrooke to Rue McTavish and continue up Rue McTavish to the venue.

Bus stops: 24 on Rue Sherbrooke at Rue Peel, 144 on Ave du Dr. Penfield at Rue Peel and des Pins at Peel, 107 on Rue Peel at Rue Sherbrooke

- Metro tickets are $3/ticket, one day passes are $9 and three day passes are $18
    - Tickets can be used on the bus and the metro
- One day and three day passes can also be used aboard the 747, which is the bus that runs from the Pierre Elliot Trudeau Airport

From the Berrie-UQAM metro stop (also the Greyhound bus Station and where the 747 airport shuttle stops):

- Get on the metro on the green line towards Angrignon, get off at the Peel stop (see metro stop above).

From the Pierre Elliot Trudeau Airport:

Shuttle Bus (recommended):

- The 747 shuttle runs between every five minutes to every hour (depends on the time of day) from the airport and stops at the Lionel-Groulx metro (Ave Atwater and Rue Saint-Jacques), several hotels places along Boulevard Rene-Leveques near hotels and at the Berri-UQAM metro (Rue Berri and Rue Ontario). It also goes back to the airport
- To go straight from the airport the easiest way is to get off at the Lionel-Groulx metro and get onto the green line towards Honore-Beaugrand and get off at the Peel Metro (see metro stop above)
    - The cost is $9, but a $9 one day pass and a $18 three day pass are also valid on the 747

Taxi:

- There is a taxi line up and it is easy to take a taxi anywhere in Montreal
    - A Taxi to the downtown area will cost $50-$60+

From Gare-Central (Via station):

- There are many exits from the train station, many of which lead into Montreal's extensive underground network and thus it is easy to get lost if you are unfamiliar with the area
- Follow signs for Boulevard Rene-Levesque to exit

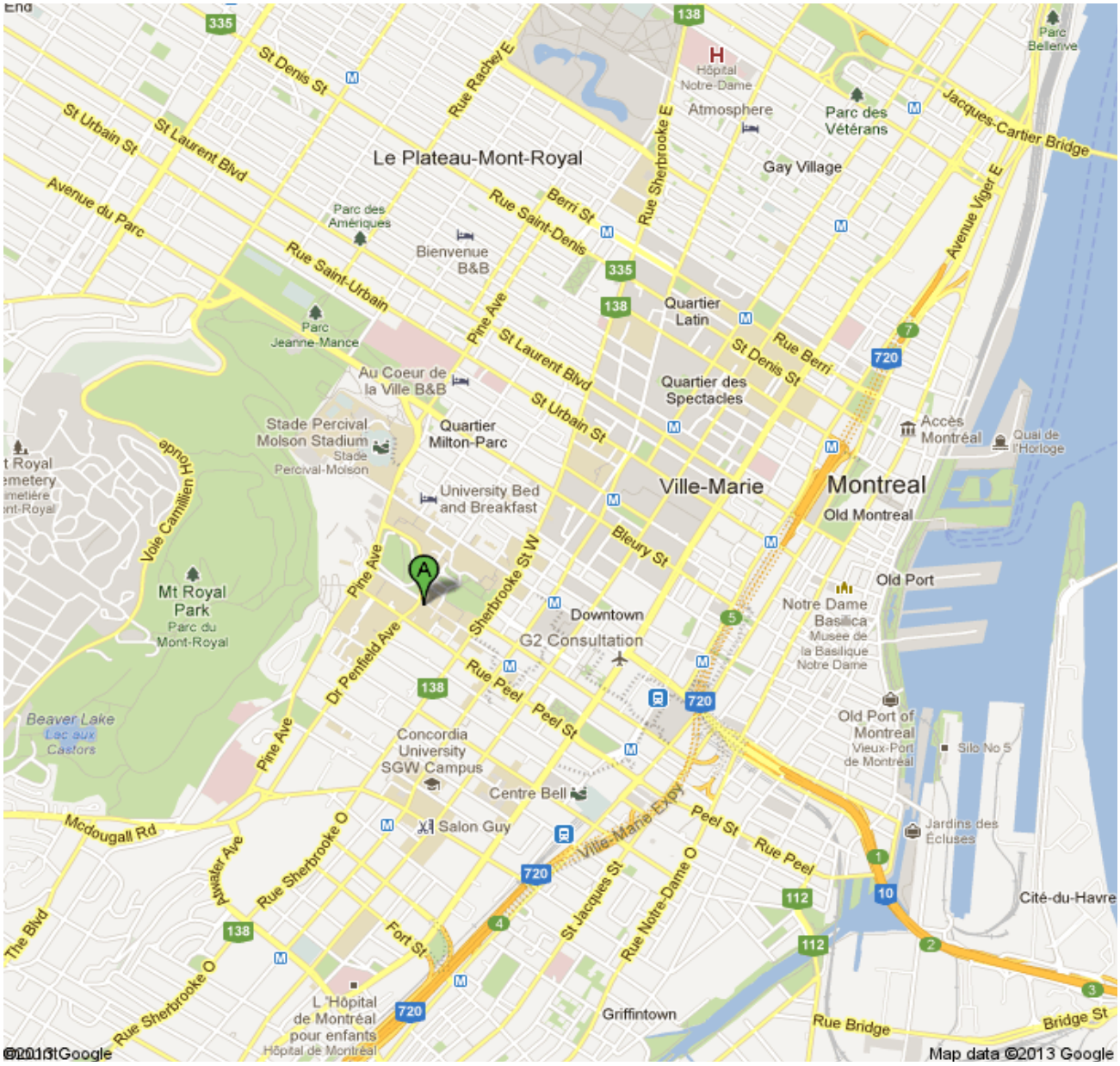
- Once on Rene-Levesque walk along it going West (should be to the left out of the train station) towards Rue Metcalfe.
- Turn right onto Rue Metcalfe and walk up hill to Rue McTavish and the conference venue.

From the MegaBus Station:

- Walk West along Rue St. Antoine to Rue Peel and get on the 107 bus going North (uphill) on Rue Peel.
- Get off at the Peel-Sherbrooke stop and walk East (right from the direction the bus was travelling) to Rue McTavish. Turn left on McTavish and walk up to the Conference Venue.

Driving:

- This is not recommended, parking in downtown Montreal is expensive and parking tickets are more expensive
- Pay parking on the street is available on many streets surrounding the venue including on Rue McTavish North of Ave du Dr. Penfield.
- There is also free street parking on the block of Rue Mctavish between Ave du Dr. Penfield and Ave des Pins but pay special attention to the signs
- There are several pay lots in downtown Montreal that generally cost $25+ for a full day of parking
- Please note that the block of Rue McTavish that the Conference Venue is located on is a pedestrian Street.



Map Showing Venue of CCTC 2013 (A).

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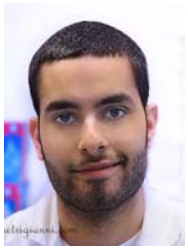
Local Organizing Committee

Rajashree Sen
(Coordinator)

Rahul Gawri
(Coordinator)

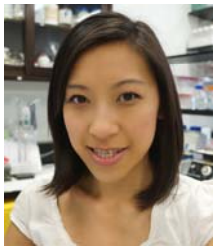
Yoon Chi
(Registration Support)

Emerson Krock
(Registration Support)



Bashar Alkhatib
(AV Support)

Derek Rosenzweig
(AV Support)



Betty Hoac
(Judges/Poster Support)

Juliana Marulanda
(Judges Support)

Surabhi Parashar
(Poster support)

Iris Boraschi-Diaz
(Poster support)

Janet Moir
(Photographer)

Yi Wen Shao
(On-site Support)

Scientific Committee

Mari Kaartinen (McGill University)
Dieter Reinhardt (McGill University)
Julie Fradette (Laval University)
Tannin Schmidt (University of Calgary)
Boris Hinz (University of Toronto)

Keynote Speaker

Gerard Karsenty (Columbia University)
" *The Contribution of Bone to Whole Organism Physiology"*

Invited Speakers

Makarand Risbud (Thomas Jefferson University)
"Gasping for Air in the Intervertebral Disc: PHD a newly emerging player"

Simon Tran: (McGill University)
"*Tissue engineering and stem cells for salivary glands* **"**

Frank Rauch: **(**McGill University**)**
"New Gene Defects Causing Fractures in Children**"**

Geoffrey Hendy: (McGill University)
"*Role of Menin in Bone Development* **"**

Tannin Schmidt (University of Calgary)
"*Boundary Lubricating Properties of Proteoglycan 4 (PRG4) on Articular Cartilage, the Ocular Surface, and Other Biomaterials***"**

Boris Hinz (University of Toronto)
"About the stress of working with myofibroblasts"

Veronique Moulin. LOEX center of Université Lava*l*
"Understanding skin fibrosis using tissue engineered models"

Keynote Speaker

Gerard Karsenty, M.D., Ph.D.

Paul A. Marks, M.D., Professor and Chairman

Department of Genetics & Development

Columbia University Medical Center

701 W 168th Street, Room 1602A HHSC

New York, NY 10032

Gerard Karsenty received his M.D. and Ph.D. from the University of Paris, France and completed his post-doctoral training at the University of Texas MD Anderson Cancer Center in 1990. His laboratory has studied every aspect of skeletal biology ranging from cell differentiation to function. His laboratory was the first one to decipher the molecular bases of osteoblast-specific gene expression, a work that culminated in his identification of Runx2 as the master gene of osteoblast differentiation. This was followed by to the identification of an entire cascade of transcription factors regulating osteoblast differentiation with his demonstration of the role in this pathway of Twist and ATF4. He also identified Gm2 as the master gene of parathyroid gland development. His laboratory has had, from its inception, a continuous interest in the physiology of the skeleton. After having elucidated the genetic bases of mineralization of the extracellular matrix in bone, (and its absence in other tissues) his laboratory has focused attention on the genetic bases of bone growth, modeling and remodeling. The overarching assumption of his work is that the appearance of bone during evolution has changed profoundly the physiology of animals because of the energetic cost that bone growth entails. Thus his group has explored in the last 10 years the hypothesis that the control of bone mass and energy metabolism must be coordinated and that this coordination is done in large part by hormones like leptin, adiponectin and osteocalcin that appear during evolution with bone. His lab has explored through genetic and molecular means every aspect of this hypothesis. At the same time a expanding his research the Karsenty lab is exploring whether there are additional connections between bone physiology and the function of other organs such as fertility. This work culminated in the discovery that bone, via osteocalcin, regulates testosterone production. For his work Dr. Karsenty has received several national and international awards.

Canadian Connective Tissue Conference 2013
Programme

Wednesday, 29.05.2013

5:30 pm-8:00 pm	Registration opens
6:00 pm-8:00 pm	Poster mounting (all posters on display all days)

Thursday, 30.05.2013

8:00 am	Registration opens (until 12:00)
8:00 am	Mounting of late posters

Welcome Remarks

8:30 - 9:00 am	**Lisbet Haglund & Monzur Murshed** (Co-chairs of CCTC 2013)

Session 1: Genetics of Connective Tissue (9:00 - 10:15 am)
Co-chairs: J-F Lavoie and Nilesh Talele

9:00 - 9:25 am	**Invited Speaker: Frank Rauch (McGill University)** ***"New Gene Defects Causing Fractures in Children"***
9:25 - 9:35 am	**Tanya Zappitelli** (University of Toronto, Toronto) *"G60S Connexin 43 mutation causes increased osteoblast activity and adipogenesis via upregulated BMP2/4 signalling*"
9:35 - 9:45 am	**Weining Yang** (Institute of Medical Science) *"Versican 3'-untranslated region (3'UTR) promotes dermal wound healing by regulating miRNA activity "*
9:45 - 9:55 am	**Kristen Fay Gorman** (University of Montreal/ Saint Justine Children's Hospital Research Center) *"Key molecules involved in cellular and biochemical endophenotypes of idiopathic scoliosis"*
9:55 - 10:05 am	**Zohreh Khavandgar** (McGill University, Montreal) "*Elastin Haploinsufficiency Delays Arterial Calcification in MGP Deficient Mice*"
10:05- 10:15 am	**Kerstin Becker** (University of Cologne, Cologne, Germany) *"Whole Genome Association and differential gene expression studies provide evidence for the importance of the Wnt Signaling pathway in Dupuytren's disease* "
10:15 - 10:30 am	**Coffee Break**

Session 2: Bone, Cartilage and tooth development (10:30 –11:45 am)
Co-chairs: Michael P Grant and Chen Li

10:30 - 10:55 am	**Invited Speaker: Geoffrey Hendy (McGill University)** ***"****Role of Menin in Bone Development* ***"***
10:55 - 11:05 am	**Nancy Karam** (Sainte-Justine University Hospital Research Center/ Université de Montréal, Montreal) "*Osteoporosis and periodontitis, Pitx1 is the link "*
11:05 - 11:15 am	**Hazem Eimar** (McGill University, Montreal) *"Cholinergic agonists with muscarinic activity favor bone mass accrual by stimulating osteoblast proliferation*"
11:15 – 11:25 am	**Élie Abed** (Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM), Montréal) " *Low Sirtuin 1 levels in human Osteoarthritis Subchondral Osteoblasts lead to abnormal SOST expression which decreases Wnt/β-catenin activity.*"
11:25 - 11:35 am	**Dareen Abd Elaziz (**McGill University, Montreal) *"Therapeutic Effects of Rapamycin on Pain and Osteolysis in a Murine Model of Bone Cancer "*
11:35- 11:45 am	**Sumeeta Warraich** (University of Western Ontario) *"Disruption of Biomineralization Pathways in Spinal Tissues from Mice Lacking Equilibrative Nucleoside Transporter*
11:45 - 2:15 pm	**Lunch, Poster viewing (Poster Session I)**

11:45 – 2:15 pm		**Poster Session I (viewing first four groups of posters)**

Session 3: Connective Tissue in Disease: Clinical and Fundamental Aspects (2:15 -3:30 pm). *Co-chairs: Corey Maggiano and Rémi Parenteau-Bareil*

2:15– 2:40 pm	**Invited Speaker: Makarand Risbud (Thomas Jefferson University)** "*Gasping for Air in the Intervertebral Disc: PHD a newly emerging player*"
2:40 – 2:50 pm	**Steven Wong** (McMaster University, Canada) *"Differential Activation of SMAD1 and SMAD2 Proteins in Lung Epithelial Cells in a Mouse Model of OSM-induced Lung ECM Accumulation"*
2:50 – 3:00 pm	**Padma Madiraju** (Jewish General Hospital, Montreal) *"The Effect of Fetuin on Mineralization of Intervertebral Disc Cells*"
3:00- 3:10 pm	**Padina Pezeshki** (University of Toronto, Toronto) *"Does Radiofrequency Ablation comprehensively destroy bone and tumour cells?"*

3:10-3:20 pm	**Michael P Grant** (Lady Davis Research Institute, Montreal) *"Extracellular calcium-sensing receptor inhibition and not activation enhances stimulation of proteoglycan synthesis in normal and osteoarthritic human chondrocytes"*
3:20-3:30 pm	**Haoran Li** (University of Toronto, Toronto) *"Anti-microRNA-378 Enhances Wound Healing Process"*
3:30-3:45 pm	**Coffee Break**

Session 4: Stem Cells in Tissue Regeneration and Tissue Engineering (3:45 -5:10 pm) *Co-chairs: Derek Rosenzweig and Elizabeth Cambridge*

3:45 – 4:10 pm	**Invited Speaker: Simon Tran (McGill University)** **"***Tissue engineering and stem cells for salivary glands* **"**
4:10 – 4:20 pm	**Jean-Michel Bourget**(Université Laval, Québec) *"Fibroblast-Derived Microstructured ECM Produced in vitro by the Self Assembly Approach as a scaffold for Vascular Media Reconstruction"*
4:20- 4:30 pm	**Nilesh Talele** (University of Toronto, Toronto) *"Extracellular matrix stiffness reversibly governs the stemness and fibrotic fate of mesenchymal stromal cells in a pro-fibrotic microenvironment"*
4:30- 4:40 pm	**Rémi Parenteau-Bareil** (Centre LOEX de l'Université Laval, Québec) *"Innervation of engineered connective tissue using human induced pluripotent stem cells"*
4:40- 4:50 pm	**Maxime Desgagné** (Centre LOEX de l'Université Laval Québec) *"Tissue-engineered human skin reconstructed with a modified self-assembly approach to shorten the production time of autologous skin."*
4:50- 5:00 pm	**Chen Li** (University of Toronto, Toronto) *"Epigenetic Imprinting Effects of the Mechanical Environment on the Fibrogenesis of Mesenchymal Stem Cells "*
5:00- 5:10 pm	**Garima Dwivedi** (Ecole Polytechnique) *"Characterization of progenitor cells isolated from the subchondral bone of rabbit trochlea and condyle"*

6:00 – 8:00 pm		**Cocktail hour and Poster session II (all posters on display)**

Friday, 31.05.2013

Session 5: Biomechanics, Mechanobiology, and Biomaterials (8:30 -9:35 am)

Co-chairs: Oladunni Babasola and Bashar Alkhatib

8:30 - 8:55 am	**Invited Speaker: Tannin Schmidt (University of Calgary)** **"***Boundary Lubricating Properties of Proteoglycan 4 (PRG4) on Articular Cartilage, the Ocular Surface, and Other Biomaterials***"**
8:55 - 9:05 am	**Sotcheadt Sim** (Biomomentum Inc/Ecole Polytechnique de Montreal, Montreal) "*Evaluation of a novel technique to map the mechanical properties of an entire articular surface in indentation*"
9:05 -9:15 am	**Hamid Mohammadi** (University of Toronto; University of Pennsylvania.) "*Remote Mechanosensing by Cells on Thin Floating collagen Matrices"*
9:15- 9:25 am	**Yousef Shafieyan** (McGill University, Montreal) "*Contrast Agent Diffusion in Compressed Articular Cartilage*"
9:25 - 9:35 am	**David Fong** (École Polytechnique de Montreal) *"Biodegradable chitosan microparticles stimulate U937-derived macrophages to activate STAT-1 and release stem cell chemokines and anti-inflammatory wound-healing factors*
9:35 - 10:00 am	**Coffee Break.**

Session 6: The Extracellular Matrix in Connective Tissues 1(10:00 -11:15 am)

Co-chairs: Elie Abed and Rahul Gawri

10:00 -10:15 am	**Message from the President: Fackson Mwale, CCTS info**
10:15 - 10:25 am	**Cui Cui** (McGill University, Montreal) *"Transglutaminase activity is required for proper fibronectin fibrillogenesis and stabilization of circulating plasma fibronectin into bone matrix*"
10:25 - 10:35 am	**Maxime Tondreau** (LOEX center of Université Laval, Quebec) " *A human tissue-engineered fibroblast-derived scaffold for vascular applications"*
10:35 – 10:45 am	**Vamsee Dhar Myneni** (McGill University, Montreal) "*Inhibition of transglutaminase enzyme activity increases adipocyte differentiation by modulating the actin cytoskeleton and fibronectin matrix assembly*"
10:45 - 10:55 pm	**Bashar Akhatib** (McGill University, Montreal) *"Chondroadherin Fragmentation as a Biochemical Marker for Early Stage Disc Degeneration*"

10.55- 11:05 pm	**Franco Klingberg** (University of Toronto, Toronto) "*Organized is More Efficient: TGF-β1 Activation from the Extracellular Matrix*"
11:05 - 11:15 pm	**Elena Zimina** (University of Toronto, Toronto) *"Kindlin-2 is a mechanosensitive regulator of the fibroblast to myofibroblast differentiation in cardiac repair*"
11:15 - 1:30 pm	**Lunch, Poster viewing (Poster Session III)**

11.15 - 1:30 pm		**Poster Session III (viewing of last four groups of posters)**

Session 7: The Extracellular Matrix in Connective Tissues 2 (1:30 – 2:50 pm)

Co-chairs: William W Du and Laurence Tessier

1:30 - 2.20 pm	**Keynote Speaker: Gerard Karsenty (Columbia University)** *"The Contribution of Bone to Whole Organism Physiology"*
2:20 - 2:30 pm	**Mohammad Atarod** (University of Calgary, Calgary) *"Mechanical Adaptations of Tissues in the Knee Joint Over Time Following ACL Injury"*
2:30 - 2:40 pm	**Mark C Blaser** (University of Toronto, Toronto) "*Versican-rich Proteoglycan Thickening in Diet-Induced Early Aortic Valve Disease in Mice*"
2:40 – 2:50 pm	**Dominique A. Behrends** (McGill University, Montreal) "*The Function of Mast Cells in Bone Regeneration*"
2:50 - 3:15 pm	**Coffee Break**

Session 8: Connective tissue remodeling 1 (3:15 - 4:20 pm)

Co-chairs: Irvens Fanélus and Justin Parreno

3:15 – 3:40 pm	**Invited Speaker: Boris Hinz (University of Toronto)** *"***About the Stress of working with myofibroblasts***"*
3:40 – 3:50 pm	**Michael Pest** (Western University, London) *"Disruption of knee joint morphology and development of oseteoarthritis in a cartilage specific MIG-6 deletion mouse"*
3.50 - 4:00 pm	**Rahul Gawri** (McGill University, Montreal) *"Link-N Peptide: Can Degenerated Human Intervertebral Discs be Repaired?*"

4:00 - 4:10 pm	**Emerson Krock** (McGill University, Montreal) "*Cytokine and Chemokine Secretion by Painful Intervertebral Discs*"
4:10- 4:20 pm	**Alexander Danco** (McGill University, Montreal) "*Effects of running exercise on disc degeneration and low back pain in mice."*

7:00 pm Banquet Dinner

Saturday, 01.06.2013

Session 9: Connective tissue remodeling 2 (9:00 - 10:15 am)

Co-chairs: Elena Zimina and Charles-Hubert Lafantaisie-Favreau

9:00 - 9:25 am	**Invited Speaker: Veronique Moulin (LOEX, Université Laval)** *"Understanding skin fibrosis using tissue engineered models"*
9:25 - 9:35 am	**Stephen M. Sims** (The University of Western Ontario, London,) "*Subcellular elevation of cytosolic free calcium is required for osteoclast migration*"
9:35 - 9:45 am	**Irvens Fanélus** (McGill University, Montreal) *"*Internalization of TGF-β receptors is impaired in scleroderma*"*
9:45- 9:55 am	**Janet Moir** (Research Institute of MUHC, Montreal) "*Bovine Intervertebral Discs Degenerated by Controlled Enzyme Treatment Responds to Physiological Load by Replacing Proteoglycans*"
9:55-10.05 am	**Heena Kumra** (McGill University, Montreal) "*The role of fibronectin in aortic wall architecture and extracellular matrix homeostasis"*
10:05-10:15 am	**Derek H. Rosenzweig** (McGill University, Montreal) "*Cartilaginous constructs using primary chondrocytes from continuous expansion culture seeded in dense collagen gels*"
10:15-10:35 am	**Coffee Break**

10:35-12:00 pm	**Closing remarks and Award ceremony**

12:00 pm	**CCTS Business meeting**

1:00 pm	**End of Conference**

Abstracts for Oral Presentations

Session 1: Genetics of Connective Tissue

Invited Lecture

New Gene Defects Causing Fractures in Children

Frank Rauch.

Shriners Hospital for Children, Montreal

The most frequent form of primary bone fragility in children is osteogenesis imperfecta (OI). In the large majority of cases, OI is caused by mutations affecting *COL1A1* or *COL1A2*, the two collagen type I encoding genes, and follow an autosomal dominant pattern of inheritance. In 10%-15% of cases, OI is caused by mutations in other genes, most of which code for proteins that participate in the processing of procollagen type I. Mutations in these genes cause recessive OI. However, for some gene defects leading to severe bone fragility in children, the relationship to collagen type I processing is not yet clear. These include a mutation in *IFITM5* with an apparently dominant negative effect, causing OI type V, and loss-of-function mutations in *SERPINF1*, which give rise to OI type VI. Homozygous mutations affecting components of the WNT signaling pathway (*LRP5*, *WNT1*) also cause severe bone fragility in children. Interestingly, heterozygous carriers of such mutations also often have osteoporosis. We recently found that a dominant gain-of function mutation in *RUNX2* can also lead to low bone density and vertebral fractures in adolescents. Due to these new genetic findings most familial cases of bone fragility can now be diagnosed with a DNA test. However, these new data also raise question with regard to the definition and classification of pediatric bone fragility disorders.

G60S Connexin 43 mutation causes increased osteoblast activity and adipogenesis via upregulated BMP2/4 signalling

Tanya Zappitelli (1) and Jane E. Aubin (1,2);

(1) Department of Medical Biophysics, (2) Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada.

Using a genome-wide ENU-mutagenesis screen, we isolated an osteopenic mutant mouse line, Gja1Jrt/+, with a G60S mutation in Connexin 43 (Cx43) that is dominant negative for gap junction formation and function (Flenniken et al., 2005). The G60S Cx43 mutation resulted in a stromal/trabecular osteoblast population that produced a bone matrix with abnormally high levels of bone sialoprotein (BSP). In younger Gja1Jrt/+ mice, the high levels of BSP along with changes in RANKL-OPG signalling, contributed to increased osteoclast number and activity resulting in early onset high turnover osteopenia (Zappitelli et al., submitted). Further, histomorphometric analysis revealed a significant increase in bone marrow adipocyte density in young Gja1Jrt/+ versus WT bones. We sought to determine the signalling mechanism by which the Gja1Jrt/+ osteoblasts upregulate expression and production of BSP protein. A QPCR array identified a number of pathways and signalling molecules that were altered in Gja1Jrt/+ versus WT stromal osteoblasts; those with largest fold-changes were the Wnt/beta-catenin and the BMP2/4 signalling pathways. Immunoblotting confirmed a significant increase in total beta-CATENIN in Gja1Jrt/+ stromal cells, however expression levels of active beta-CATENIN and two of its direct target genes, Axin2 and Nkd1, were not significantly different in mutant versus WT cells. Results of treatment with a potent Wnt signalling inhibitor, IWP-2, further confirmed that Wnt/beta-catenin signalling was normal in Gja1Jrt/+ stromal cells. On the other hand, we found that BMP2/4 signalling was upregulated in Gja1Jrt/+ stromal/trabecular osteoblasts based on increased expression of Bmp2, Bmp4, and Tcf7 genes, and increased phosphorylation of Smad1/5/8 proteins. The use of Noggin, a BMP signalling inhibitor, in stromal cultures was effective in reducing to WT levels both BMP2/4 signalling and Bsp expression level in Gja1Jrt/+ stromal cells. We next sought to determine whether the upregulation of BMP signalling was also the mechanism underlying the increase in Gja1Jrt/+ bone marrow adipogenesis. We found that expression of Pparg2, the master adipogenic regulator, as well as adipocyte markers aP2, LPL and adipsin, were significantly increased in Gja1Jrt/+ bone marrow extracts and in Gja1Jrt/+ cultures in which Bmp2 expression was increased. We conclude that the G60S Cx43 mutation does not affect Wnt/beta-catenin signalling in stromal cells, but does upregulate BMP2/4 signalling, which is at least partly responsible for the increased production of BSP in osteoblasts and the increased bone marrow adipogenesis in Gja1Jrt/+ versus WT mice.

Versican 3'-untranslated region (3'UTR) promotes dermal wound healing by regulating miRNA activity

Weining Yang, Albert J Yee.

Institute of Medical Science

Versican is an extracellular chondroitin sulfate proteoglycan. While functioning as a structural molecule, versican can also regulate a variety of cellular activities. This study was designed to explore the role of versican in the process of wound repair. To elevate levels of versican, we ectopically expressed versican 3'-untranslated region (3'UTR) as a competitive endogenous RNA to modulate expression of versican by regulating miRNA functions. We demonstrated that the wound closed faster in transgenic mice expressing the versican 3'UTR than in wild type mice. We stably expressed versican 3'UTR in NIH3T3 fibroblasts and found that the 3'UTR-transfected cells could survive longer in serum-free medium than the vector-transfected cells. The 3'UTR-transfected cells could migrate faster and had a greater ability to invade through Matrigel than the control cells. Morphologically, the 3'UTR-transfected cells displayed less capacity in adhesion than the control. Interestingly, we found that the 3'UTRs of versican and b-catenin shared common miRNAs including miR-135a, miR-185, miR-203*, miR-690, miR-680, and miR-434-3p. Luciferase assay showed that all of these miRNA could target the 3'UTRs of both versican and b-catenin, when the luciferase constructs contained fragments harboring the miRNA binding sites. As a consequence, expression of versican and b-catenin was up-regulated as compared with the controls by ectopic transfection of the versican 3'UTR, which was confirmed in vitro in the NIH3T3 fibroblasts transfected with versican 3'UTR and in vivo in the versican 3'UTR transgenic mice. Transfection with siRNAs targeting the versican 3'UTR abolished the 3'UTR's effects on cell migration and invasion. Taken together, these results demonstrate that versican play important roles in wound repair and that versican mRNAs could compete with endogenous RNAs for regulating miRNA functions.

Key molecules involved in cellular and biochemical endophenotypes of idiopathic scoliosis

Gorman, Kristen Fay:1,3; Lakshmi Suvarnan:1,3, Saadallah Bouhanik:1, Cédric Julien:1,3, Anita Franco:1, DaShen Wang:1, Ginette Larouche:1, Ginette Lacroix:1, Alain Moreau:1,2,3

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Objectives: We previously reported a hypofunctionality of G-inhibitory proteins in idiopathic scoliosis (IS) patients, contingent upon a cellular responsiveness to osteopontin (OPN). Moreover, a differential defect has allowed us to define three endophenotypes with distinctive biochemical activities and clinical profiles. The goal of this study was to identify genomic expression patterns specific to these endophenotypes.

Methods: Microarrays were performed on osteoblasts from 15 IS patients and 5 controls. Raw data were normalized and filtered using a Kruskal-Wallis test followed by a false recovery rate (FDR)-correction, and a threshold of at least a 3-fold differential expression. Genes were considered significant if their FDR-corrected p-value was ≤ 0.05. This filtered dataset was explored using clustering algorithms, and 50 candidate genes were selected for single-gene analysis via quantitative real time PCR (qPCR) in an age-matched expanded cohort. Confirmed results are currently being tested using qPCR in PBMCs (Peripheral Blood Mononuclear Cells). These results will determine whether differential gene expression is systemic or tissue specific.

Results: There are 175 genes that are significantly differentially expressed from the microarrays. Importantly, these reflect the previously described biochemical dichotomy among IS patients regarding OPN sensitivity or resistance. Of the 50 genes chosen as a confirmation set, less than 15 are significantly differentially expressed. Further testing of this confirmed gene set in lymphocytes is ongoing.

Conclusions: Genes identified here are important candidates that will allow us to better understand the different pathways involved in the IS associated signalling defect. This will allow us to better direct therapeutic efforts to different prognoses and co-morbidities.

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Elastin Haploinsufficiency Delays Arterial Calcification in MGP Deficient Mice

Zohreh Khavandgar 1†, Hassem Roman 2†, Jingjing Li 3, Sara Lee2, Hojatollah Vali1,2, Juergen Brinckmann 4, Elaine C Davis 2 and Monzur Murshed 1,3

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Matrix gla protein (MGP) is a potent inhibitor of extracellular matrix (ECM) mineralization. MGP-deficiency in humans leads to Keutel syndrome, which is hallmarked by abnormal soft tissue calcification. MGP-deficient (Mgp-/-) mice show progressive deposition of hydroxyapatite minerals in the arterial walls and die within 2 months of age. The mechanism of MGP anti-mineralization function is not fully understood. We examined the progression of vascular calcification and expression of several chodro/osteogenic markers in the thoracic aorta of Mgp-/- mice at various ages. Although cells with chondrocyte-like morphology have been reported in the calcified aorta, our gene expression data suggest that chondro/osteogenic markers are not upregulated in the arteries prior to the initiation of calcification. Interestingly, the arterial calcification in Mgp-/- mice appears first in the elastic lamellae. Considering the known mineral scaffolding function of elastin (ELN), a major elastic lamellae protein, we hypothesize that elastin content in the lamellae is a critical determinant for arterial calcification in Mgp-/- mice. To investigate this, we generated Mgp-/-;Eln+/- mice with a reduced elastin levels in the blood vessels. Skeletal preparations and micro-CT analyses show that decreased elastin levels significantly reduce arterial calcification in these mice. Our data suggest that MGP deficiency leads to altered vascular ECM remodelling that in turn initiates arterial calcification.

Whole Genome Association and differential gene expression studies provide evidence for the importance of the Wnt Signaling pathway in Dupuytren's disease

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Dupuytren's disease (DD) is a multifactorial fibromatosis that causes progressive and permanent contracture of the palmar fascia with subsequent flexion contracture of affected fingers. A strong genetic predisposition exists but little is known about the epidemiology and the molecular etiology and pathogenesis of the disease. It is the most frequent genetic disorder of connective tissue with a prevalence of about 3% in Germany and up to 40% in parts of Scandinavia.We have collected clinical data and samples from over 830 DD patients in order to investigate the genetics of this disease. We evaluated the importance of different risk factors and found that a genetic predisposition had the strongest influence on the age at the first surgical treatment compared to environmental factors. Patients with a positive family history were on average 5.2 years younger than patients without known family history (P 2.2x10-08). We have performed a genome wide association study (GWAS) with 565 unrelated DD patients and 1,219 controls. Data for 5,204,451 single-nucleotide polymorphisms (SNPs; 186 cases genotyped with Affymetrix Genome-Wide Human SNP Array 6.0 and 379 cases, 1,219 controls genotyped with Axiom CEU 1 Array; data imputed with HapMap CEU reference panel) were analyzed for association with DD. SNP rs2290221 on chromosome 7p14 showed the strongest association signal with a P-value of 2.2x10-10 and odds ratio of 2.13. It is located intronic of the genes for secreted frizzled-related protein 4 (SFRP4) and ependymin related protein 1 (zebrafish) (EPDR1). Moreover, we identified SNPs in four further regions with significant association with DD. A whole transcriptome analysis with samples from primary tissues from 12 DD patients and 12 normal fascia controls revealed changes in growth factors and molecules involved in cellular adhesion and upregulation of the Wnt/β-catenin signaling pathway in the disease tissue. Remarkably, these findings clearly corroborate our and other GWAS results. Using disease tissue derived fibroblasts grown on collagen gels, we have further demonstrated that Wnt3a induced the expression of alpha smooth muscle actin (αSMA). Moreover, RNAi mediated knock down of glycogen synthase kinase 3 beta (GSK3β), nucleoredoxin (NXN) or NADPH oxidase 4 (NOX4) attenuated Wnt3a induced αSMA expression. Taken together, the Wnt signaling pathway may be a key player in the susceptibility to fibromatosis as observed in DD in the context of aging and aging associated diseases.

Session 2: Bone, Cartilage and tooth development

Invited Lecture

Role of menin in bone development

Geoffrey N. Hendy, Ippei Kanazawa, Lucie Canaff, Aarti Angrula, JIngjing Li, and Monzur Murshed

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Objective: The tumor suppressor menin is expressed in bone but little is known about the *in vivo* role of menin in the skeleton.

Methods and Materials: We conditionally inactivated *Men1* in postnatal mature osteoblasts by crossing *osteocalcin*-Cre mice with floxed *Men1* mice to generate mice lacking *Men1* exon 3 to 8 in osteoblasts ($Men1_{ob}^{-/-}$ mice).

Results: Nine-month-old $Men1_{ob}^{-/-}$ mice displayed significant reduction in bone mineral density by dual-energy X-ray absorptiometry and in trabecular bone volume and cortical bone thickness by micro-computed tomography (CT) analysis. Micro-CT images indicated abnormality of trabecular bone formation in $Men1_{ob}^{-/-}$ mice. By histomorphometric analysis bone volume/total volume, osteoblast and osteoclast number, as well as mineral apposition rate (MAR) were significantly reduced in $Men1_{ob}^{-/-}$ mice. The mRNA expression of osteoblast genes, OPG, RANKL, BMP-2, Runx2, Osx, Dlx2, Dlx5, and cyclin-dependent kinase inhibitors, p15, p18, p21 and p27, were all reduced, whereas that of cyclin dependent kinases, CDK2 and CDK4, were increased in isolated osteoblasts from $Men1_{ob}^{-/-}$ mice compared to controls. These data are consistent with the menin-deficient osteoblasts having reduced responsiveness to TGF-β and/or BMP-2 and loss of Smad signaling. In contrast to the knockout mice, 12-month-old transgenic mice overexpressing the human menin cDNA in osteoblasts ($Men1_{ob}^{TG/+}$ mice) driven by the 2.3 kb *Col1a1* promoter, showed a gain of bone mass by micro-CT and histomorphometric analysis. Osteoblast number and MAR were significantly increased in $Men1_{ob}^{TG/+}$ mice.

Conclusion: Taken together, depletion of menin in the osteoblast leads to decreased osteoblast and osteoclast numbers as well as impaired bone remodeling, resulting in a reduction in trabecular and cortical bone whereas overexpression increases bone volume by enhancing bone formation. Therefore, maintenance of menin expression and function in the osteoblast is important to avoid decreased bone mass.

Funding CIHR, FRSQ, RSBO.

Osteoporosis and periodontitis, Pitx1 is the link

Nancy Karam MS1,3, Benoit St Jacque Ph.D 3, Alain Moreau PhD1,2,3,

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Pitx1 over-expression in transgenic Col1a1-Pitx1 mice causes an osteoporosis like phenotype. Because osteoporotic patients often exhibit dental problems, we characterized the Col1a1-Pitx1 mice to elucidate the possible links between osteoporosis and periodontitis. The study of dental phenotypes of osteoporotic mice over-expressing Pitx1 gene was performed by laboratory methods (X-ray, DEXA-scan, biomechanical tests, histology). The mandibles of the Col1a1-Pitx1 mice compared to the wild-type show a decrease of bone mass density by 15.3% (p=0.049) for the upper mandible, and 18.4% (p=0.014 .10-2) for the lower mandible. This decrease was accompanied by a significant decrease of the bone mineral content by 30.5% (p=0.03) for the upper mandible and 26.2% (p=0.07 .10-3) for the lower mandible. In addition, Col1a1-Pitx1 mice exhibit a dramatic reduction in the thickness of the cortical bone by 66.7% (p=0.049 .10-6) for the upper mandible and 62% (p=0.028 .10-35) for the lower mandible. This decrease was accompanied by spontaneous fracture in 30% of cases.Taken together; these data suggest that Col1a1-Pitx1 transgenic mice exhibit a reduced biomechanical resistance. This was shown as a 37.7% (p=0.07) decrease in the stiffness, accompanied by a significant decrease of the 'work to ultimate time point' by 56.5% (p=0.026). In addition, these mice exhibit severe malocclusion. Their teeth are yellowish and show atypical form. We note that 22% of these mice lose at least one molar. Besides that, they show severe signs of periodontitis. These signs were shown by a significant furcal bone loss by 57.9% (p=0.01 .10-8) for the first molar of the upper mandible and by 33.8% (p=0.02 .10-12) for the first molar of the lower mandible. This loss was accompanied by a significant increase in the Alveolar Crest Height loss by 488.8% (p=0.07 .10-4) for the first molar of the upper mandible and by 129% (p=0.02 .10-2) for the first molar of the lower mandible. In order to understand what is going on at the cellular level, histological assays were performed. The Hematoxilin-Eosin coloration shows a bacterial infection, leading to subsequent inflammation and a wavy appearance of the teeth, resulting from an external resorption. This phenotype is accompanied by a decrease in the number of odontoblasts with a disorganized pattern. Our results show that there is a significant similarity in the dental and periodontal phenotypes between mice over expressing Pitx1 and osteoporotic patients. We suggest that Pitx1 expression might be an indicator of predisposition for periodontal disease in osteoporotic patients.

Cholinergic agonists with muscarinic activity favor bone mass accrual by stimulating osteoblast proliferation

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The muscarinic activity of the parasympathetic nervous system (PNS) has been shown to regulate bone mass by suppressing the sympathetic nervous system (SNS). Although, muscarinic receptors are expressed in osteoblasts, their physiological role has never been reported at the bone level. We demonstrated that muscarinic receptors, particularly cholinergic receptor, muscarinic 3 (ChRM3), are expressed in bone and primary osteoblasts. Using DNA transfection techniques, we showed that selective stimulation of ChRM3 in MC3T3.E1 preosteoblasts promotes cell proliferation in vitro. Finally, we showed that treatment of mice with cholinergic agonists that stimulates muscarinic receptors promote bone mass accrual by increasing osteoblast numbers. For this experiment, we used two different cholinergic agonists: neostigmine that is unable to cross the blood-brain barrier and works only peripherally; and donepezil, a centrally acting cholinergic agonist that crosses the blood-brain barrier. Both agonists increased bone mass that was accompanied by a boosting in osteoblast numbers with no changes in osteoclast numbers. The body mass index, body weight and visceral fat pad weight were increased in donepezil-treated mice, but not in neostigmine-treated mice. In agreement with these findings, we observed a decreased epinephrine levels in donepezil-treated mice; however, epinephrine levels remained unchanged in neostamine-treated mice. These findings suggest that neostigmine promotes bone mass accrual without affecting the sympathetic tone. Our results represent the first experimental evidence that peripheral stimulation of the muscarinic receptors in osteoblasts by pharmacological approach is able to increase bone mass accrual and becomes a potential treatment for osteoporosis.

Low Sirtuin 1 levels in human Osteoarthritis Subchondral Osteoblasts lead to abnormal SOST expression which decreases Wnt/β-catenin activity.

Élie Abed, Denis Couchourel, Aline Delalandre, Nicholas Duval, Jean-Pierre Pelletier, Johanne Martel-Pelletier, & Daniel Lajeunesse

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Background: Wnt/β-catenin (cWnt) signaling plays a key role in osteogenesis by promoting the differentiation and mineralization of osteoblasts, activities altered in human osteoarthritis subchondral osteoblast (OA Ob). Sclerostin has been shown to alter cWnt signaling. Sirtuin 1 (Sirt1) acts as a novel bone regulator and represses Sclerostin levels in Ob. However the role of Sirt1 and Sclerostin in OA Ob remains unknown.

Objectives: To explore the role played by Sirt1 and Sclerostin on the abnormal mineralization and cWnt signaling in OA Ob.

Methods: Primary human normal and OA Ob were prepared from tibial plateaus. SOST levels were evaluated by immunohistochemistry, the expression and production of genes by qRT-PCR and WB analysis. Their inhibitions were performed using siRNA. cWnt signaling was measured by the TOPflash TCF/lef luciferase reporter assay. Mineralization was determined by alizarin red staining.

Results: Sclerostin levels were significantly increased in OA Ob compared to normal and were linked with elevated TGF-β1 levels in these cells. Sirt1 expression was significantly reduced in OA Ob and was not modified by TGF-b1. In contrast, specific inhibition of Sirt1 increased TGF-β1 and Sclerostin expressions in OA Ob, while stimulating Sirt1 activity with β-Nicotinamide mononucleotide reduced TGF-β1 expression and increased mineralization in OA Ob. Reduced cWnt signalling, β-catenin levels, and mineralization in OA Ob were all modified corrected via reducing Sclerostin expression.

Conclusion: These data indicate that Sclerostin is responsible for the reduced cWnt and mineralization of human OA Ob, which in turn is linked with abnormal Sirt1 levels in these pathological cells.

Therapeutic Effects of Rapamycin on Pain and Osteolysis in a Murine Model of Bone Cancer

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In advanced breast cancer, bone metastases occur in 70% of patients. Managing the devastating pain associated with the disease is difficult. Rapamycin is an immunomodulatory drug used to prevent rejection in organ transplantation. Rapamycin was shown to reduce pain in inflammatory and neuropathic models and to decrease osteolysis associated with metastatic breast cancer in pre-clinical models.

The aim of this study was to evaluate the effectiveness of Rapamycin in reducing pain associated with experimental osteolytic metastases. A mouse model of bone cancer pain was induced by intra-tibial injections of murine mammary carcinoma cells (4T1) in BALB/c mice. Four groups received inoculation of 4000 4T1 cells (cancer) or saline (sham). The 3 cancer groups were treated intraperitoneally with Vehicle, Rapamycin or Pamidronate, which is currently used to reduce bone loss in bone cancer patients.

Radiographs taken on week 5 after caner inoculation showed that bony mixed lesion areas are significantly different between cancer and sham and both drugs reduced the bony lesions. Measures of evoked pain behavior including sensitivity to mechanical, thermal and cold stimuli were evaluated weekly. By week 3, the cancer/Vehicle-treated group showed significant differences in sensory thresholds compared to sham. Rapamycin was able to decrease cancer-induced mechanical hypersensitivity at week 3, decrease cancer-induced hypersensitivity to heat stimuli at week 3 & 5 and reduce hypersensitivity to cold at week 5. Spontaneous pain behaviors were assessed in an open field. For limb use score, significant differences between sham and cancer groups were evident only at week 5 but both drugs had no effect. Guarding of the affected limb was evident at week 3 in the cancer group and both drugs were able to significantly reduce guarding but the drug effect diminished later.

Our data suggest that Rapamycin may have efficacy in the management of pain as well as osteolysis associated with metastatic breast cancer. The mechanisms underlying these effects remain to be investigated.

Disruption of Biomineralization Pathways in Spinal Tissues from Mice Lacking Equilibrative Nucleoside Transporter 1

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Background: Recent studies have suggested a role for adenosine metabolism in the regulation of biomineralization. Equilibrative nucleoside transporter 1 (ENT1) is a membrane protein involved in the bi-directional transport of adenosine across the plasma membrane. In mice lacking ENT1 (ENT1-/-), we have reported the development of ectopic mineralization of paraspinal tissues in the cervical-thoracic region at 2 months of age, which extended to the lumbar and caudal regions with advancing age. Histological examination revealed large, irregular accumulations of eosinophilic material in spinal fibrocartilaginous tissues including ligaments, entheses and intervertebral discs. Plasma adenosine levels were greater in ENT1-/- mice than in wild-type controls and elevated plasma levels of inorganic pyrophosphate (PPi) in ENT1-/- mice indicated generalized disruption of pyrophosphate homeostasis. This skeletal phenotype closely resembles a human disorder known as diffuse idiopathic skeletal hyperostosis (DISH). DISH is a relatively common non-inflammatory spondyloarthropathy, characterized by ectopic calcification of spinal tissues. Its etiology is unknown and there are no specific treatments.

Objectives: The objectives of the present study were i) to characterize expression of adenosine transporters and receptors in spinal tissues; and ii) to investigate whether loss of ENT1 alters the expression of genes that regulate biomineralization, and/or genes involved in adenosine metabolism and signaling.

Methods and Results: Using quantitative RT-PCR, we compared gene expression in intervertebral disc tissues obtained from 6-month-old male ENT1-/- and wild-type mice. No differences were observed in genes encoding nucleoside transporters, adenosine receptors or enzymes involved in adenosine metabolism. In contrast, intervertebral discs from ENT1-/- mice exhibited reduced expression of genes involved in suppressing biomineralization, including: matrix gla protein (Mgp – an inhibitor of soft tissue mineralization); ectonucleotide pyrophosphatase/phosphodiesterase 1 (Enpp1 – responsible for the production of PPi, which inhibits mineralization); progressive ankylosis protein (Ank – a putative PPi transporter); and osteopontin (Spp1 – an inhibitor of mineralization). Unexpectedly, expression of alkaline phosphatase (Alpl – responsible for the hydrolysis of PPi to Pi) was also reduced in ENT1-/- discs. Of note, no differences were observed in the expression of these genes in tissues, including knee and liver, which do not exhibit ectopic mineralization in ENT1-/- mice.

Conclusions: These findings suggest that alterations in regulatory proteins that normally prevent soft tissue mineralization contribute to the ectopic calcification of spinal tissues in ENT1-/- mice. ENT1-/- mice may be a useful model to investigate the etiology of and therapies for DISH and related disorders of mineralization in humans. *Supported by CIHR*

Session 3: Connective Tissue in Disease - Clinical and Fundamental Aspects

Invited Lecture

Gasping for Air in the Intervertebral Disc: PHD a newly emerging player

Makarand V. Risbud, PhD

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One overriding aspect of intervertebral disc cell biology is that cells of the nucleus pulposus and cells residing in the inner annulus are removed from the blood supply and therefore exist in a unique hypoxic niche. Several studies have shown that HIF-a, a key regulator of hypoxic response, is robustly expressed in disc cells and controls expression of a number of genes important in disc cell survival and function. Recent work from our laboratory has focused on mechanisms governing stabilization and turnover of HIF-1α and HIF-2a in nucleus pulposus cells. It is known that PHD proteins hydroxylate specific prolyl residues in the oxygen-dependent degradation domain of HIF-α subunits. Unlike articular chondrocytes, our studies indicate that PHD2 controls to a limited extent HIF-1α degradation even under hypoxic conditions indicating preservation of PHD2 enzymatic activity at low O_2 tension. This observation highlights the unique physiology of the nucleus pulposus cells and suggests that there is very low cellular utilization of O_2, an adaptive response to the hypoxic niche. Moreover, the limited involvement of PHD2 in HIF-1a degradation implies that it is not the primary regulator of HIF-1α turnover. I would further elaborate on these concepts that HIF-α levels are regulated primarily by oxygen-independent pathways in nucleus pulposus cells.

Differential Activation of SMAD1 and SMAD2 Proteins in Lung Epithelial Cells in a Mouse Model of OSM-induced Lung ECM Accumulation

Steven Wong; Rebecca Rodrigues; Sean Lauber; Jessica Guerette; Carl D. Richards.

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Background: Evidence suggests that TGFβ is a primary regulator of lung extracellular matrix (ECM) remodeling in diseases such as pulmonary fibrosis. However, other cell signaling molecules including gp130 cytokines have been shown to induce ECM remodeling. Overexpression of Oncostatin M, one member of the gp130 cytokine family, induces ECM remodeling and other effects in mice consistent with the phenotype of pulmonary fibrosis. The objective here was to examine the involvement of SMAD pathways in this model, as well as to determine if OSM-induced ECM remodeling requires SMAD3, IL-6, or IL-13.

Methods: Mice from C57Bl/6 and Balb/c background were intubated with adenovirus vector encoding OSM for 7 days to induce transient pulmonary OSM overexpression. Lung homogenates were collected for protein and RNA analysis via immunoblot and qRT-PCR, respectively. Hydroxyproline assay was performed using lung homogenates to quantify total collagen. Through histology, lungs were examined using picrosirius red for collagen, and immunohistochemistry for smooth muscle actin, phosphorylated SMAD2 (pSMAD2), and phosphorylated SMAD1/5/8 (pSMAD1/5/8). In addition, epithelial cell responses to OSM and other cytokines were examined in vitro in A549 epithelial cells via immunoblots of cell protein lysates.

Results: Overexpression of OSM induced ECM remodeling of the lungs, as seen by increased amounts of collagen and smooth muscle actin. ECM remodeling occurred due to OSM overexpression in gene deficient mice lacking either SMAD3, IL-6, or IL-13. Immunohistochemistry analysis showed OSM overexpression to cause an increase in phosphorylated STAT3 and pSMAD2 expression but a suppression of pSMAD1/5/8 expression in the airway epithelial cells. Total lung mRNA levels of typical SMAD1 activators BMP-2 and -4 were downregulated at Day 7 of AdOSM-treated mice. Furthermore, in vitro data using A549 epithelial cells showed that while TGFβ induces SMAD2 phosphorylation, OSM and TGFβ together suppress SMAD1/5 phosphorylation. Conclusions: Overexpression of OSM induces rapid ECM remodeling in mouse lungs. Data suggests that this process does not require SMAD3, IL-6, or IL-13. The OSM-induced response is correlated by an activation of STAT3 and SMAD2 associated pathways and a suppression of SMAD1 associated pathways in lung epithelial cells.

The Effect of Fetuin on Mineralization of Intervertebral Disc Cells.

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Calcification of the cartilaginous endplate of the intervertebral disc (IVD) can act as a significant barrier to nutrient transport and is thought to be one of the causes of disc degeneration. It has been shown that IVD degeneration is associated with ongoing mineral deposition. Fetuin-A acts as a systemic physiological inhibitor of ectopic calcification, and is known to antagonize mineralization of soft tissues. It accomplishes this by forming soluble colloidal complexes, called calciprotein particles, with calcium and phosphate ions thus preventing the formation of insoluble calcium phosphates. Calciprotein nanoparticles were previously described in both physiological mineralization of bone and in pathological calcification. The purpose of the present study was to determine how Fetuin-A influences calcifying bovine IVD cells. Coccygeal IVDs from the tails of adult bovine steers (2–4 years old) were used for cell isolation immediately after transportation to the laboratory from the abattoir. IVDs were separated into annulus fibrosus (AF) and nucleus pulposus (NP) regions and cells were enzymatically isolated from the tissue pieces. NP cells were cultured in osteogenic differentiation medium. After culturing for six days, the medium was supplemented with β-glycerophosphate with or without Fetuin (1mg/ml) for 21 days. The proteoglycan (measured as GAG) content of the culture medium and alkaline phosphates activity in the cell lysates was determined every 3 days for 21 days. Results are normalized to total protein. Gene expression of collagen types I, II and X and aggrecan and the markers of bone formation, such as osteopontin and osteocalcin were determined by RT-PCR. NP and AF cells cultured in mineralization supportive medium readily induced mineral formation. However, when the cells were exposed to Fetuin-A, mineralization was significantly suppressed. Our studies indicate the presence of autocrine factors produced by disc cells in vivo that prevent mineralization and preserve matrix integrity. Previous studies have shown that Fetuin-A can act as a calcification inhibitor and prevent the development and progression of extraosseous calcification processes. Here we show that Fetuin-A is able to stimulate the synthesis of aggrecan while suppressing calcification in the disc. Fetuin-A and other peptides like Link N supplementation could be used in unison for optimal repair of the degenerated disc.

Does Radiofrequency Ablation comprehensively destroy bone and tumour cells?

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Purpose: The use of radiofrequency ablation (RFA) in the treatment of skeletal metastases has been rising, yet its impact on bone is not well understood. This study aims to evaluate the effect of RFA on bone marrow, osteocytes, osteoclasts and tumour cells in a rabbit femoral tumour model using a novel bipolar cooled RF probe.

Methods: Twelve New Zealand White Rabbits received a 200 µl injection of VX2 suspended tumor cells into one femur. The animals were MR imaged on day 14 and femurs (24 in total) were divided into four groups: tumour bearing BCRF treated, healthy BCRF treated, tumour bearing sham and healthy sham. Post treatment, MR imaging was repeated on day 28, followed by euthanasia and dissection. For histological analysis, the samples were fixed in 10% formalin, decalcified in EDTA and sectioned. Staining using H&E (for general evaluation), AE1/AE3 (for evaluation of VX2 tumor necrosis), TRAP (for evaluation of bony osteoclasts) and TUNEL (for determination of osteocyte ablation) was conducted.

Results: Histological examination demonstrated good correlation among the ablation regions as compared to MRI (Gd enhanced FIESTA and SPGR sequences). Large zones of RF ablation (average volume of 12.9±5.5 cm3) extending beyond the femur cortex (corresponding to the probe tip design) were measured post treatment on the MR images and confirmed with H&E staining. The RFA treated tumour involved specimens demonstrated a significant reduction in tumor volume compared to sham femora ($p<0.05$), yet a small number of viable tumour cells remained within the ablation volume. In contrast, the bone marrow was comprehensively ablated post RFA. TRAP staining demonstrated a significant reduction of osteoclast number (both large and small) post RFA in both the tumour involved and healthy groups ($p<0.05$). TUNEL staining revealed areas of patchy cortical osteocyte necrosis within the ablation zone.

Conclusions: Histology verified the large region of effect created via RFA as seen on MR imaging. While osteoclasts were found to be very susceptible to RFA, a small number of tumour cells and a larger number of osteocytes in the treated regions remained viable. As the RF treatment zone did not encompass the full extent of the lesion, it is possible that the sporadic VX2 cell viability may be explained by local tumour cell migration. The more limited destruction of osteocytes by RFA may be desirable in restoring bone health.

Extracellular calcium-sensing receptor inhibition and not activation enhances stimulation of proteoglycan synthesis in normal and osteoarthritic human chondrocytes

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Introduction: Osteoarthritis (OA) affects millions of individuals, and, although, the mechanism(s) of OA onset is unclear, the biological outcome is cartilage degradation. There is currently no treatment that will prevent or repair joint damage. The extracellular calcium-sensing receptor (CaSR) is a G protein-coupled receptor (GPCR). It is the principle regulator of parathyroid hormone (PTH) synthesis and secretion, and functions to maintain calcium homeostasis. 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.

Materials and Methods: Articular cartilage was isolated from 5 donors undergoing total hip replacement. Cells were recovered from the cartilage of each femoral head by sequential digestion with Pronase followed by Collagenase, and expanded in DMEM supplemented with 10% FBS. OA and normal chondrocytes (PromoCell, Heidelberg, Germany) were transferred to 96 well plates in culture medium containing 1mM CaCl2 (control), 5 mM CaCl2, 5 μM cinacalcet (agonist), or 5 μM antagonist, and replenished every third day for a duration of 12 days. The sulfated glycosaminoglycan (GAG, predominantly aggrecan) content of the medium was analyzed using the 1,9-dimethylmethylene blue (DMMB) dye-binding assay. All experiments were performed in duplicate.

Results: The cumulative production of GAG increased throughout the 12 day culture period in control medium. When normal chondrocytes were cultured in medium supplemented with high calcium (5 mM CaCl2) or cincalcet (CaSR agonist), GAG synthesis was diminished. 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 synthesis and that antagonizing the receptor may support a role in cartilage regeneration.

Anti-microRNA-378 Enhances Wound Healing Process

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Background: Delayed or impaired wound healing is a major public health issue worldwide, especially in patients with diabetes mellitus and vascular atherosclerosis. Wound healing is achieved by complex physiological processes, including hemostasis, inflammation, re-epithelialization, vascularization, and tissue remolding. Many factors affect these processes. MicroRNA has emerged as a key regulator of wound healing. Our previous studies revealed that microRNA-378 (miR-378) plays a role in modulating cell proliferation, apoptosis, migration and invasion.

Methods: In this study, we developed an anti-miR-378 sponge construct expressing multiple tandem microRNA binding sites. With highly matched sequence, this homological antisense transcript sufficiently blocked the process of precursor microRNA. CD1 transgenic mice were generated to express the anti-miR-378 unit by microinjection of transgene fragments into fertilized zygotes. Positive transgenic mice along with control group were subject to skin biopsy, causing a pair of full-thickness, excisional wound on the back of neck. Wound sizes were measured everyday thereafter, and tissue samples were collected for immunohistochemistry examination. Meanwhile, mouse fibroblast cell line NIH/3T3 was transfected with anti-miR-378 and subject to migration, differentiation and angiogenesis assays.

Results: Anti-miR-378 sponge could block mature miR-378 functions in vitro and in vivo. Compared to wildtype mice, enhanced wound healing process was seen in anti-miR-378 transgenic mice. In addition, we found that levels of vimentin and integrin beta-3, two modulators that are important in wound healing process, elevated remarkably in the transgenic mice. Wound scratch and transwell migration assays showed a greater mobility in the anti-miR-378-transfected NIH/3T3 cells, which was due to up-regulation of vimentin and integrin beta-3. Both molecules were confirmed as targets of miR-378, and thus their expression could be rescued by anti-miR-378. Overexpression of vimentin could also contribute to fibroblast differentiation, and up-regulation of integrin beta-3 by anti-miR-378 was responsible for angiogenesis.

Conclusion: We demonstrated that knockdown of miR-378 by endogenous integrated antisense fragments could increase the expression of its target proteins, vimentin and integrin beta-3, which enhanced wound healing in vivo and accelerated fibroblast migration and differentiation in vitro. These results add a new layer of knowledge in would repair by microRNA regulation

Session 4: Stem Cells in Tissue Regeneration and Tissue Engineering

Invited Lecture

Tissue engineering and stem cells for salivary glands

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For functional restoration of damaged salivary glands, our group is studying two experimental approaches. The first approach is building an artificial salivary gland using “tissue engineering”, which is a field of research aiming at repairing damaged tissues and creating replacement organs (bioartificial). The second approach is using “(stem or progenitor) cell-based therapy”. This presentation provides an overview of the key findings from these two experimental approaches. Also, we describe recent findings using bone marrow cell extract to repair salivary glands damaged by irradiation or Sjogren’s syndrome.

Fibroblast-Derived Microstructured ECM Produced in vitro by the Self Assembly Approach as a scaffold for Vascular Media Reconstruction

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Cardiovascular diseases are the leading cause of death in industrialized countries. There is a lack of an optimal prosthesis for the surgical replacement of small caliber blood vessels. Tissue-engineered blood vessels produced by the self-assembly approach are demonstrating impressive results in human. However, some modifications of the assembly technique could lead to substitutes having improved physiological properties. Features such as alignment of cells within native tissues are important for organ functionalities such as resistance and contractility. This alignment can be reproduced in vitro by contact guidance. Recently, we adapted the original self-assembly technique of tissue engineering by using a decellularized matrix scaffold (dMS) produced by self-assembly using human fibroblasts (Bourget et al, Biomaterials, 2012). Using this scaffold, we improved the mechanical and contractile properties of the reconstructed vascular media. In the present study, we used a microfabricated pattern of hills and valleys produced by photolithography and hot embossing on a tissue culture substrate to produce aligned dMS. Smooth muscle cells (SMCs) were seeded on those aligned dMS and rolled to form a reconstructed media with physiological alignment of SMCs and ECM. SMCs were grown on top of aligned or regular dMS, in standard culture medium containing ascorbic acid and serum. SMC alignment was monitored by time-lapse imaging using DsRed expressing cells. After a week in culture, cell sheets were rolled around a mandrel to form a tissue-engineered vascular media (TEVM). After maturation, concentric layers of cell sheets adhered together and were cut into 5-mm long rings for analysis. Those rings were tested for contraction capabilities and mechanical resistance. Histological and immunofluorescent staining of SMC and ECM markers were also performed to verify cell distribution, alignment and matrix microstructure. Anisotropy of the construct was also monitored by polarized light microscopy. Time-lapse imaging demonstrated that the aligned matrix can direct migration and alignment of SMCs. Circumferential alignment of the dMS resulted in a tissue engineered media with improved ultimate tensile strength. Contractility of aligned constructs was also higher than media produced without dMS but was similar for aligned and unaligned constructs. Histological staining demonstrated a good cohesion for these constructs and immunofluorescent staining showed alignment of cells and ECM within the tissue. By controlling the direction of collagen assembly using microfabrication, we produced stronger tissues without changing their composition or wall thickness. Alignment of cells is an important factor for tissue function, and recreating such geometry will eventually allow tissue-engineered blood vessels to display propeties matching their physiological counterparts.

Extracellular matrix stiffness reversibly governs the stemness and fibrotic fate of mesenchymal stromal cells in a pro-fibrotic microenvironment

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Delivery of mesenchymal stromal cells (MSCs) to chemical and mechanical microenvironment of fibrotic or cancerous tissue bears the risk that MSCs will turn into fibrogenic myofibroblasts and worsen the disease rather than resolving. Myofibroblasts are highly contractile cells due to the expression of α-smooth muscle actin (α-SMA) in stress fibers. It is unclear, which factors determine whether MSCs promote or resolve fibrosis and whether the myofibroblastic character of MSCs is a determinant in this process. MSC niches are characterized by soft extracellular matrix (ECM) in contrast to fibrotic tissue which are composed of highly dense and collagenous ECM. We hypothesize that the stiffness of fibrotic ECM promotes MSC-to-myofibroblast differentiation by controlling the maturation of ECM adhesions and α-SMA stress fiber formation, thereby altering their clonogenicity and multipotency. To delineate whether myofibroblast differentiation is a common possible trait of MSCs, we tested whether MSCs obtained from human adipose tissue, umbilical cord and bone marrow (hBMSCs) differentiate into myofibroblasts under profibrotic conditions (+TGF-β1). To investigate the effect of ECM stiffness on myofibroblast character, we plated hBMSCs on physiological-soft and fibrosis-stiff elastomeric substrates under physiological and pro-fibrotic conditions. Reversibility of the contractile myofibroblast phenotype was assessed by enriching α-SMA-positive and α-SMA-negative MSC populations by FACS that were then subject to fibrosis-inducing and suppressing culture conditions. To delineate the mechanisms involved in myofibroblast phenotype reversibility, we assessed myofibroblast characteristics by lowering ECM stiffness, inhibiting TGF-β1 signalling with SB431542 and specifically targeting α-SMA and myosin II as components of the contractile cytoskeleton. To test the impact of the myofibroblast phenotype on MSC stemness, we performed multi-colour flow cytometry and tri-lineage differentiation assays. Lastly, as a proof-of-principle model to establish a non-myofibroblastic source of MSCs, we explanted and passaged rat bone marrow MSCs on soft substrates in comparison with standard stiff tissue culture plastic. The fibrotic myofibroblast character was assessed by ECM contraction assays, Western blotting and immunofluorescence. De novo myofibroblast differentiation was observed in all MSCs plated on stiff tissue culture plastic, which was aggravated upon stimulation with pro-fibrotic TGF-β1. Culture on soft elastomeric substrates inhibited the spontaneous myofibroblast differentiation of hBMSCs even in the presence of TGF-β1. hBMSC myofibroblasts preserve a MSC surface marker repertoire but are incapable of tri-lineage differentiation. Plating on soft ECM, inhibition of TGF-β1, and α-SMA, and myosin II targeting resulted in reversion of enriched hBMSC myofibroblasts to α-SMA-negative cells. Our study of myofibroblast suppresion offers strategy for effective cell therapy.

Innervation of engineered connective tissue using human induced pluripotent stem cells

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The development of tissue-engineered (TE) models has been widely used to study various organs and diseases such as kidney, cardiovascular diseases, psoriasis and much more. Innervations of such TE structures to recapitulate peripheral nerves migration in vitro represent promising tools for studying numerous neuropathies and neuroinflammatory diseases. Historically, the primary source of peripheral neurons has been the dorsal root ganglion of rodents. Since induced pluripotent stem (iPS) cells and neuronal differentiation protocols are now well defined and readily available, these TE models will gain much accuracy to recapitulate neurodegenerative diseases using iPS cells derived from patients. This study is a proof of principle showing that human iPS cells can be successfully differentiated into sensory neurons used to promote axonal migration in a tridimensional TE connective tissue. iPS cells were generated, cultured in suspension to acquire a neural crest stem cell (NCSC) identity and differentiated into peripheral neurons. In the first stage of differentiation, the NCSC spheres were marked for p75, HNK-1, MASH 1, Sox 10, Ngn1 and Ngn2 to assess their NCSC phenotype. The final stage of neuronal differentiation was characterized by staining of TrK family receptors, TujI, peripherin, CGRP and TRPV1. CGRP was also quantified by ELISA to confirm the functionality of nociceptive peptidegic neuron. Differentiated neurons were then cultured for 21 days on a TE connective tissue made of a collagen-chitosan scaffold. Axonal migration was shown in the whole thickness of the TE connective tissue using confocal imaging. Differentiated neurons were also seeded underneath a TE skin equivalent and were shown to migrate through the dermis to reach the subepidermal layer. Using iPS cells obtained from patients with neuroinflammatory diseases or peripheral neuropathies could be a powerful strategy to recapitulate these disease phenotypes in vitro and better understand the causes and the evolving process of the pathologies. In addition, such models could be very useful to screen new drugs and develop new strategies to treat these diseases.

Tissue-engineered human skin reconstructed with a modified self-assembly approach to shorten the production time of autologous skin.

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PROBLEMATIC: The self-assembly approach of the LOEX can produce very promising skin substitutes for the treatment of severely burned patient. Preservation of stem cells in skin substitutes is very important for the long term regeneration of the skin. The self-assembly method currently used allow the preservation of stem cells in the basal layer of the epidermis unlike other production methods. However, the production time (45 days) is a factor that could be improved.

HYPOTHESIS: A new self-assembly method involving earlier seeding of epithelial cells during skin production could reduce the time required to produce reconstructed skin. However, stem cells must be kept in the basal layer of the epidermis to ensure the long term renewal of the epidermis.

METHODOLOGY AND RESULTS: The improved method proposed here aims to reduce the current production time by 7 days. This method is compared with the original method which imply culturing autologous fibroblasts and keratinocytes, separately, superimposing three layers of fibroblasts (after 21 days), culturing 7 days before seeding keratinocytes on the top layer, to grow the reconstructed skin submerged (for keratinocyte proliferation) and then at the air-liquid interface (for maturation of the epidermis). The improved method proposed consists in seeding keratinocytes on a fibroblast sheet 7 days prior to the stacking with 2 other fibroblast sheets (total time reduced by 7 days). The results are promising : the histological staining confirm the proper organisation of the epidermis with all expected layers (stratum basale, spinosum, granulosum and corneum). Immunohistological analyzes confirm the persistence of stem cells in the basal layer (immunodetection of keratin 19 (K19)). Moreover, the structure and the differenciation of the different layers of the skin is well preserved (immunodetection of K14 and integrin alpha-3 (basal layer), K10 (suprabasal layers), Ki67 (proliferation)) as well as electron microscopy (ultrastructure and a properly structured basement membrane). The basement membrane is essential for proper adhesion of the epidermis to the dermis. Laminin and collagen IV immunodetection (basement membrame components) showed uniform labeling. The quality of these reconstructed skin will be further assessed by analyzing their structural stability (contraction of skin substitutes in vitro) and their long-term persistence by preclinical studies in vivo.

CONCLUSION : This modified self-assembly approach allows reduction of the production time without affecting the quality of the reconstructed skin thus allowing faster treatment of the patient.

Epigenetic Imprinting Effects of the Mechanical Environment on the Fibrogenesis of Mesenchymal Stem Cells

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Background: When routine repair mechanisms of the body fail to regenerate large area skin burn wounds, mesenchymal stem cell (MSC) therapy is considered. MSCs can give rise to fibroblastic cells that facilitate wound healing. However, engrafted MSCs are prone to become myofibroblasts when exposed to high mechanical tension and pro-fibrotic cytokines in the wound microenvironment. Myofibroblast activity increases wound stiffness and activate healthy into contractile precursor cells, resulting in pathological tissue remodelling and development of hypertrophic scars.

Objective: To test if the mechanical conditions of cell culture prime MSCs towards or protect MSCs against the myofibroblast fate by inducing epigenetic changes through fibrosis-associated microRNAs.

Methods: Rat bone marrow-derived MSCs were isolated and sub-cultured on skin-soft (elastic modulus of 5 kPa) or fibrosis-stiff silicone substrates (100 kPa) for three passages before transferring to their respective other substrates for two additional passages. Myofibroblast character was evaluated before and after substrate switching to verify if MSCs acquire a 'memory' through mechanical priming. The expression of fibrosis-associated microRNAs was assessed using qRT-PCR.

Results: After three passages, expression levels of the myofibroblast marker smooth muscle actin (SMA) in MSCSs are higher on stiff than on soft substrates. High SMA levels are maintained even after switching MSCs from stiff to soft substrates for another two passages. Conversely, switching from soft to stiff maintains low SMA levels. Consistently, MSCs preserve their level of ED-A fibronectin production after substrate switching. MiR-21 is responsive to mechanical stimuli as stiff substrates upregulate and maintain miR-21 expression.

Conclusion: Continuous culture on soft substrates imprints a mechanical memory that protects MSCs from activation by elevated mechanical stress, such as engraftment into stiff dermal scars. By demonstrating that culture on skin-soft substrates protects MSCs against myofibroblast activation, we are providing novel methods to expand 'high-quality' MSCs prior to body engraftment.

Support: Ontario graduate scholarship. CIHR training program in regenerative medicine.

Characterization of progenitor cells isolated from the subchondral bone of rabbit trochlea and condyle

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Purpose: Bone marrow stimulation initiates repair by fracturing or drilling into subchondral bone at the base of a debrided cartilage defect, typically leading to the formation of a fibrocartilagenous repair tissue. Incomplete regeneration, high inter-individual variability and poorer outcome in older animals are some of the shortcomings of this procedure. Cartilage repair outcomes are affected by defect location and age, suggesting that the structural and biological characteristics of underlying subchondral bone strongly influence the repair response. The aim of this study was to carry out a comprehensive analysis of the progenitor cells present in the subchondral bone of rabbit condyle and trochlea, taking into account location and age.

Materials and methods: Progenitor cells were isolated from the distal femurs of 2 female New Zealand White rabbits, aged 4 months (young) and > 10 months old (retired breeder) using collagenase digestion to obtain the first cell population and subsequent outgrowth from the digested bone explants to obtain the second cell population. Cells were isolated separately from trochlear and condylar regions of subchondral bone. Cell yield was determined at the end of P0. Stemness of cultures was verified by means of CFU-F assay, flow cytometry and multilineage differentiation. Cells were differentiated into a cartilaginous phenotype in 3D culture as pellets by addition of TGF-β3. Distribution of GAGs and collagen type I and II in pellets was characterized.

Results: The growth rate as well as the total cell yield at the end of P0 was found to be significantly lower for the older animal. P0 cell yield was also significantly lower for condylar samples in comparison to trochlear samples, observed for both young and old animals. The progenitor cells isolated from the explants as well as from collagenase digestion demonstrated clonogenic potential and expressed cell surface markers characteristic of bone marrow stem cells (CD44+ and CD34-). On stimulation, the progenitor cells differentiated into cartilaginous phenotype. The collagenase-derived cultures exhibited a more complete differentiation into cartilaginous phenotype than explant-derived cultures, evidenced by higher Safranin-O staining. A greater expression of cartilaginous phenotype was similarly observed for cultures originating from the trochlea versus condyles. The latter is consistent with our previously published results of more hyaline repair in the rabbit trochlea than in the condyle (Chen in Press).

Conclusions: The progenitor cells isolated from distal femur had stem cell characteristics. An age-related difference was observed in the proliferation capacity of the progenitor cells isolated from young and old animal. Irrespective of the age, trochlear cell yield was superior compared to condylar. Finally, our preliminary results show that progenitor cells isolated from trochlea exhibit greater chondrogenic potential compared to cells isolated from condyles and therefore are more promising source for cartilage repair.

Session 5: Biomechanics, Mechanobiology, and Biomaterials

Invited Lecture

Boundary Lubricating Properties of Proteoglycan 4 (PRG4) on Articular Cartilage, the Ocular Surface, and Other Biomaterials

Tannin Schmidt

Proteoglycan 4 (PRG4), also known as lubricin, is a glycoprotein originally discovered in synovial fluid and is critical for normal joint health. PRG4 is present at the surface of articular cartilage where it functions in a dose-dependent manner, both alone and synergistically with hyaluronan, as a boundary lubricant to reduce friction during articulation. PRG4 was also recently discovered in cells on the surface of the eye, and demonstrated to function as a boundary lubricant at the ocular surface and may therefore be critical for ocular health as well.

Current research interests include the study of boundary lubrication of articular cartilage, the ocular surface, and other biomaterials. For synovial joint lubrication, relevant areas include the study of normal, injured, and diseased synovial fluid, where composition and interactions of mechanically relevant biomolecules can be altered, as well as biomaterials used in total knee replacement. Indeed, PRG4 composition in synovial fluid can be diminished after a traumatic knee injury and with osteoarthritis (OA), thus potentially contributing to joint degradation in OA. For ocular surface lubrication, relevant areas include the study of lubricating macromolecules present at ocular surfaces and the role they may play in maintaining ocular surface integrity. Altered PRG4 expression may play a role in the development and persistence of dry eye disease, and potential damage to the ocular surface. Recombinant human PRG4 has recently been expressed and is currently being studied for potential therapeutic biomaterial applications as well.

Collectively, this work could ultimately lead to the development of new cell, protein or tissue based biomaterials that may: 1) halt or reverse the progressive loss of cartilage after an injury or with aging and arthritis; 2) prolong the lifetime of total knee replacement biomaterials; 3) provide a therapeutically effective treatment for those who suffer from symptoms associated with dry eye disease.

Evaluation of a novel technique to map the mechanical properties of an entire articular surface in indentation

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Purpose: Mechanical testing of articular cartilage is suggested by the FDA for products intended to repair or replace knee cartilage [1]. It can be done in different experimental configurations including unconfined compression, confined compression, tension, shear and indentation. Indentation has many practical advantages including minimal disruption of the articular surface prior to testing, the ability to perform histological analyses after testing, and testing multiple sites. One limitation of indentation is the need to position each measurement where the surface of the cartilage needs to be perpendicular to the indenter tip. The objective of this study was to investigate the ability of a novel indentation mapping technique to automatically characterize mechanical properties of the entire articular surface with rapidity, precision and reproducibility. Ovine joints were chosen because of their current use in articular cartilage repair studies and murine knee joints were also tested given their use in many disease and developmental studies.

Methods: Our indentation technique calculates a normal vector to the surface at each position by measuring the contact coordinates at each point on the surface and at 4 nearest-neighbour points. Then, the device moves the x, y and z axes simultaneously to perform an indentation along the normal vector. The resulting force along the normal vector is measured with a 6 degree of freedom load cell. The device used is the Mach-1 v500css from Biomomentum Inc. and the spherical indenter has a radius of 0.5 mm. A thickness measurement was also done at the same position as the indentation using the needle technique adapted from a technical note published in 1995 [2].

Results: We demonstrated reproducibility among random sample orientations on bovine femoral condyles with an ICC (2, 1) of 0.98 for the normal vector angle, the maximum load and the equilibrium load of indentation. This study has shown that mechanical properties could be obtained over the entire surface with high resolution (≈ 150 measurements/articular surface), requiring around 1 minute per indentation and 30 seconds per thickness measurement. Mappings for the thickness, load at 20% strain and Young's modulus (for an elastic model assuming Poisson's ratio = 0.5) have been done.

Conclusions: This study demonstrates the ability of this novel normal indentation mapping technique to characterize the mechanical properties of an articular cartilage surface automatically and rapidly with precision and reproducibility. The technique works with any type of articular cartilage as thin as murine or thick as bovine cartilage and with any type of articular surfaces curvatures such as the tibial plateau or femoral condyles. It is a promising tool for research studies that need to characterize the mechanical properties of the articular surface. *Ref [1] Guidance Document from the FDA Ref [2] Jurvelin, J.S., Rasanen T., Kolmonen P. and Lyyra T. J. Biomechanics, Vol. 28, No. 2. pp. 231-235, 1995*

Remote Mechanosensing by Cells on Thin Floating collagen Matrices

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The mechanical properties of the extracellular matrix impact many cellular functions but the contribution of matrix deformations to cellular mechanosensing, especially at sites beyond the immediate cell-matrix interface, is not defined. We examined remote mechanosensing with a novel cell culture model that employs collagen gels supported cirumferentially by nylon mesh grids floating on culture medium. This model obviates mechanical interference from the rigid underlying foundation of tissue culture plastic and enables assessment of remote, in-plane mechanosensing. We found that 3T3 cells rapidly (<3h) formed cellular extensions whose lengths and number per cell depended on the grid opening size. When the opening sizes were 200 μm or 500 μm wide, the mean number of extensions per cell and the sum of cell extension lengths was not significantly different ($p>0.6$). In contrast, in larger grids (1700 μm width), the mean number of extensions per cell, the mean extension length and the sum of cell extension lengths decreased (40-60%; $p<0.0001$). In grids of 200 μm and 500 μm widths, cell-generated deformation fields extended to, and were resisted by, the grid boundaries. However, in grids of 1700 μm width, the deformation field did not extend to the grid boundaries. Generation of cellular extensions required the ß1 integrin, focal adhesion kinase, filamin A and non-muscle myosin II activity. The new model demonstrates that in the absence of a rigid underlying foundation, cells sense remotely the presence of physical boundaries through creation of deformation fields in thin matrices, which affects the formation of cell extensions.

Contrast Agent Diffusion in Compressed Articular Cartilage

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Objective: Early detection of degenerative changes in cartilage is essential for effective treatment. Contrast agent-based magnetic resonance (MR) or x-ray computed tomography (CT) imaging protocols have been developed for this purpose, where solute transport properties are used to assess tissue composition. However, since a long time is required to attain equilibrium, the use of contrast agents for direct measurement of partition coefficients (an equilibrium-based measure of matrix density and composition) is limited. Non-equilibrium transport, such as diffusion, of contrast agents through cartilage therefore presents new possibilities for imaging protocols. We therefore aimed to study the effects of static compression on diffusion of sodium iodide through articular cartilage. Static compression occurs physiologically and results in changes in extracellular matrix (ECM) density, and here provided a means for nondestructive modification of tissue structure.

Methods: Osteochondral cores were drilled from bovine humeral heads and 600-900 μm thick cartilage disks were sliced from the middle zone using a microtome. Explant disks (5 mm in diameter) were punched from dissected cartilage and immersed in a solution of 2M sodium iodide in PBS overnight. Disks were then removed from absorption baths and smaller disks (3 mm in diameter) were punched from them. Explants were then transfered to a custom-made diffusion apparatus in which 7 mL PBS (initially free of sodium iodide) was circulated around the statically compressed explants. Samples of 400 μL were taken from the diffusion apparatus at regular time intervals and sodium iodide concentration was measured using a microplate reader. Experiments included 30 explants compressed in the range from 0 to 50%. Diffusion coefficients were calculated by fitting desorption bath solute concentration versus time to theoretical curves. GAG content and fluid fraction of all explants were evaluated after diffusion experiments.

Results: Static compression was associated with decreased diffusivity of sodium iodide. Increasing compression from 0 to 50% caused changes in diffusivity from 1095 to 262 μm2/s. Diffusion coefficients increased as fluid volume fraction increased and GAG weight fraction decreased.

Conclusion: Consistent with previously reported diffusivities for small solutes, diffusivity of sodium iodide (as a relatively small contrast agent) decreases with increasing static compression. Alterations in fluid and GAG contents of cartilage ECM are consistent with this behaviour. Findings suggest that measurement of contrast agent diffusivities during CT or MR imaging could be used to assess focal changes in matrix properties in articular cartilage.

Biodegradable chitosan microparticles stimulate U937-derived macrophages to activate STAT-1 and release stem cell chemokines and anti-inflammatory wound-healing factors

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Purpose: Marrow stimulation is a cartilage repair therapy whose success depends on the attraction of bone marrow mesenchymal stem cells (BMSCs) to the cartilage defect. Chitosan, a polysaccharide composed of glucosamine and ~20% N-acetyl glucosamine (GlcNA), was shown to improve cartilage repair and attract more arginase-1+ macrophages to repairing bone marrow stimulation sites than surgery-alone. Macrophages support many wound healing functions, such as stem cell attraction through the release of chemokines. We tested the hypothesis that chitosan potentiates the release of stem cell chemotactic factors from macrophages that are primed towards distinct states of differentiation, in a structure-specific manner.

Methods and Materials: PMA-differentiated human U937 macrophages (D-U937) were primed for 24 hours, with and without chitosan microparticles (80M: 80% degree of deacetylation (DDA), 240 kDa; 98M: 98% DDA, 150 kDa), as well as IFN-γ/LPS (M1: classically activated D-U937), IL-4 (M2a: alternatively activated D-U937) or IL-10 (M2c: deactivated D-U937). Cells were analyzed for viability, chitosan uptake, and time dependent STAT-1 and STAT-6 activation, while conditioned media (CM) was analyzed for the release of cytokines and chemokines. Migration of primary human BMSCs (N=4 donors) to macrophage CM was determined by a transwell chemotaxis assay.

Results: Macrophages were successfully polarized towards M1, M2a but not M2c phenotypes. 80M chitosan specifically stimulated the release of chemokines CXCL10/IP-10, CCL2/MCP-1, and anti-inflammatory factors IL-1ra and IL-10. BMSCs migrated to CM from non-polarized and M2a-polarized cells, with and without chitosan stimulation, but failed to migrate to M1 macrophage CM. M1 macrophages stimulated by 80M chitosan, but not 98M chitosan, showed partial restoration of stem cell chemotactic activity. To determine the molecular mechanism behind increased release of CXCL10/IP-10 and IL-1ra, we show that chitosan induces a delayed activation of STAT-1, but not STAT-6. STAT-1 activation and CXCL10/IP-10 release were positively influenced by increasing chitosan GlcNA content.

Conclusions: Our findings show for the first time that chitosan can guide macrophages to release therapeutic wound repair factors under different polarization states. We further show that chitosan can be optimized based on GlcNA content in order to induce chemokine release and selectively improve macrophage capacity to support BMSCs attraction. 348

Session 6: The Extracellular Matrix in Connective Tissues 1

Transglutaminase activity is required for proper fibronectin fibrillogenesis and stabilization of circulating plasma fibronectin into bone matrix

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Fibronectin (FN) is a ubiquitous glycoprotein capable of regulating cell behavior and assembling into extracellular matrix (ECM) where it facilitates deposition of other ECM components, including type I collagen. Plasma FN (pFN), synthesized by hepatocytes, constitutes 90% of the FN in bone, while the rest is cellular FN (cFN) secreted by osteoblasts. Hepatocyte-specific deletion of FN resulted in defective mineral-to-matrix ratio and decreased biomechanical properties of bone, while osteoblast-specific deletion of cFN affected mainly osteoblast cell numbers. This suggests pFN and cFN have different roles in bone. FN is a substrate of transglutaminase (TG) enzymes which create covalent isopeptide bonds within and between proteins. But this modification's role in FN's function is not known. We hypothesized that TG activity stabilized circulating pFN into bone matrix. To study this in vitro, we used MC3T3-E1 osteoblast cultures as a model. Monodansylcadaverine (MDC) - a competitive inhibitor of crosslink formation - was added to cell culture, it covalently incorporated into FN fibrils and significantly affected FN solubility, demonstrating that FN crosslinking by TG was required to generate DOC-insoluble FN matrix. The irreversible TG activity inhibitor NC9 decreased FN matrix assembly and fragmented the FN network. To investigate if this was caused by poor integration of pFN into matrix, an exogenous biotinylated pFN (biot-pFN) was added to culture media for tracking purpose. NC9 dramatically decreased biot-pFN assembly, which was confirmed by increased biot-pFN left in culture media. Addition of biot-pFN to the cell cultures together with MDC, followed by immunoprecipitations, confirmed that biot-pFN was a substrate of TG. Co-localization of biot-pFN together with cFN (EDA-FN) revealed that pFN constituted the majority of FN matrix, and these two FN forms underwent separate fibrillogenesis processes having occasional overlap within the matrix. The tracking of NC9 (which binds irreversibly to active TGs and contains a dansyl group) showed that the crosslinking enzyme was active in the ECM and integrated into DOC-insoluble matrix. NC9 treatment of the osteoblast cultures also inhibited type I collagen deposition and osteoblast mineralization. In vivo analysis of mice lacking two TG enzymes (TG2 and Factor XIIIA, Tgm2-/-; F13a1-/-) showed increased FN solubility in bone and increased FN levels in serum. These mice were also osteopenic with decreased bone mineral density and biomechanical properties and increased bone resorption. This FN assembly defect might contribute to changes in bone matrix quality and development of osteopenia in Tgm2-/-; F13a1-/- mice.

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A human tissue-engineered fibroblast-derived scaffold for vascular applications

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Rationale : There is currently a lack of clinically available small-diameter vascular graft (<6 mm). Indeed, the saphenous vein and internal mammary artery, routinely used for coronary artery bypass surgery, are more and more unavailable or inadequate due to prior use or atherosclerosis. Synthetic vessels, very appropriate for large-diameter applications, are unsuitable for small-diameter grafts due to the thrombosis that always occurs in small biosynthetic vessels. We have therefore created a tissue-engineered blood vessel that supports the growth of an endothelium. However, the current methodology requires about 2 months to tissue-engineer a vessel with a patient's own autologous cells.

Objective: To develop a hybrid tissue-engineered vessel, combining a readily available decellularized scaffold with subsequently seeded autologous vascular cells for an optimal functionality.

Methods: Briefly, fibroblasts were cultured with ascorbic acid for 3 weeks to enhance significantly the secretion of collagen leading to the formation of cellular sheets. The sheets were rolled around a 4.7-mm mandrel and allowed to fuse during a maturation phase. The vessels were decellularized in deionized water with no other agent and the decellularized fibroblast vessels were then preserved at 4°C. Vessels were cannulated and seeded with a cellular suspension of human smooth muscle cells. After 24hrs, the vessels were placed in a circulatory bioreactor.

Results: We have first studied the maturation phase and were able to determine, using histological and mechanical analyses, the optimal time needed for the production of the decellularized scaffolds. An immunohistological analysis of the decellularized tissue revealed the absence of actin, suggesting an efficient elimination of the fibroblastic debris. Recellularization by smooth muscle cells showed that the cells grew almost entirely on the luminal surface with minimal invasion of the matrix. The smooth muscle cells presented a contractile phenotype in response to vascular agonists as confirmed by myograph studies. We have also confirmed the feasibility of endothelializing recellularized vessels to form an inner endothelium.

Conclusion: We have successfully created a human hybrid tissue-engineered vessel made from decellularized fibroblast-derived extracellular matrix that is recellularized with vascular smooth muscle cells. After optimisation of the culture aspect preclinical studies may be undertaken.

Inhibition of transglutaminase enzyme activity increases adipocyte differentiation by modulating the actin cytoskeleton and fibronectin matrix assembly

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Adipose tissue is a specialized form of connective tissue whose function is to store and release lipids. Pre-adipocytes are cells of mesenchymal origin which upon differentiation acquire a rounded morphology through changes in cell-matrix interactions. Transglutaminases (TGs) are protein-crosslinking enzymes that have role in cell differentiation, matrix synthesis, cell-matrix interactions and matrix stabilization of many mesenchyme-derived cells; however, whether TGs contribute to adipogenesis is not known. The aim of this study was to investigate TGs in adipose tissue and adipocytes. We analyzed mouse white adipose tissue (WAT), 3T3-L1 cell line and mouse embryonic fibroblasts (MEFs) from C57BL/6 mice. Analysis of isopeptide bonds in WAT showed a pericellular localization in tissue stroma. A differentiation treatment (dexamethasone, methylisobutylxanthine [IBMX] and insulin) of 3T3-L1 cells increased TG activity by 8-fold in 24 hours. TG activity reached a maximum by day 2, and then gradually decreased during lipid accumulation phase. TG activity was promoted by IBMX via the PKA pathway, and by insulin via the P13K pathway. Inhibition of TG activity, with traceable TG inhibitor NC9, increased lipid accumulation in a dose- and time-dependent manner, suggesting that TG activity is an early (within 4 days) negative regulator of adipogenesis. The increase in lipid accumulation upon inhibiting TG activity was confirmed in MEFs. The increase in lipid accumulation appeared to be attributable to faster differentiation since NC9 also significantly increased expression of PPARγ at days 1 and 2. Immunofluorescence microscopy and Western blot analysis showed that NC9 integrated onto the cell surface and reduced focal adhesion formation and actin stress fibers. NC9 inhibitor also significantly inhibited ROCK kinase activity. ROCK kinase inhibitor promoted lipid accumulation and had an additive effect together with NC9. NC9 reduced cell adhesion and actin stress fiber formation, and decreased crosslinking of fibronectin, the latter being the main TG substrate in many tissues. Two TG enzymes were expressed in adipocytes, TG2, and the cellular form of Factor XIIIA. FXIIIA mRNA and protein levels followed the pattern of TG activity during differentiation, with highest expression and protein levels at the early phase followed by a gradual decrease in mRNA levels and degradation of the protein product. In conclusion, TG activity, likely arising from Factor XIIIA, is found in adipocytes at an early phase of differentiation where it modulates adhesion and stability of actin cytoskeleton via fibronectin crosslinking. This role is likely important in adipocyte proliferation.

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Chondroadherin Fragmentation as a Biochemical Marker for Early Stage Disc Degeneration

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Disc degeneration has been strongly associated with back pain. At present, little is known about the molecular mechanisms involved in the degeneration of intervertebral discs and how these may differ from normal turnover of the tissue. As a result of this, a biomarker for disc degeneration has not yet been identified and we propose chondroadherin (CHAD) fragmentation as a potential marker. The aims of this study were to determine whether CHAD fragmentation is unique to disc degeneration, to characterize the cleavage site within CHAD at which fragmentation occurs, and to identify the protease capable of CHAD cleavage at the characterized site.

CHAD fragmentation was studied using SDS-PAGE and western blotting in combination with specific antibodies. Characterization of the cleavage site was achieved by fractionating a degenerate surgical disc sample using SDS-PAGE. Gel portions containing the CHAD fragment were excised and identified by mass spectrometry. An anti-neoepitope antibody was raised to recognize the cleavage site sequence. Protease digests of macroscopically normal disc tissue were performed with proteases known to be upregulated during disc degeneration. Digest tissue was extracted and fractionated using SDS-PAGE. Immunobloting was performed with an anti-CHAD antiserum and the anti-neoeptiope antibody.

Evidence for proteolytic degradation of CHAD was observed in adult discs showing degeneration but not in tissue from a macroscopically normal disc. Furthermore, the higher the degree of degeneration seen in the disc, the higher the amount of CHAD fragmentation. Upon analysis with the anti-neoepitope antibody, it was apparent that CHAD fragmentation occurred at the same cleavage site in degenerate discs from apparently healthy donors, surgical samples from adults with disc degeneration, and adolescents with premature degeneration due to scoliosis. Normal tissue samples showed no anti-neoepitope antibody binding, confirming that CHAD fragmentation at this site was not present in the healthy disc. A single protease was identified that was capable of CHAD cleavage at an identical site to that seen in all tissue donors with disc degeneration as confirmed by the anti-neoepitope antibody.

CHAD fragmentation can be used as a biomarker to distinguish normal aging from disc degeneration. This evidence can be used to develop a potential immunoassay to screen the serum of at-risk patients and detect early disc degeneration.

Organized is More Efficient: TGF-β1 Activation from the Extracellular Matrix

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Background: Current pharmaceutical and translational research targets proteases, integrins or TGF-β1 to attenuate fibroproliferative diseases such as fibrosis/cancer. However, none of the above strategies have been successfully implicated in resolving persistence of fibrosis, thereby requiring a mechanistic explanation of fibrotic persistence. Deregulated and stiffened matrix along with persistent TGF-β1 secretion are the hallmarks of all fibrosis, and hence we hypothesize deregulated assembly of ECM proteins results in enhanced TGF-β1 activation; thereby leading to persistence of fibrosis. To investigate the phenomenon, we focus on a human patient derived-dermal fibroblasts (hDF) cell model and latent transforming growth factor β1 binding protein (LTBP1) as ECM molecule that tether the highly profibrotic TGF-β1 to the matrix. Objective: To test whether deregulated LTBP-1 matrix assembly into pre-stressed ECM fibrils improves mechanical activation of tethered TGF-β1 by myofibroblasts.

Methods: First, we tested whether fibroblasts increase LTBP-1 matrix assembly upon TGF-β1 stimulation. Second, we fabricated compliant and stiff cell culture substrates to investigate the effect of matrix stiffness on matrix remodeling. Third, we expressed and purified LTBP-1 in HEK cells and hDFs to establish a fibroblast-free, fibrotic and totally cell free systems to visualize deregulated matrix assembly and TGF-β1 release. Fourth, we strategically knocked down focal adhesion kinase to disrupt matrix assembly in fibrotic system. Fifth, we produced fibroblast derived LTBP-1 matrix on silicone substrate, specifically detached the cells, and stretched the underlying silicone to create a proof-of-concept pre-stressed ECM fibril model. In all the assay, live imaging was done with LTBP-1-eGFP construct, and release of TGF-β1 was assessed by TMLEC reporter luciferase assays.

Results: 1. Our results demonstrate significantly increased amount and matrix assembly level of LTBP-1 during fibroblast-to-myofibroblast differentiation. 2. Differentiated myofibroblasts were able to assemble LTBP1 in deregulated fashion in fibroblast-free, fibrotic and cell free systems. 4. When ECM assembly was disrupted, e.g. in a knock-out model of focal adhesion kinase, myofibroblasts released less TGF-β1 than from fibrillar structured ECM as compared to control. 5. Finally, the proof-of-model concept model demonstrated successful mechanical release of TGF-β1 upon pre-stressing the LTBP-1 matrix. Conclusion: Myofibroblasts remodel pre-existing or supplemented LTBP-1 into fibrillar structures. The organization level of the TGF-β1 complex determines the amount of active TGF-β1 released from the ECM.

Significance: Translation of our findings to in vivo treatment of fibrosis possibly involves pharmaceutical targeting of the integrins that are responsible for latent TGF-β1 complex pre-stressing. Similarly, our approach highlights the important microenvironmental regulation of diseases.

Kindlin-2 is a mechanosensitive regulator of the fibroblast to myofibroblast differentiation in cardiac repair

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Cardiac fibroblasts are able to respond to the mechanical stress and to differentiate into myofibroblasts. Myofibroblasts are highly contractile cells that are responsible for the tissue repair after the myocardial infarction. In a pathological situation excessive differentiation of the cardiac fibroblasts results in cardiac fibrosis. Mechanical signals in fibrosis result from the local ECM stiffening. Integrin receptors are involved at the initial sensing step at the site of ECM binding. Integrin adaptors are the main candidates for an intracellular transmission of extracellular cues. Kindlin-2 is the adaptor for beta1 and beta3-integrins. Our findings suggest that cardiac fibroblasts differentiation is controlled by kindlin-2 in mechanosensitive way. We demonstrated that kindlin-2 is upregulated in cardiac fibroblasts upon myofibroblast activation in vivo and in vitro. Kindlin-2 accumulates in focal adhesions of primary human cardiac myofibroblasts. We subjected primary human cardiac fibroblasts to different mechanical conditions to model the long-term and short-term mechanical challenges of fibroblasts upon cardiac repair and fibrosis. Kindlin-2 levels were increased in primary human cardiac fibroblasts cultured on fibrosis-stiff silicone substrates in compare with fibroblasts cultured on soft substrates; moreover the levels of kindlin-2 and differentiation marker alpha-SMA were co-regulated. The short term mechanical stimulation of the cardiac fibroblasts was performed by fibronectin-coated ferromagnetic microbeads or by stretching the cells on deformable silicone membranes. It results in a portion of kindlin-2 translocating from the focal adhesions to the nucleus. Downregulation kindlin-2 in primary cardiac fibroblasts regulates the myofibroblast differentiation by controlling the level of alphaSMA promoter activity. Taken together our observations show that mechanical stress controls the expression and localization of kindlin-2 in cardiac fibroblasts. Hence kindlin-2 is novel mechanosensor within the myocardium that contributes to the myofibroblast differentiation.

Session 7: The Extracellular Matrix in Connective Tissues 2

Keynote Lecture

The Contribution of Bone to Whole Organism Physiology

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The mouse genetic revolution has shown repeatedly that most organs have more functions than expected. This has led to the realization that, in addition to a molecular and cellular approach, there is a need for a whole-organism study of physiology. The skeleton is an example of how a whole-organism approach to physiology can broaden the functions of a given organ, reveal connections of this organ with others such as the brain, pancreas and gut, and shed new light on the pathogenesis of degenerative diseases affecting multiple organs.

Mechanical Adaptations of Tissues in the Knee Joint Over Time Following ACL Injury

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Background: Rupture of the anterior cruciate ligament (ACL) can lead to the development of osteoarthritis (OA). This may be due to altered mechanics, altered biology, or a combination of both factors. The main objective of this study was to investigate the in vivo mechanics of the ovine stifle joint over time following ACL injury. The ovine stifle joint is a promising biomechanical model of human knee joint.

Methodology: The six-degree-of-freedom (6-DOF) in vivo kinematics of the stifle joint of sheep (N=5) was first measured during "normal gait" using a kinematic linkage. Each sheep then underwent an arthroscopic stifle surgery in which their ACL was fully transected. The in vivo gait kinematics was measured again over time, 4 weeks and 20 weeks following ACL transection. The animals were then euthanized and their limbs disarticulated from the hip and mounted on a unique robotic testing platform. The robot was programmed to reproduce all the previously recorded in vivo kinematics (normal, 4 weeks, and 20 weeks gait paths) and gait loads were simultaneously recorded using a universal force/moment sensor. The load borne by each tissue (collateral ligaments, posterior cruciate ligament, and menisci) was determined using the principle of superposition.

Results: Our results indicate significant inter-subject variability in tissue loads and tissue adaptations following ACL injury. The medial and lateral collateral ligament (MCL and LCL) forces increased considerably short-term following ACL injury but returned to within normal ranges, or decreased dramatically compared to normal conditions for some subjects, in the longer term. The posterior cruciate ligament (PCL) behavior was also quite variable between different subjects. For some subjects the PCL forces decreased following ACL injury and continued to decrease over time; whereas, for other subjects PCL forces increased remarkably following ACL injury and did not return to normal ranges in longer term. All subjects, however, demonstrated a dramatic increase in meniscal loads, both short and long term, compared to normal conditions. For some subjects, the meniscal loads doubled or even tripled following injury. This highlights the significant role of menisci following ACL injury and may help explain the meniscal failures often reported following chronic ACL deficiency.

Conclusion: This is the first in vivo study quantifying the mechanical load redistribution in various key tissues in the joint following ACL injury over time. Our next step will be to study the corresponding biological changes to attempt to determine cause and effect with respect to post-traumatic OA.

Versican-rich Proteoglycan Thickening in Diet-Induced Early Aortic Valve Disease in Mice

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Aortic valve disease (AVD) is marked by dysregulated connective tissue synthesis and calcification, however its early pathogenesis is poorly studied. Investigating early AVD may identify novel biomarkers and/or treatment techniques for use prior to the development of an untreatable calcific burden. We examined the effects of a mildly atherogenic diet on early AVD development in mice, and the involvement of proteoglycans (PG), Sox9, and TGFβ1 therein.

Male wild-type (WT) C57Bl/6J mice were fed control or BioServ F3282 (high-fat/high-carbohydrate, HF/HC) diets for four months. Aortic valve function was examined by high-resolution echocardiography. Aortic valve sections were stained with Movat's Pentachrome, and immunostained for versican, decorin, biglycan, perlecan, Sox9, and α-smooth muscle actin (αSMA). Valve interstitial cells (VICs) from healthy porcine aortic valves were treated with 5 ng/ml TGF-β1 for 5 days. PG synthesis was measured by Alcian Blue guanidine-HCL, while mRNA levels of versican, decorin, and biglycan were determined by qPCR. HF/HC mice became obese with mild hypercholesterolemia (4.7 ± 1.0 vs. 3.1 ± 0.4 mmol/L total cholesterol, $p < 0.05$) and decreased aortic valve opening area ($p < 0.01$). Distal valve thickening was found in HF/HC leaflets (84.4 ± 10.4 vs. 37.3 ± 2.7 μm, $p < 0.05$), due to PG deposition (11435 ± 7681 vs. 5448 ± 2948 μm2, $p < 0.01$) not increased collagen synthesis (1729 ± 815 vs. 1771 ± 663 μm2, $p = 0.87$). Only versican was increased in HF/HC leaflets (2.38–fold, $p < 0.05$), while there was no change in decorin or biglycan ($p = 0.69$ & 0.59 respectively); perlecan was not expressed in valvular tissue. HF/HC leaflets were αSMA-negative and displayed a 3.42-fold increase in Sox9 expression ($p < 0.01$). Differential PG expression was mirrored in culture, where TGF-β1 induced a 2.14-fold increase of total PG synthesis ($p < 0.01$) but only versican mRNA was significantly increased by TGF-β1 treatment (1.53-fold, $p < 0.05$), while decorin mRNA was reduced by 48.7% ($p < 0.0$ 1) and biglycan mRNA was unaffected ($p = 0.35$).

These studies have shown that a high-fat diet lacking added cholesterol induces changes in the cells, ECM, and functional properties of a WT mouse aortic valve. Diet-induced de novo proteoglycan synthesis is versican-rich, and corresponds to increased Sox9 expression. These early changes occur prior to any substantial myofibroblast activation or fibrosis, and provide new insight into the involvement of proteoglycans and chondrogenic processes in the pathogenesis of AVD. 398

The Function of Mast Cells in Bone Regeneration

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Bone fractures are currently treated with surgical fixation or prolonged immobilization. To avoid complications associated with delayed healing or non-union there is a pressing need to develop improved adjunct therapies that accelerate bone regeneration at a fracture site. Mast cells are gaining recognition for their critical role in the maintenance of tissue integrity, particularly in bone where they influence angiogenesis and the recruitment and function of osteoblasts and osteoclasts. Mast cell-induced bone regeneration could therefore represent a novel idea for skeletal regeneration and reconstruction. Mast cell proliferation, function and phenotype are mediated primarily by the c-kit receptor and defects in the receptor inevitably lead to a state of severe mast cell deficiency. This research is built on the hypothesis that c-kit deficiency in mice leads to impaired fracture healing.

All animal procedures were performed in accordance with a protocol approved by the McGill Animal Care Facility. Cortical window defects measuring 1mm x 2mm were generated at the mid-femoral diaphysis of 30 c-kit deficient mice age 4-6 months and 30 age-matched wild type C57Bl6 mice. Cohorts of 10 mice were euthanized at two, four or six weeks after surgery to quantify bone regeneration in the defect using micro computed tomography (micro CT). After micro CT the bones were embedded in polymethylmethacrylate for histological analysis. Five micron sections were stained with Von Kossa to identify mineralised tissue, with alkaline phosphatase (ALP) to identify osteoblast activity, with tartrate resistant acid phosphatase (TRAP) to identify osteoclast activity, or with modified toluidine blue to visualize mast cells.

Micro CT analysis of femora harvested two, four and six weeks after surgery revealed decreased cortical bone regeneration, in association with a reduction in cortical bone healing in the c-kit deficient mice compared with age matched wild type mice. At two weeks post-operative the callus in c-kit deficient mice was larger but the concentration of bone was lower than seen in the wild type mice. This was accompanied by decrease ALP staining and increased TRAP staining in the mutant mice compared with wild type mice at the same time point.

Healing of a 1mm x 2mm window defect in the mid-femoral diaphysis was reduced in c-kit deficient mice when compared to age and strain matched wild type mice. The results suggest mast cells migrate to sites of bone healing and could therefore be genetically modified to deliver therapeutic agents to expedite fracture healing and bone repair.

Session 8: Connective tissue remodeling 1

Invited Lecture

ABOUT THE STRESS OF WORKING WITH MYOFIBROBLASTS

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Organs lose their structural integrity upon injury. To rapidly restore the mechanical stability of tissues, different cell types are activated to acquire a reparative phenotype - the myofibroblast. Hallmarks of the myofibroblast are secretion of extracellular matrix (ECM), development of adhesion structures with the ECM, and formation of actomyosin contractile stress fibers. These cytoskeletal features enable the myofibroblast to remodel and contract the ECM but also to adapt its activity to changes in the mechanical microenvironment. Rapid repair comes at the cost of tissue contracture due to the inability of the myofibroblast to regenerate tissue. When contracture and ECM remodeling become progressive and manifest as organ fibrosis, stiff scar tissue obstructs and ultimately destroys organ function.

In addition to being a consequence of myofibroblast activities, the mechanical properties of scarred fibrotic organs promote myofibroblast contraction and activation. I will provide an overview how mechanical stress controls the myofibroblast phenotype and activity. Acute responses to stress comprise structural rearrangement of cell adhesions and of the contractile cytoskeleton, leading to adaptation of cell contractile activity. Long-term consequences of mechanical stimulation are mediated by mechanical activation of the pro-fibrotic growth factor TGF-β1 from the ECM by integrin-mediated cell contraction. We propose that interfering with the specific mechano-perception and –transmission machinery of myofibroblasts provides multiple targets for possible anti-fibrosis therapies; the most promising approaches will be discussed.

Disruption of Knee joint morphology and development of Osteoarthritis in a Cartilage Specific MIG-6 deletion mouse.

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Introduction: Osteoarthritis (OA) is a degenerative joint disease with no effective treatment. Previous studies by our lab have shown that the signalling molecule transforming growth factor alpha (TGFα) is upregulated in both animal models of OA and in a subset of human cases (Appleton et al, 2007;2010). TGFα signals via epidermal growth factor receptor (EGFR) in chondrocytes. Mitogen-inducible gene 6 (MIG-6) has been shown to attenuate signalling by EGFR. Ubiquitous deletion of MIG-6 in mice causes the development of joint deformities and OA (Zhang et al, 2005; Jin et al, 2007), however it is not known which cell type is responsible for these defects. Purpose: To assess the consequences of increased EGFR signalling specifically in cartilage by removing negative regulation via MIG-6 using a transgenic in vivo mouse knock out model.

Methods: MIG-6 was selectively removed in cartilage using transgenic mice (MIG-6fl/fl) and the Col2a1 driven expression of Cre (Col2-Cre+/-). Mice were aged up to one year to assess spontaneous development of OA and joint deformation. Grip force and gait were evaluated for pain response. MicroCT, whole skeletal staining (alizarin red/alcian blue), and histological staining were used to assess altered joint morphology. OA severity was scored on safranin O/fast green stained frontal and saggital sections of the tibiofemoral joint. Molecular changes in protein expression and localization in joint tissues were also examined by immunohistochemistry. Results: In both male and female knockout (KO) knee joints, extensive joint deformities in the tibiofemoral joint were observed as early as 12 weeks of age, including ectopic development of cartilaginous and partially calcified tissues. Small calcified nodules were also observed on the thoracic spine of three 36-40 week old female KO animals. Heterozygous (HET) and wild type (WT) animals did not show evidence of joint deformity by visual inspection, microCT scans or histology. Gait analysis and grip strength showed no statistically significant difference between genotypes for walking patterns and grip strength respectively. KO, HET and WT animals were not significantly different in size or weight.

Conclusions: Negative regulation of EGFR signalling by MIG-6 in Col2a1 expressing tissues is essential for management of normal joint morphology and may be involved in the regulation of tissue calcification.

Link-N Peptide: Can Degenerated Human Intervertebral Discs be Repaired?

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Introduction: Back pain is a fairly common problem affecting a large portion of the population across all ages. Link-N peptide represents a 16 amino acid sequence from the N-terminus of the Link protein that stabilizes the proteoglycan aggregates present in cartilage and disc. We evaluated its regenerative potential in human intervertebral discs.

Methods: Cells were isolated from lumbar discs and exposed to Link-N for 48 hours. The discs were prepared for organ culture and injected in NP with 35SO4 with 1mg of Link-N/disc. Link-N was conjugated with 5-TAMRA dye and its distribution in the medium and within the disc after injection was studied to see whether Link-N is retained in the disc.

Results; Proteoglycan synthesis increased in a dose dependent manner with the maximal response at 1000ng/ml of Link-N in human disc cells. Discs injected with Link-N showed increased proteoglycan synthesis compared to adjacent level control discs. Injected discs continued to show an increase in proteoglycan synthesis one week post injection, suggesting a sustained effect of Link-N in increasing proteoglycan synthesis. The fluorescent peptide was found to localize in NP and NP/iAF junction but its lateral diffusion in the disc was restricted by collagen fibers. Loss of Link-N appears to occur by diffusion through the endplates.

Conclusions: In this work we show that Link-N can promote proteoglycan synthesis in human disc cells cultured in 3D constructs and in adult human disc cells in their native environment. Link-N has definitive regenerative effect and significant cost advantage over growth factors such as BMP7, TGFβ and GDF5.

Cytokine and Chemokine Secretion by Painful Intervertebral Discs

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Introduction: Discogenic low back pain, the pain associated with intervertebral disc (IVD) degeneration, represents an enormous socio-economic cost in developed countries. Whereas pain-free IVDs are largely aneural, painful, degenerating IVDs are often innervated, suggesting a relationship between discogenic low back pain and increased IVD innervation. Cytokines, chemokines and neurotrophins such as IL-1β, IL-6, IL-8, MCP-1, TNF-α and NGF are soluble factors related to nerve growth and nociception but their precise role in IVD innervation leading to discogenic pain is unknown. Increased expression of these factors in degenerating innervated discs could result in activation of pain-sensing fibers, resulting in discogenic pain.

Objective. Analyze levels of pro-nociceptive and pro-inflammatory cytokines, chemokines and neurotrophins secreted by painful vs. control IVDs. \\Methods: Degenerating IVDs from patients suffering from low back pain were surgically removed and healthy IVDs were harvested from human organ donors through Transplant Quebec. These discs were then cultured for 48 hours and the conditioned media was collected. Levels of cytokine, chemokine and neurotrophin levels in the conditioned media were assessed using RayBiotech cytokine arrays according to manufacturer's instructions and NGF and TNF-α levels were quantified with commercially available ELISA kits.

Results: Cytokine arrays and ELISAs showed increased levels of IL-6, RANTES, MCP-2, MCP-3, Gro-α, IL-5, TNF-α and NGF in conditioned media collected from cultured degenerating painful IVDs compared to cultured non-degenerating IVDs.

Conclusion: Secreted levels of pro-inflammatory and pro-nociceptive cytokines and chemokines and neurotrophic factors related to neurite growth are increased in painful degenerating IVDs, possibly contributing to the molecular mechanisms leading to neuronal ingrowth and nociceptor activation that contribute to discogenic low back pain.

Future Directions: Neurons will be cultured in conditioned media collected from cultured painful IVDs or from healthy IVDs. Neurite growth will be measured to analyze the effect of the different media. Antagonists to inflammatory and neurotrophic factors found at increased levels will be added to cultures to elucidate the effect of individual factors.

Effects of running exercise on disc degeneration and low back pain in mice.

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Chronic low back pain (LBP) affects over 10% of Canadians and seriously decreases quality of life. The aging intervertebral disc (IVD) is prone to degeneration, frequently contributing to low back pain. Physical activity influences IVD physiology; for example, animal studies have shown that cyclic loading increases proteoglycan synthesis in the disc, suggesting a role for running exercise in disc repair. Our laboratory has characterized a rodent model of LBP, the aging SPARC-null mouse, which displays age-dependent IVD degeneration accompanied by axial and radiating LBP that is pharmacologically reversible. Behavioural signs of axial LBP correlate with severity of disc degeneration, whereas signs of radiating LBP correlate with disc height. We aimed to determine whether habitual running exercise would delay disc degeneration and decrease pain behavior in the aging SPARC-null mouse.

After two months with free access to running wheels, SPARC-null mice show significantly reduced cold sensitivity in the hindpaw compared to baseline ($p<.05$) and control group ($p<.05$), indicating reduced radiating LBP. Axial LBP was unaffected, suggesting the effect was specific to radiating pain. In older animals, cold sensitivity was significantly reduced compared to control group after four months ($p<.05$), suggesting exercise is still beneficial in animals with more advanced disc degeneration. If running wheels are withdrawn from the home cage, SPARC-null mice continue to show reduced cold sensitivity for two months ($p<.01$). Only after three months without wheel access do they return to baseline behaviour, strongly suggesting that the effect of running exercise on behavioural signs of radiating LBP is not an acute effect of exercise, but rather the consequence of an underlying anatomical or physiological change.

X-ray analysis showed that SPARC-null animals display significant improvement in lumbar disc height after habitual exercise ($p<.01$). However, exercise had no effect on the severity of disc degeneration in SPARC-null mice by histological analysis. Therefore, running exercise specifically improves radiating LBP and disc height, but does not improve axial LBP or histological signs of disc degeneration. Overall, these results suggest that habitual running exercise has an overall positive effect on LBP and IVDs in mice. Improving our understanding of this relationship will hopefully lead to improved therapies and preventions for the LBP patient.

Session 9: Connective tissue remodeling 2

Invited Lecture

Understanding skin fibrosis using tissue engineered models

Veronique J Moulin

LOEX, Université Laval

Fibrosis is the main characteristic of numerous pathologies including scleroderma, hypertrophic scars or pulmonary fibrosis. In our lab, we use hypertrophic scar pathology as a model of local fibrosis and scleroderma as a fibrosis reaching the whole body. We have isolated pathological cells from skins and, using three-dimensional models, we study several mechanisms that can be at the origin of the fibrosis.
Hypertrophic scars are a pathological process characterized by an excessive deposition of extracellular matrix components and a high vascularization, resulting in elevated and red scars. This pathology is usually described as a disorder of the dermis and no consideration of any role for the epidermis is mentioned. However, we reported that hypertrophic scar keratinocytes induce formation of a fibrotic dermal matrix. We further investigated dermal-epidermal interactions during fibrosis formation and found that the equilibrium between matrix formation and degradation can by highly modulate by keratinocytes.
Systemic sclerosis or scleroderma (SSc) is an incurable connective tissue disease. One of the main characteristic of this pathology is fibrosis of the skin and internal organs that is often prominent, causing dysfunction of tissues, leading to organ failure and death. Using reconstitution of skin fibrosis development using a tissue engineering approach, we have determined that the fibrotic phenotype of systemic sclerosis fibrosis varies with disease duration.

Fibrosis is a complex mechanism and tissue engineered models can be a mean to further understand this pathological mechanism.

Subcellular elevation of cytosolic free calcium is required for osteoclast migration

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Osteoclasts are multinucleated cells responsible for the resorption of bone and other mineralized tissues during development, physiological remodeling and pathological bone loss. Osteoclasts have the unique ability to resorb substrate while concurrently migrating. However the subcellular processes underlying migration are not well understood. It has been proposed that, in other cell types, cytosolic free Ca2+ concentration ([Ca2+]i) regulates cell protrusion as well as retraction. Integration of these distinct events would require precise spatiotemporal patterning of subcellular Ca2+. The large size of osteoclasts offers a unique opportunity to monitor patterns of Ca2+ during migration. We used ratiometric imaging to map [Ca2+]i within rat osteoclasts. Migration was characterized by lamellipodial outgrowth at the leading edge along with intermittent retraction of the uropod. Migrating osteoclasts displayed elevation of [Ca2+]i in the uropod, preceding retraction. Dissipation of this [Ca2+]i gradient by loading osteoclasts with the Ca2+ chelator BAPTA abolished uropod retraction. In contrast, elevation of [Ca2+]i using ionomycin initiated prompt uropod retraction. To investigate downstream effectors, we treated cells with calpain inhibitor-1, which impaired uropod retraction. However, lamellipodial outgrowth at the leading edge of osteoclasts was unaffected by any of these interventions, indicating that the signals regulating outgrowth are distinct from those triggering retraction. The large size of mature, multinucleated osteoclasts allowed us to discern a novel spatiotemporal pattern of Ca2+ involved in cell migration. Whereas localized elevation of Ca2+ is necessary for uropod retraction, lamellipod outgrowth is independent of Ca2+ – a heretofore unrecognized degree of specificity underlying the regulation of osteoclast migration.

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Internalization of TGF-β receptors is impaired in scleroderma

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Scleroderma is a complex connective tissue disease characterized by autoimmunity, vasculopathy and fibrosis. Transforming growth factor-beta (TGF-β) plays a key role in scleroderma by stimulating dermal fibroblasts to increase the production of extracellular matrix, mainly type 1 collagen. Internalization of TGF-β signaling receptors from the cell surface via clathrin-coated pits promotes TGF-β signaling, whereas internalization through caveolae facilitates receptor degradation and thus inhibition of TGF-β signaling. This study was undertaken to determine whether the internalization and the degradation of TGF-β receptors are impaired in SSc dermal fibroblasts. Internalization of TGF-β receptors was performed by monitoring the uptake of 125I-TGF-β1 in SSc and normal dermal fibroblasts. To assess endogenous receptor degradation, cell surface receptors were affinity labelled using 125I-TGF-β1 followed by cross-linking. TGF-β receptors levels were then analyzed by SDS-PAGE and autoradiography. Interaction between caveolin-1 and TGF-β receptors was determined by immunoprecipitation of cell lysates with an anti-caveolin-1 antibody followed by Western blot analysis with an anti-type II receptor antibody. Internalization studies revealed that the uptake of 125I-TGF-β is markedly reduced in SSc fibroblasts compared with normal fibroblasts. Interestingly, degradation of type II receptor was shown to be impaired SSc fibroblasts. Moreover, the interaction of caveolin-1 with type II receptor was diminished in SSc skin fibroblasts, when compared to normal skin fibroblasts. Our findings suggest that impairment of caveolin-1 and type II receptor interaction as well as the resistance of type II receptor to degradation may contribute to aberrant TGF-β signaling and the production of collagen by fibroblast in SSc.

Bovine Intervertebral Discs Degenerated by Controlled Enzyme Treatment Responds to Physiological Load by Replacing Proteoglycans

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Intervertebral disc (IVD) degeneration is a common cause of back pain. It has a negative impact on the quality of life of the patient and on the health care system. It is crucial to understand the interplay between mechanobiology, disc composition and metabolism in order to understand the underlying cause of disc degeneration and to be able to study ways to regenerate the disc. To address some of these questions, the bioreactor culture system allows measurement of numerous parameters of interest.

Intact bovine discs were prepared as described (Jim et al. Eur Spine J 20:1244-1254 2011). The discs were injected with either trypsin (high or low dose) to deplete proteoglycans (PGs) from the matrix or, as control, with buffer alone. The discs were loaded under cyclic dynamic load ranging from 0.1 to 0.3MPa for 4 hrs/day over 14 days or were cultured without external load for the same period. Cell viability at the end of culture period was assessed using the Alamar Blue Assay. PG content was measured using the DMMB assay and by Safranin O staining of paraffin sections. In a separate set of discs, stress profilometry was performed at 0.6MPa static load and the internal pressure was measured.

Bovine discs treated with trypsin maintained high cell viability over the14 days both loaded and unloaded. Unloaded discs lost about 40 to 60% of their proteoglycan content whereas discs loaded under low dynamic load completely replenished the proteoglycan content. No significant change in the load profiles were found for the low trypsin dose, however, the discs treated with a high dose of trypsin showed an 11 % significant reduction of the internal pressure ($p=0.026$).

This study shows that physiological load has the ability to stimulate PG synthesis and to restore PG content after 14 days of axial dynamic loading. The bioreactor can also be used to evaluate changes in mechanical properties of the disc following either biologically-induced injury, or biomechanical stimuli to initiate biological change.

The bioreactor provides an experimental platform useful to evaluate whether biologic repair is feasible over a range of loading conditions, or is impaired outside this range. This knowledge may provide direct patient benefit in relation to lifestyle choices following a biological repair procedure. The isolation technique and culture system also provides an experimental platform where numerous parameters can be evaluated in an in vivo model, e.g. effect of growth factors, oxygen tension, and glucose levels.

The role of fibronectin in aortic wall architecture and extracellular matrix homeostasis

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Fibronectin is an extracellular glycoprotein which exists in two different forms: soluble plasma fibronectin and insoluble cellular fibronectin. The latter is mainly secreted by cells of mesenchymal origin and is assembled into an insoluble fibrillar network. Fibronectin participates in fundamental developmental and physiological events such as cell adhesion, migration and proliferation. In recent years, numerous studies have demonstrated that the fibronectin network forms the foundation for the deposition of many extracellular matrix proteins in vitro such as fibrillin-1, collagen I and III, thrombospondin-1, tenascin-C, fibulin-1 and LTBPs. We and others have also demonstrated the requirement for continued fibronectin polymerization for fibrillin-1, collagen-1 and thrombospondin-1 matrices homeostasis. Therefore, fibronectin is thought to be a "master organizer" of extracellular matrix. In the present study, we investigate fibronectin's capacity to regulate the extracellular matrix composition and stability in vivo. As the fibronectin null mouse is embryonically lethal, we generated a conditional and tamoxifen-inducible fibronectin knockout mouse model in smooth muscle: FN flx/flx; SMA-cre-ER/+ mouse. Experimental mouse pups were injected subcutaneously at P1 and for 5 consecutive days with tamoxifen and were analyzed at one month of age. Successful fibronectin deletion in smooth muscle cells was observed at the genomic DNA level by PCR. Preliminary histological analysis of aorta shows disrupted architecture of the vessel wall; aortic smooth muscle cell attachment to elastic lamellae was highly disrupted as observed by hematoxilin and eosin staining. Fibrillin expression and the structure of elastic lamellae will be analyzed by immunohistochemistry, Hart's staining and electron microscopy to investigate if their ultra-structure is altered. Our preliminary findings suggest that fibronectin is a critical matrix protein in the postnatal development and homeostasis of blood vessels.

Cartilaginous constructs using primary chondrocytes from continuous expansion culture seeded in dense collagen gels

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Cell-based therapies such as autologous chondrocyte implantation (ACI) require in vitro cell expansion. However, standard culture techniques require cell passaging, leading to dedifferentiation into a fibroblast-like cell type. Primary chondrocytes grown on continuously expanding culture dishes (CE culture) limits passaging and protects against dedifferentiation. We tested whether CE culture chondrocytes were advantageous for producing mechanically competent cartilage matrix when seeded in dense collagen gels. Primary chondrocytes were grown either in CE culture or passaged twice on static silicone dishes (SS culture; comparable to standard methods) and then seeded in dense collagen gels and cultured for three weeks. Compared to gels seeded with SS culture chondrocytes, CE chondrocyte-seeded gels had significantly higher chondrogenic gene expression after two and three weeks in culture correlating with significantly higher aggrecan and type II collagen protein accumulation. There was no obvious difference in glycosaminoglycan (GAG) content from either culture condition, yet CE chondrocyte-seeded gels were significantly thicker and had a significantly higher dynamic compressive modulus than SS chondrocyte-seeded gels after three weeks. Chondrocytes grown in CE culture and seeded in dense collagen gels produce more cartilaginous matrix with superior mechanical properties, making them more suitable than SS cultured cells for tissue engineering applications.

Abstracts for Poster Presentations
(In numerical order corresponding to order of display)

Theme	Group	Theme Name	Poster Numbers
Theme 1	Group 1	Connective Tissue in Disease - Clinical and Fundamental Aspects	P1 - P6
	Group 2	Connective Tissue in Disease - Clinical and Fundamental Aspects	P7 - P13
Theme 2	Group 1	Stem Cells in Tissue Regeneration and Tissue Engineering	P14 – P19
Theme 3	Group 1	Genetics of Connective Tissue Disease	P20 – P25
Theme 4	Group 1	Bone Cartilage and Tooth Development	P26 – P31
Theme 5	Group 1	Biomechanics, Mechanobiology, and Biomaterials	P32 – P39
	Group 2	Biomechanics, Mechanobiology, and Biomaterials	P40 – P46
Theme 6	Group 1	Extracellular Matrix in Connective Tissues	P47 – P52
	Group 2	Extracellular Matrix in Connective Tissues	P53 – P59
Theme 7	Group 1	Connective Tissue Remodeling	P60 – P65

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Poster numbers P32 to P65 - evaluation on Friday 31/05/2013

P1 - Latent TGF-β1 Activation by Human Cardiac Fibroblast Contraction Requires αvβ3 and αvβ5 Integrins

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Pathological remodelling of the heart tissue by myofibroblast contraction is a hallmark of cardiac fibrosis and contributes to heart failure. Myofibroblasts differentiate from cardiac fibroblasts under the action of TGF-β1, which is secreted into the extracellular matrix as large latent complex. Integrin-mediated traction forces activate TGF-β1 by inducing a conformational change in the latent complex. In cardiac fibrosis, the latent TGFβ1 binding integrins αvβ3 and αvβ5 are upregulated but whether they contribute to latent TGF-β1 activation and myofibroblast differentiation is yet unknown.

To test whether pro-fibrotic culture conditions affect the expression of integrins αvβ3 and αvβ5, we assessed protein levels and localization in human cardiac in conjunction with the myofibroblast marker α-smooth muscle actin (α-SMA). Integrin overexpression, function-blocking peptides, antibodies, and shRNA tested the implication of both integrins in myofibroblast differentiation. We quantified active TGF-β1 produced by myofibroblast contraction under the same conditions to assess whether TGF-β1 activation by integrins αvβ3 and αvβ5 contributes to myofibroblast differentiation.

Expression of integrins αvβ3 and αvβ5 was co-upregulated with α-SMA in pro-fibrotic culture conditions. Integrin overexpression led to increased levels of active TGF-β1 and α-SMA expression. Blocking both integrins lead to significantly reduced TGF-β1 activation by cardiac fibroblast contraction and loss of α-SMA expression. Addition of exogenous active TGF-β1 rescued myofibroblast activation in integrin-blocking conditions. Our results demonstrate that both, αvβ3 and αvβ5 integrin are important for myofibroblast differentiation through activating latent TGF-β1. Pharmacological targeting of these integrins is a possible strategy to selectively block TGF-β1 activation by fibrotic fibroblasts and progression of fibrosis.

P2 - Macrophage Adhesion Through Cadherin-11 Promotes Myofibroblast Persistence

E Cambridge and B Hinz

University of Toronto.

Chronic pulmonary fibrosis is characterized by the co-existence of macrophages which produce pro-fibrotic TGFß-1 and myofibroblasts, which excessively secrete and contract extracellular matrix in response to TGFß-1. We propose that binding of macrophages to myofibroblasts via cadherin-11-mediated adherens junctions establishes a 'TGFß-1 niche' that contributes to the persistence of both cell types in fibrosis. To test this hypothesis we used primary mouse lung (myo)fibroblasts and bone marrow-derived macrophages that were stimulated to attain the M2a 'fibrosis' subtype. Expression and localization of adhesion proteins and cell differentiation markers was determined using Western blotting and immunofluorescence. To quantify cell-cell adhesion, we seeded macrophages onto monolayers of either myofibroblasts or fibroblasts and used suspension aggregation assays. To quantify activation of TGFß-1 in mono- and heterocellular co-cultures we used TGFß-1 reporter cells that were either cultured with experimental cells or incubated with conditioned media.

Adhesion assays demonstrated that macrophages bind to myofibroblasts faster and to a higher extent than to fibroblasts. To elucidate molecular mechanism of binding, we screened different adhesion proteins and identified the adherens junction protein cadherin-11 as a novel mediator of macrophage-myofibroblast attachment. Expression of cadherin-11 was measured in both macrophages and fibroblastic cells and increased upon myofibroblast differentiation. Immunofluorescence of heterocellular co-cultures demonstrated cadherin-11 accumulation in junctions between macrophages and myofibroblasts. Mono-cultures of macrophages produced high amounts of TGFß-1 of which only 10% was biologically active. In direct co-culture with myofibroblasts a greater percentage of total TGFß-1 was biologically active. Blocking adherens junction formation in heterocellular co-cultures and macrophage stimulation with myofibroblast conditioned media indicated that cadherin-11 mediated heterocellular contacts potentially facilitate TGFß-1 activation. We conclude that cadherin-11 junctions keep macrophages and myofibroblasts in close proximity to generate a microenvironment that is rich in active TGFß-1.

P3 – H3K9 Demethylation by LSD1 Contributes to IL-1-Induced mPGES-1 Expression in OA Chondrocytes

Fatima Ezzahra El Mansouri and Hassan Fahmi

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Introduction: Microsomal prostaglandin E synthase-1 catalyzes the terminal step in the biosynthesis of PGE2, which plays a critical role in the pathophysiology of osteoarthritis.

Objective: To investigate the role of histone H3 (H3K9) methylation in interleukin-1b (IL-1)-induced microsomal prostaglandin E synthase-1 (mPGES-1) expression in human osteoarthritic (OA) chondrocytes.

Methods: Chondrocytes were stimulated with IL-1 and the expression of mPGES-1mRNA was analyzed using real-time reverse transcriptase-polymerase chain reaction. H3K9 methylation and the recruitment of the histone demethylase LSD1 to the mPGES-1 promoter were evaluated using chromatin immunoprecipitation assays. The role of LSD1 was further evaluated using the the amino oxidase inhibitor tranylcypromine (a potent inhibitor of LSD1 activity).

Results: Treatment with IL-1 induced mPGES-1 expression in a time dependent manner. The induction of mPGES-1 expression by IL-1 was associated with H3K9 demethylation at the mPGES-1 promoter. These changes were concomitant with the recruitment of the histone demethylase LSD1. Treatment with tranylcypromine inhibited IL-1-induced H3K9 demethylation as well as IL-1-induced mPGES-1 expression.

Conclusion: These results indicate that H3K9 demethylation by LSD1 contributes to IL-1-induced mPGES-1 expression and suggest that this pathway could be a potential target for pharmacological intervention in the treatment of OA and possibly other arthritic diseases.

P4 - Investigating PPARdelta: A Novel Target for Osteoarthritis Therapy

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Introduction: Osteoarthritis (OA) is a degenerative joint disorder characterized by the breakdown of articular cartilage. Recent findings from our laboratory indicate that activation of the transcription factor PPARdelta induces the expression of enzymes involved in proteoglycan breakdown and can lead to cartilage degeneration in OA, prompting us to speculate whether inhibition of PPARdelta, can protect from cartilage breakdown in OA. Objective: To investigate the role of PPARdelta in Osteoarthritis through all encompassing in-vitro, ex-vivo and in-vivo models of disease.

Methods: Human chondrocytes and cartilage explants were treated with pharmacological agonist (GW501516) and antagonists of PPARdelta (GSK3787/0660) to evaluate changes in gene expression (qPCR), and histology (Safranin-O, immunohistochemistry) consistent with OA, and potential recovery. Our in-vivo approach uses the Cre-Lox system to inactivate PPARdelta specifically in the cartilage of using a surgical model of OA. Mutant and control mice aged 20 weeks will be compared 8 weeks after a destabilization of medial meniscus (DMM) surgery. In order to assess the progression of OA between groups, histopathological scoring (OARSI) and immunohistochemistry for known markers of OA, (MMP 13, ADAMTS 5, cartilage matrix breakdown products) were performed. To investigate changes in joint loading during OA, mutant and control mice were compared through gait analyses using the CatWalk system which measures loads on individual limbs, stride length, stride pattern and velocity of movement.

Results: Our data indicate upregulation in gene expression of catabolic enzymes after agonist treatment, as well as pathophysiological markers of OA such as Aggrecan and Collagen II fragments in histological analyses. In-vivo data indicates chondroprotection in mice with cartilage specific deletion of PPARdelta versus wild-type DMM mice.

Conclusions: Our study will provide a comprehensive understanding of this gene's role in OA from molecular and functional levels. Our data suggests that inhibition of PPARdelta is a valuable approach to treat OA.

P5 - Expression of PPARa, b, g, and H- and L-PGDS during Osteoarthritis in the Spontaneous Hartley Guinea Pig and the Experimental Dog Models

Sarah-Salwa Nebbaki, Fatima Ezzahra El Mansouri, Hassan Afif, Mohit Kapoor, Mohamed Benderdour, Jean-Pierre Pelletier, Johanne Martel-Pelletier, And Hassan Fahmi

Objective: To investigate the expression of peroxisome proliferator-activated receptor (PPAR) a, b, g, and hematopoietic and lipocaline-type prostaglandin D synthase (H- and L-PGDS) over the course of osteoarthritis (OA) in the spontaneous Hartely guinea pig and the anterior cruciate ligament transection dog models.

Methods: Guinea pigs were sacrificed at 2 (control group), 4, 8, and 12 (n = 5 per group) months of age. Non-operated (control) and operated dogs were sacrified at 4, 8 and 12 weeks post-surgery. Cartilage was evaluated histologically using the Osteoarthritis Research Society International (OARSI) guidelines. The expression of PPARa, b, g, and H- and L-PGDS were evaluated by real-time PCR and immunohistochemistry. The non-parametric Spearman test was used for correlation analysis.

Results: PPARa, b and g were detected in medial tibial plateau from control animals in both the spontaneous and surgical models. The levels of PPARa and b did not change over the course of OA, whereas PPARg levels decreased during the progression of the disease. We also showed that the expression of H-PGDS remained unchanged, whereas that of L-PGDS increased over the course of OA. PPARg levels correlated negatively, whereas L-PGDS levels correlated positively, with the histological score of OA.

Conclusion: The level of PPARg decreased, whereas that of L-PGDS increased during the progression of OA. These data suggest that reduced expression of PPARg may contribute to the pathogenesis of OA, whereas enhanced expression of L-PGDS may be part of a reparative process.

P6 - Implication of Mitogen-Activated Protein Kinase in Naproxen Induced Type X Collagen Expression in Human Bone Marrow Derived Mesenchymal Stem Cells

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PURPOSE: Several studies have shown that type X collagen (COL X), a marker of late stage chondrocyte hypertrophy, is expressed in mesenchymal stem cells (MSCs) from osteoarthritis (OA) patients. In a recent study, we found that Naproxen, but not other NSAIDs, can significantly induce COL X expression in bone marrow derived MSCs from healthy donors and OA patients. The purpose of this study was to investigate the intracellular signaling pathways that mediate Naproxen induced COL X expression in MSCs from normal and OA patients.

METHODS: Normal human MSCs were purchased from Lonza (Switzerland). These cells were grown in media supplemented with or without Naproxen (control). Osteoarthritis patient's MSCs were harvested from aspirates from the intramedullary canal of donors (60–80 years of age) undergoing hip replacement surgery for OA, using a protocol approved by the Research Ethics Committee of the Jewish General Hospital. Since the bone marrow is well vascularized, the concentration of Naproxen (100 μg/ml) supplemented into the media was based on circulating levels in the blood. Protein expression and phosphorylation were determined by immunoblotting using specific antibodies (COL X, p38, phosphorylated-p38, JNK, phosphorylated-JNK, ERK, phosphorylated-ERK). GAPDH was used as a house keeping gene.

RESULTS: We first determined basal phosphorylation levels of the three major members of mitogen-activated protein kinase family (ERK, JNK and p38) in MSCs from both normal and OA patients. The phosphorylation of MAPKs in MSCs from OA patients was significantly higher than in normal. Values represent the mean (%) ± standard deviation of 7 donors (p-ERK, 307.4 ± 69.31, $p < 0.0002$, p-JNK 943.4 ± 92.02, $p < 0.0001$, p-P38 124.8 ± 26.44, $p < 0.0479$) as percentage to normal human MSCs (100%). Incubation of normal MSCs with Naproxen 100 ug/ml for different periods of time had significantly increased the phosphorylation of ERK at one hour (153.1 ± 12.42, $p < 0.05$) and reached to below control levels at 6 hours (50.09 ± 25.83, $p < 0.05$). Naproxen also significantly increased the phosphorylation of JNK in the first 30 minutes and reached to control levels by 1 hour. Similarly, the phosphorylation of p38 increased significantly at 30 minutes and was sustained up to 6 hours. Furthermore, we also found that Naproxen significantly stimulated the expression of COL X in normal MSCs treated for one, two and three days.

CONCLUSION: This study shows that Naproxen can induce COL X expression in normal MSCs probably through the activation of MAPK pathway.

P7 - Chemically Modified N-acylated Hyaluronic Acid Fragments as Novel Anti-Inflammatory Agents

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Hyaluronic acid (HA) which is widely distributed in the connective and epithelial tissue is a high viscosity polymer, with a kDa of > 1000, composed of disaccharides of D-glucoronic acid and D-N-acetyl glucosamine linked via an alternating β-1, 4 and β-1, 3 glycosidic bonds. Lower molecular mass HA fragments are known to induce inflammation, primarily through the CD44 receptor. It is not known if the inflammatory properties of these fragments can be modified by altering the N-acetyl groups in the glucosamine moieties of HA.

N- deacetylation of HA via the hydrazinolysis reaction resulted in free $-NH_2$ functional groups, which were then acylated by reacting with acetic anhydride or butyric anhydride, to yield re-acetylated HA (AHA) and partially butyrylated HA (BHA). Several other N-acyl derivatives were also synthesized. The chemically modified polymers were obtained in good yield with a degree of deacetylation of approximately 21%. There was bond cleavage of the HA chain causing significant reduction of the molecular weight to <30 – 214 KDa. The total NH_2 and N-acetyl units in AHA was 6.2% and 93.8% respectively while the total NH_2, N-acetyl and N-butyl units in BHA was 4.2%, 75% and 20.8 % respectively. We studied the effect of these polymers on cytokine elaboration by cultured human macrophages (THP-1 cells) as a model of inflammation. The amount of 1L1-β elaborated was quantified using the human IL1-β ELISA kit. IL1-β response was dependent on the concentration of AHA, with a maximal response at 500 ug/ml, which was comparable to LPS. The addition of 500 ug/ml of BHA and 500 ug/ml of native HA did not stimulate significant amount of 1L1-β over the untreated control THP1 macrophages. Furthermore, the simultaneous addition of 500 ug/ml of BHA and AHA significantly diminished the amount of IL1-β elaborated, when compared to AHA alone. Similar results were obtained for IL6, IL8, MCP1, and TNF-α, assayed by different ELISA kits.

This study -indicated that lower molecular mass AHA stimulated the elaboration of pro-inflammatory cytokines in a human macrophage system, and that this inflammatory response can be significantly reduced in the presence of BHA. We postulate that: (i) HA fragments may contribute to elaboration of pro-inflammatory cytokines; and (ii) certain synthetic N-acyl modifications of the glucosamine moieties of HA, such as BHA, have the potential of being developed as a class of novel anti-inflammatory agents.

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P8 - Link N Exerts its Growth Factor Like Effects Through BMP Signaling

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In normal healthy intervertebral disc, tissue integrity is maintained by homeostasis between the synthesis and breakdown of extracellular matrix. This equilibrium is regulated intricately by the physiological growth factors, mainly TGF-β1, BMP-2 and catabolic cytokines like IL-1β and TNF-α. Intervertebral disc degeneration is associated with loss of this balance where cytokine induced degradation of matrix by metalloproteinases supersedes the synthesis of proteoglycans. Link N, the amino terminal peptide of link protein has been shown to act as a growth factor and was known to stimulate proteoglycan and collagen synthesis in articular cartilage. Link N was also shown to increase accumulation of proteoglycans, type II and type IX collagens in pellet cultures of disc cells. Link N was also found to increase the expression of aggrecan and suppress metalloproteinase expression. However, the molecular basis of Link N action is not known. Earlier studies have shown that growth factors TGF-β1 as well as BMP-2 can stimulate the synthesis of matrix components like proteoglycans and collagens in disc cells. Inasmuch as the actions of Link N resemble that of growth factors, we have now examined whether Link N action proceeds via TGF or BMP signaling pathway. Growth factor signaling with TGF family of ligands involves phosphorylation of R-Smad proteins (2 and 3), whereas with BMP ligands the signaling starts with the phosphorylation of R-Smad proteins (1,5 and 8). Inhibitory Smads 6 and 7 antagonize the BMP signaling while only Smad 7 blocks the TGF signaling pathway. We now find that Link N addition to human nucleus pulposus cells did not promote the phosphorylation of Smads 2 & 3, similar to BMP-2. This suggested that Link N signaling does not proceed in a fashion similar to TGF-β, which stimulates the phosphorylation/ activation of Smads 2 & 3. Further, similar to BMP-2, Link N promoted the phosphorylation of Smad 1/5, even though the magnitude of this effect is smaller than that of BMP-2. Interestingly, I-Smad 6 expression, which is relevant for BMP signaling pathway, is stimulated strongly by Link N after 1h incubation. Co-Smad 4, which is common for both BMP and TGF signaling mechanisms, is unaffected by Link N. These results suggest that Link N exerts its growth factor-like effects via BMP signaling mechanism rather than TGF- β.

P9 - Effect of Link N Treatment on Pain Related to Intervertebral Disc Degeneration

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Introduction: Low back pain associated with intervertebral disc (IVD) degeneration is an insidious disorder that by age 70 affects about 60% of the population. Previous studies have shown that discogenic back pain was due to the invasion of nociceptive nerve fibres into the aneural inner annulus fibrosus (AF) and nucleus pulposus (NP) of the IVD during degeneration. It was also shown that co culturing degenerative NP cells with neural cells increased the expression of nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) in neural cells. Nerve fibers growth by NGF and BDNF stimulation has a role in painful IVD. We previously showed that Link N can stimulate extracellular matrix biosynthesis and is a potential stimulator of IVD repair in vivo and in vitro. We also showed that Link N could regenerate damaged AF and NP cells by inhibiting the expression of proteinase gene expression. In the current study, the specific aim is to determine the effect of link N on NGF and BNDF expression and also other pain stimulants such as substance P in injured bovine IVD and also in primary neuronal cells isolated from bovine thalamus.

Materials and Methods: Coccygeal IVDs from the tails of adult bovine steers (20 to 25 months) were used for disc isolation immediately after transportation to the laboratory from the abattoir. The isolated discs were preconditioned for 24h in DMEM supplemented with 10%FBS. After 24 h discs were punctured and injected with Link N (1ug/mL). Live dead assay for all discs (control, capsaicin 1ug/ml treated, punctured and punctured with Link N injection) was done after 4 days of culturing. Spent media was collected at various time points and substance P in the media was concentrated by Solid Phase Extraction using C-18 Columns. Substance P in the samples was assayed by Elisa. RNA was isolated from AF, NP and endplate tissue samples from the discs. Gene expression for NGF, BNDF and neurokinin 1 receptor (NK1-receptor, NK1R) was determined by RT PCR.

Results: Preliminary data indicated that in punctured and Link N treated discs; Link N can suppress substance P from disc cells after 4h of incubation (76 pg/mL) as compared to the untreated punctured disc (92pg/mL). The suppression of substance P by Link N was sustained after 24 hours, when compared to control.

Conclusion: Link N may help deplete substance P availability to local nerve endings and relieve pain.

P10 - Normative Values of T1ρ MRI for Intervertebral Disc at Different Ages & Different Severity of Degenerative Disc Disease: Validation of T1ρ MRI as a Tool to Quantify Early Degenerative Disc Disease.

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Introduction. Current diagnostic modalities are not able to diagnose the advent of early disc degeneration, nor are they able to provide insight into the biochemical composition of human IVDs. The aim of this study was to provide normative values for T1ρ of human IVD and correlated these values to biochemical composition at different age groups as well at different severity of disc disease.

Materials and Methods. Twelve human lumbar spines totaling 47 IVDs were harvested from levels T11-12 to L5/S1. Standard T2 and T1ρ magnetic resonance images were acquired for all discs both in sagittal and axial planes. Average T1ρ values were recorded for specific zones of interest (SZI) in NP and AF for each disc. A core biopsy was taken representing this zone of interest and proteoglycan content was quantified using DMMB analysis. Correlation between T1ρ values, Pfirrmann grade and proteoglycan concentration was performed.

Results. Lower T1ρ values are observed in the AF than in the NP region of healthy discs. With increasing Pfirrmann grade, T1ρ values in both the AF and NP decreased. T1ρ in the NP decreased more prominent than those of the AF and begin to converge with AF with increasing Pfirrmann grades. The T1ρ values at the punch location that was used for performing DMMB of the NP were within 5%, which was validated for 12 IVDs. DMMB analysis for proteoglycans showed lower concentrations at lower T1ρ intensity associated with increasing Pfirrmann grades.

Conclusion. Having a diagnostic tool allowing us to detect early biochemical changes in otherwise healthy looking disc (Pfirmann 1-2) is crucial in the hope that we can alter the natural history of degenerative disc disease. We have demonstrated that we can map in 3D the health of IVD using axial and sagittal T1ρ MRI images that these T1ρ values grossly correlate with establish DDD grading schemes as well as loss of proteoglycan concentration.

P11 - Solute Interactions with Cracked and Intact Surfaces of Injured Articular Cartilage: Toward the Design of Cartilage-Specific Functional Contrast Agents

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Objective: Current cartilage-specific contrast agents can detect cartilage degradation only after a significant change in glycosaminoglycan (GAG) content. Differential adsorption of solutes to cracked and intact surfaces of injured cartilage (due to altered properties at fissures) may provide another tool for functional assessment of cartilage. Macromolecules with differential surface interactions which can be conjugated with CT or MRI contrast agents may be of interest in designing cartilage-specific imaging tools. We therefore aimed to study the adsorption of native and aldehyde-modified dextrans and a chondroitin sulfate (CS)-binding peptide (sequence: YKTNFRRYYRF) to cracked and intact surfaces of cartilage as a potential mechanism for customized contrast agent function. Dextrans are biodegradable and pharmacologically inert and have been widely used in other medical applications. In addition, they are available commercially in different molecular weights and have numerous hydroxyl groups that can be easily conjugated to CT or MRI contrast agents or desired functional groups. Dextran-aldehyde and a CS-binding peptide were selected since aldehyde modification enhances molecular binding to cartilage surfaces and chondroitin sulfate is a major component of cartilage.

Methods: Osteochondral cores from bovine knees were incubated in chondrocyte culture medium (DMEM with nonessential amino acids, HEPES, FBS and penicillin-streptomycin-amphotericin). Injury was induced with single unconfined axial compressions and positive controls were prepared by slicing the surface of explants. Explants (injured, positive control, and uninjured control) were equilibrated with solutes (4 μM) for 20-22 hr. In desorption experiments, explants were then transferred to culture medium (blank) for 20-22 hr. 200 μm thick sections were then cut perpendicular to the articular surface and imaged using an inverted fluorescence microscope for visual assessment and quantification of solute adsorption to cracked and intact surfaces.

Results: Adsorption at cracked surfaces was significantly ($p<0.01$) less than at intact surfaces for injured and sliced explants for all solutes both after absorption and desorption. Dextran-aldehyde and CS-binding peptide showed higher adsorption strengths (fluorescence intensity after desorption divided by intensity after absorption) which could be beneficial in clinical applications since lower concentrations would be required.

Conclusion: This study demonstrates that differential solute-surface interactions may open new avenues for development of contrast agents for more sensitive functional assessment of cartilage. In addition, characterization of solute interactions with cracked and intact surfaces of injured cartilage may enhance our understanding of the biochemical and structural alterations at fissures.

P12 - Effects of Osteopathy on Hand, Global and Work Disabilities in Patients with Systemic Sclerosis: A Series of Single Case Studies.

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Introduction: In systemic sclerosis (SSc), a chronic autoimmune rheumatic disease, hand contractures are common and associated with hand, global and work disabilities. There are no known effective treatments, although there have been a few promising studies with manual therapies. The role of osteopathy to reduce hand contractures and disability in SSc has not been studied to date. Our aim was to explore the effects of osteopathy on hand, global and work disabilities in SSc patients.

Materials and methods: A series of single case studies using an A1-B-A2 design was undertaken. Six SSc patients with hand contractures and self-reported limitations in vocational activities were recruited among subjects enrolled in the Canadian Scleroderma Research Group cohort at 2 sites (Jewish General Hospital, Montreal and Centre hospitalier universitaire de Sherbrooke, Sherbrooke). Patients received 9 weekly semi-standardized sessions of osteopathy, targeted on upper limbs, thorax and cranial base. Repeated measures were taken, twice a week for self-reported symptoms (numerical rating scales) and once a week for range of motion of fingers (delta Finger-to-palm), hand disability (Cochin Hand Functional Disability Scale) and global disability (Health Assessment Questionnaire - Disability Index). Upper body skin score (subscore of the modified Rodnan skin score), work disability (Arthritis Work Instability Scale - modified) and health-related quality of life (SF-36v.2) were measured at baseline (A1), week 9 (B) and at 1-month follow-up (A2). Data for each variable with repeated measures were represented on simple line graphs and visually interpreted, completed by the Sheward's two Standard Deviation Band. The Wilcoxon signed rank test was used to identify statistically significant differences for the variables measured at three time points (baseline, end of treatment, 1-month follow-up).

Results: All subjects were female; mean age 50 ± 10 years; mean disease duration 7,42 ± 1,95 years; 4 with limited and 2 with diffuse skin involvement. Subjects showed improvement in most self-reported symptoms (n=6), range of motion of fingers (n=6), hand disability (n=6) as well as global disability (n=5) during the treatment phase (B) compared to baseline (A1), and improvement was maintained in most outcomes at 4 week follow up (A2). Despite the small sample, skin thickness and work disability showed a strong trend towards improvement (p=0,06). The SF-36v.2 Physical Component Summary score improved significantly (p=0,02) and this was maintained in follow-up (p=0,03).

Conclusion: These findings suggest that osteopathy may be effective in reducing disability from hand contractures in SSc. A randomized controlled trial is needed to confirm these results.

P13 - Preventing the Development of Osteoarthritis Following Reconstruction of the Anterior Cruciate Ligament

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Introduction: Osteoarthritis (OA) is a degenerative joint disease involving the breakdown of the cartilage that covers the ends of the articular joint-bone interface. Severe injury to the knee joint such as cruciate ligament tears and/or menisci damage often results in accelerated development of OA. It is thought that there may be some type of injury-induced, mechanical abnormality of the injured joint and subsequent interplay between altered mechanics and/or biological changes, such as inflammation, which may lead to cartilage damage. Severely injured anterior cruciate ligaments (ACL's) are often replaced using tendon autografts with the aim to restore the biomechanical function, however OA may still develop in spite of a successful reconstructive surgery. It remains to be determined whether predominantly biological or biomechanical origins may cause OA development and to look for possible interventions to block the initiation of the disease.

Hypothesis: Early inflammation following idealized ACL reconstruction surgery initiates the degeneration of the knee joint. This hypothesis will be evaluated by the following specific objectives: 1) determine improvement in kinematics, 2) determine changes in cartilage damage, 3) evaluate inflammation treatment, and 4) define metabolomic biomarkers of osteoarthritis in an idealized ACL reconstruction model.

Methods: An ovine model will be used for the idealized ACL reconstruction. The animals will be allocated into three groups: experimental 1 (idealized ACL reconstruction surgery), experimental 2 (idealized ACL reconstruction surgery + dexamethasone treatment group), and controls (no surgery + no dexamethasone). The surgeries will be accomplished via arthrotomy to the right stifle joint, with the left joint serving as the control. For evaluating kinematics, an in vivo motion analysis system for measuring gait will be used. Kinematics will be collected pre-surgery, three, and ten weeks post-surgery. The animals will be sacrificed at ten weeks after autograft surgery and tissues, synovial fluid, and blood will be collected. Synovial fluid and blood will be collected at various time points before (pre-surgery) and after surgery (three and ten weeks) and stored at -80°C. Gross morphological grading will be conducted for gross cartilage defects, osteophyte formation, and meniscal damage. The mRNA expression of inflammatory cytokines will be measured by real-time polymerase chain reaction. Metabolomic analysis will be performed using the Luminex multiplex assay to identify potential biomarkers.

Significance: By developing an understanding of biological and biomechanical changes with OA, treatments may be developed to treat conditions such as OA and possibly prevent or slow damage caused to the articular cartilage.

P14 - Role of Seaweed Laminaran on Collagen Deposition During Production Of Tissue - Engineered Skin

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Our laboratory has developed a technique to reconstruct in vitro skin from human cells, using a tissue-engineering method called "self-assembly method" that uses the ability of fibroblasts to deposit the collagen I they secrete. The time for making this skin, about 6-7 weeks, is a drawback for any clinical use. Previous studies have shown that fibroblasts cultured with laminaran (LM) produce more collagen I and MMP that cultured fibroblasts without LM. However, it has also been shown that the whole quantity of collagen I produced by the cells is not deposited to form dermis but some remains in the culture medium. Our hypothesis is that the addition of LM in the culture medium can increase the production of collagen I but also can increase its deposition on the cells, potentially accelerating the rate of production of self - assembled dermis. Dermal fibroblasts were cultured in the presence of LM for 7 or 28 days. Deposited collagen I by cells cultured for 7 days was quantified by Western blot and normalized using total actin. The dermis reconstructed by fibroblasts cultured for 28 days was analyzed by histology and the thickness of the dermis was evaluated according to the amount of LM added into the culture medium.

In both cases, the LM seems to increase the collagen deposition and the thickness of the dermis. According fibroblast populations, however, the results were variable and must be confirmed by repeating the experiments with other fibroblast populations.

If this hypothesis is confirmed, the LM could be used to increase the speed of the production of reconstructed self-assembly dermis. It could also be used in cosmetic (wrinkles) or therapeutic (ulcers) creams.

P15 - The Dedifferentiated Phenotype of Chondrocytes is Regulated by Actin Polymerization

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Clinically relevant cell-based bioengineered articular cartilage constructs requires access to a large quantity of cells. Traditional passaging of chondrocytes in monolayer may be utilized to achieve a greater number of cells, however, this results in chondrocyte dedifferentiation. Dedifferentiated cells have reduced expression of aggrecan and type II collagen concomitant with enhanced expression of fibroblast matrix markers, type I collagen and tenascin C. Passaged cells also have an altered cytoskeleton which results in tissue contraction of bioengineered articular cartilage constructs. The aim of this study was to further investigate the phenotype of passaged chondrocytes and to elucidate how the actin cytoskeleton may regulate the dedifferentiated phenotype of cells. It was hypothesized that passaged cells acquire a myofibroblast-like phenotype which correlates with the polymerization status of actin. It was revealed that dedifferentiated (passaged) cells consist of stress fibers as opposed to cortical actin as seen in primary chondrocytes. Passaged cells had lower levels of g-/f-actin and expressed higher levels of contractile proteins alpha smooth muscle actin, transgelin, and vinculin as compared to primary chondrocytes. Furthermore passaged cells, but not primary chondrocytes, contracted stress-relaxed type I collagen gels. Exposure of passaged cells to latrunculin B resulted in an increased g-/f-actin ratio, reduced expression of type I collagen, tenascin C, alpha smooth muscle actin (aSMA), transgelin (TAGLN), and vinculin (VNC). In addition, exposure of cells in type I collagen stress-relaxed gels resulted in decreased matrix contraction as well as reduced levels for aSMA, TAGLN, and VNC. Passaged cells acquire an altered actin cytoskeleton which regulates gene expression in passaged cells. A further understanding of the signalling mechanisms governing dedifferentiation of cells may lead to the identification of the methodology to the reacquisition of the differentiated chondrocyte state and a suitable cell source for the development of large, non-contractile, bioengineered articular cartilage constructs.

P16 - A Continuous Expansion Culture Method for Preservation of Human Articular Chondrocyte Phenotype During Population Expansion

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Standard culture methods for population expansion of autologous articular chondrocytes cause loss of chondrogenic phenotype and dedifferentiation to fibroblast-like cells. We have previously developed a novel continuous expansion (CE) culture system which can significantly block bovine articular chondrocyte dedifferentiation. Here, human articular chondrocytes (HACs) were cultured on the high-extension silicone rubber culture surface, which was stretched continuously to 600% of its initial surface area. This maintained HACs at high density while limiting contact inhibition and reducing cellular passaging. Proliferation in CE culture was on par with cells passaged on static silicone dishes (SS culture). Gene expression analysis as well as biochemical assays and immunofluorescence microscopy of follow-on pellet cultures showed blockade of HAC dedifferentiation in CE versus SS cultures. CE culture also yielded cells with a more chondrocyte-like morphology. CE culture HACs displayed a trend for higher RNA-level expression of chondrogenic markers collagen type II and aggrecan while displaying a trend for a negligible increase in expression of collagen type I RNA. Pellet cultures from CE cultured HACs contained more sulphated glycosaminoglycan and collagen type II than pellets from SS culture. HACs grown in CE culture may therefore be more suitable for use in cell-based therapies.

P17 - Membrane Culture and Reduced Oxygen Tension Enhances Cellular Preconditioning of Equine Cord Blood Mesenchymal Stromal Cells for Direct Use in Tissue Engineering or Cytotherapeutic Strategies.

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Cartilage injuries are the main contributor to equine lameness, loss of training days and premature retirement. Joint cartilage has poor intrinsic repair potential and current treatment modalities are insufficient for long term relief. This project explores factors effecting preconditioning of equine cord blood mesenchymal stromal cells (eCB-MSC) to chondroprogenitor cells for subsequent use in tissue engineering or cytotherapeutic strategies. In vitro chondrogenesis or cell preconditioning is traditionally done in aggregate pellet culture at normoxia (21% oxygen). Objective 1 compares the traditional pellet culture to a novel membrane culture method, whereby cells are seeded on top of PTFE membranes and grown at high cell density. Objective 2 compares effects of differentiation under normoxic or hypoxic (5% oxygen) culture conditions. Preliminary results indicate improvement in matrix morphology and accumulation in the membrane culture method and under hypoxic conditions. Future prospects are to evaluate the combined influence of the novel culture method under low oxygen tension. The overall goal is to produce high quality tissue or pre-chondrocytes for direct applications in treating articular damage in horses.

P18 - Phenotypic and Immunomodulatory Properties of Equine Cord Blood-Derived Mesenchymal Stromal Cells (eCB-MSC)

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The observation that mesenchymal stromal cells (MSC) can secrete trophic and immunomodulatory factors in vitro suggests that cytotherapeutic treatment modalities may be used to modulate the immune system. MSC polarisation into either a pro-inflammatory (MSC type-1) or anti-inflammatory phenotype (MSC type-2) is a new paradigm of MSC function. The marker expression pattern associated with each phenotype seems variable. Toll-like receptor 3 and 4 has been associated with anti-inflammatory and pro-inflammatory responses, respectively. The hypothesis of this project is that undifferentiated equine cord blood-derived cells have a consistent cryo-tolerant phenotype and exhibit immune-regulatory properties that correlate to Toll-like receptor expression. Selected surface markers will be assessed with flow cytometry and correlated with gene expression using qPCR. The immunoregulatory properties will be assessed in mixed lymphocyte reaction assays. Preliminary data suggests that the nucleated cell fraction of equine umbilical cord blood (eCB) express MHC I, MHC II, CD11a/18 and CD90, but lose expression of all these markers except for CD90 after culture in plastic culture ware (e.g putative eCB-MSCs). Mixed lymphocyte reaction showed that eCB-MSCs inhibit lymphoproliferation suggesting immune-suppressive abilities. Correlation study of mixed lymphocyte reactions and Toll-like receptor expression is ongoing.

P19 - Reg1 Increases Proliferation of Bone Marrow Derived Mesenchymal Cells and Induces Early Mineralization by Osteoblasts.

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Reg proteins are implicated in the regeneration/healing of multiple tissues such as pancreas, stomach, intestine and skin and in a number of cancers. Their role and expression in skeletal tissues remains to be established. In this study we ask whether Reg proteins could modulate the proliferation, differentiation and mineralization of osteoblast precursors and the osteoblasts themselves. We generated transgenic mice overexpressing Reg1 under the control of collagen type1a 1 promoter (Col1a1-Reg1). The mice obtained did not show any obvious morphological abnormalities. However, cultures of bone marrow derived colony forming unit (CFU) show an average increase of 35% in the number of CFU obtained in the Reg1 overexpressing animal compared to its wild type littermate (95% confidence interval: 2% – 67%). In addition, bulk culture of bone marrow derived mesenchymal cells isolated from Col1a-Reg1 animals showed a two-fold increase in proliferation index as assessed by Ki67 marker. We then assessed the ability of these CFU to differentiate into osteoblasts by assessing their ability to form minerals following osteogenic differentiation. The quantification of the number of CFU forming minerals at 2 weeks of differentiation was reduced (by 30% to 50%) in cultures derived from Reg1 overexpressing animals compared to their wild type littermate. In osteoblasts, the proliferation index (Ki67) was similar in both Col1a1-Reg1 and wild type cultures. We then assessed the effect of Reg1 on the ability of osteoblasts to produce minerals. Using Alizarin red staining one week following the induction of mineralization, we observed the presence of minerals in osteoblasts derived from Col1a1-Reg1 animal, but none in those derived from wild type littermates. In addition ALP activity was higher in Col1a1-Reg1 osteoblast cultures. At three weeks post mineralization, minerals were present in both cultures with staining more abundant in Col1a1-Reg1 cultures. In addition, early mineralization of wild type osteoblasts was induced by mineralization media conditioned by Col1a1-Reg1 osteoblast cultures for 18hrs. Furthermore, addition of human recombinant Reg1a protein to wild type osteoblast culture in mineralization condition resulted in the formation of Alizarin red positive nodules. Taken together, these results show dual effects of Reg1 protein on osteoblasts and osteoblast precursor cells. These findings may lead to the treatment of non-healing fractures or osteoporosis.

P20 - Specificity of miR-378a-5p Targeting Rodent Fibronectin

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One criterion for microRNA identification is based on their conservation across species, and prediction of miRNA targets by empirical approaches using computational analysis relies on the presence of conservative mRNA 3'UTR. Because most miRNA target sites identified are highly conserved across different species, it is not clear whether miRNA targeting is species-specific. To predict miRNAs targeting, we aligned all available 3'UTRs of fibronectin and observed significant conservation of all 20 species. Twelve miRNAs were predicted to target most fibronectin 3'UTRs, but rodent fibronectin showed potential binding sites specific for five different miRNAs. One of them, the miR-378a-5p, contained a complete matching seed-region for all rodent fibronectin, which could not be found in any other species. We designed experiments to test whether the species-specific targeting possessed biological function. After confirming repression of rodent fibronectin by miR-378a-5p, we found that expression of miR-378a-5p decreased mouse breast cancer cell proliferation, migration, invasion, and colony formation, resulting in inhibition of tumor growth. Induced expression of fibronectin produced opposite results, while silencing fibronectin displayed similar effects as miR-378a-5p. Unexpectedly, exogenous addition of fibronectin to the cultures produced opposite results as compared with induced expression of fibronectin. Tumor formation assay confirmed that enhanced expression of fibronectin in the stromal tissues as a background environment suppressed tumor growth while increased fibronectin expression inside the tumor can promote tumor growth. This was likely due to the different signaling direction, either inside-out or outside-in signal. Our results demonstrated that species-specific targeting by miRNA could also exert functional effects. Thus, one layer of regulation has been added to the complex network of miRNA signaling.

P21 - MicroRNA miR-24 Enhances Tumor Invasion and Metastasis by Targeting PTPN9 and PTPRF to Promote EGF Signaling

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MicroRNAs are known to play regulatory roles in gene expression associated with cancer development. We analyzed microRNA miR-24 levels in patients with breast carcinoma and found that miR-24 was higher in breast carcinoma samples than in breast benign tissues. We generated constructs expressing miR-24 and studied its functions using both in vitro and in vivo techniques. We found that the ectopic expression of miR-24 promoted breast cancer cell invasion and migration. In vivo experiments indicated that the expression of miR-24 enhanced tumor growth, invasion to local tissues, metastasis to lung tissues, and decreased overall mouse survival.

In the miR-24 expressing cells and tumors, EGFR was highly phosphorylated, while expression of the phosphatases PTPN9 and PTPRF were repressed. We confirmed that miR-24 could directly target both PTPN9 and PTPRF. Consistent with this, we found that the levels of pEGFR were higher while the levels of PTPN9 and PTPRF were lower in the patients with metastatic breast carcinoma. Ectopic expression of PTPN9 and PTPRF decreased pEGFR levels, cell invasion, migration, and tumor metastasis. Furthermore, we found that the levels of MMP2, MMP11, pErk, and ADAM15 were up-regulated, while the levels of TIMP2 were downregulated, all of which supported the roles of miR-24 in tumor invasion and metastasis. Our results suggest that miR-24 plays a key role in breast cancer invasion and metastasis. miR-24 could potentially be a target for cancer intervention.

P22 - Transcriptome Profiling of Genes Regulated by 17-beta Estradiol in Human Osteoblasts Derived From Idiopathic Scoliosis Patients.

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Adolescent idiopathic scoliosis (AIS) is the most common form of spinal deformity affecting up to 4% of schoolchildren worldwide. The etiology and molecular mechanisms of AIS are not clear; currently the consensus on AIS is of a multifactorial etiology, but the involvement of genetic factors is widely accepted. Several physiopathological, clinical and molecular observations suggest that hormones such as melatonin, estrogens and growth hormones play a critical role in bone mass acquisition and consequently in the progression of AIS, but the precise mechanisms involved are yet unclear. The role for estrogen seems possible due to its interaction with many factors that influence the development and progression of this spinal deformity. Additionally, estrogens impact bone remodeling and growth as well as bone acquisition, all of which are affected in AIS. To study the role of estrogen in AIS, six unrelated individuals with AIS and 6 controls (non-AIS individuals), all French Canadian females from Quebec were studied. Gene expression profiling was preliminarily investigated by microarray analysis in RNA samples from Osteoblasts derived from control (non AIS) and AIS patients. Osteoblasts were cultured for 16 h without or with increasing amounts of Estradiol .Data analysis was performed in R version 2.10.1 (Bioconductor packages oligo and limma). Selected genes with change fold greater than 1.5 were further investigated by RT-qPCR. Microarray analysis revealed several genes that are differentially regulated in AIS osteoblasts compared to control. Many of these genes are involved in different physiological signaling pathways. When we compared the transcriptome profiling of estrogen –regulated genes between non-AIS and AIS osteoblasts, several genes were up-and down-regulated in response to estrogen. W e considered five genes for further anaylsis.We are interested in these genes cause three of these genes were previously associated with AIS.Insilico analysis,show that these genes have several estrogen reponse elements in their promoters which confirm the fact that these genes are estrogen regulated. We successfully cloned these genes in PGL3 vector which includes a luciferase coding sequence. The luciferase activity will be measured in the presence or absence of estrogen. More than one gene is likely responsible for AIS, and some of these genes are estrogen–regulated. In the absence of specific causative gene(s) for AIS, our study of gene expression by microarray pointed out putative biological pathways and genes to be carefully investigated.

P23 - Use of Next-Generation Sequencing to Identify New Skeletal Disease Genes.

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Through the use of exome sequencing in individuals with Mendelian skeletal diseases, we have identified the genes causing dysosteosclerosis, a form of osteopetrosis (*SLC29A3* gene, see HMG 2012;21:4904), genitopatellar syndrome (*KAT6B* gene, see AJHG 2012;90:282), osteogenesis imperfecta and early-onset osteoporosis (*WNT1* gene, NEJM in press), Yunis-Varón syndrome which presents with features of cleidocranial dysplasia (*FIG4* gene, AJHG in press), in addition to different forms of spondylo(epi)metaphyseal dysplasia and of brittle bone disease. We have also developed a next-generation sequencing panel providing higher coverage sequencing for 35 genes known to cause low or high bone mineral density (Osteoporosis Int. 2013, Feb 27). Our findings provide new insights in the regulation of skeletal development and maintenance, and shed light on genes not previously thought to be implicated in these processes. Additionally, applying whole exome sequencing at a larger scale in all individuals with even mild skeletal diseases will allow us to better understand the full phenotypic spectrum of known disease genes. We hypothesize that combinations of relatively common variants in genes such as those encoding matrix proteins predisposing to osteoporosis or other common skeletal diseases.

P24 - Phenotypic Differences in PTHrP+/- Mice on C3H and C57Bl6 Backgrounds

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Introduction: Osteoporosis is a chronic disease characterized by reduced bone mineral density and deterioration of bone micro-architecture. The properties of human bone, including its mass, micro-architecture, biomechanical properties, remodeling properties, susceptibility to fracture and capacity for repair, have been attributed to a combination of genetic background, hormone levels and age. Previous work showed that ablation of one copy of the parathyroid hormone related peptide (PTHrP) gene resulted in haploinsufficiency characterized by osteopenia in 4-month-old mice on a C57Bl6 background. We aim to compare the bone phenotype of 4 and 12 month old male and female mice heterozygous for the PTHrP (PTHrP+/-) gene and maintained for 20 generations on C57Bl6 or C3H backgrounds.

Methodology: Femurs are harvested from PTHrP+/+ and PTHrP+/- mice between 4 and 12 months of age. Samples are fixed in 4% paraformaldehyde overnight, and stored in PBS at 4Â°C for subsequent analysis. The samples are imaged by micro CT scanner (Skyscan 1172) and analyzed by using NRecon and CTAn software. The regions of interest of the images are defined and used to quantify the bone mass and structural properties of the cortical bone in diaphysis and trabecular bone in metaphysis. Cortical bone perimeter, area, and thickness are used describe the cortical structure; percentage of bone volume to tissue volume (BV/TV) is used to quantify trabecular bone mass.

Results: The significantly decreased trabecular bone mass in PTHrP+/- on C57Bl6 background reported by previous authors is not found in C3H strain. C3H strain has consistently higher trabecular and cortical bone mass than the otherwise matched mice on C57Bl6 background. C57Bl6 PTHrP+/- mice have increased bone perimeter and lower cortical bone thickness compared to their WT counterparts. C57Bl6 is able to maintain the cortical thickness through aging while its trabecular bone is susceptible to age related bone loss. On the contrary, decreasing cortical thickness due to aging is more prominent in C3H while age related trabecular bone loss was only observed in C3H female mice and absent in C3H male ones. Also of interest, male gender in C57Bl6 mice leads to a consistently greater bone mass than their otherwise matched female counterparts. However, difference of bone mass due to gender was only observed in the trabecular bone of 12 month old mice on C3H background.

Conclusion: Different genome-determined osteogenic mechanism exists in C3H and C57Bl6 strains that have interactions with PTHrP signalling, aging and sex hormone and affects bone phenotype.

P25 - Human V-ATPase a2 P405L Mutation Results in Cutis Laxa Possibly by Reducing V-ATPase Assembly and/or Stability

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Introduction & Objectives: V-ATPase is an evolutionarily conserved enzyme that couples the energy of ATP hydrolysis to proton translocation across membranes. The 100-kDa "a" subunit is encoded by four isoforms (a1-a4) in humans. Mutations in a3 and a4 result in osteopetrosis and distal renal tubular acidosis, respectively. Only recently was it discovered that mutations in a2 underlie autosomal recessive cutis laxa type 2A (ARCL2A). This genetic disorder affects elastic fiber biosynthesis, and is characterized by wrinkly loose skin, developmental delay, and abnormal N- and O- glycosylation. Here, we have studied an ARCL2A-causing missense mutation (a2 P405L) in a S. cerevisiae yeast model and report its effects on V-ATPase structure and function.

Methods and Results: The human a2 P405L mutation was reconstructed in the yeast a2 ortholog Vph1p at residue 416 using site-directed mutagenesis. Topological examination of the Vph1p P416L missense mutation shows that it is located in the middle of the first transmembrane helix. Vph1p P416L had defective growth on selective media and no detectable V-ATPase activity. The mutant phenotypes were recessive, as co-expression of wild type Vph1p successfully restored growth. Immunoblots revealed reduced expression of mutant Vph1p in the whole cell extract and microsomal membranes, barely detectable levels of mutant Vph1p on purified vacuoles, and wild-type levels of other V-ATPase subunits (A and B). These results suggested that a protein folding defect and subsequent accelerated degradation was underlying the disease-causing effects. Further experiments assessing whether osmolyte chemical chaperones, known to assist in protein folding, can correct this defect and rescue V-ATPase growth are underway.

Conclusion: ARCL2A is likely a new addition to the growing list of genetic disease where degradation of mutant protein underlies the "loss of function" phenotype. Potential rescue with chemical chaperones could represent a therapeutic option for this disease, and other V-ATPase-associated genetic diseases.

P26 - The Regulatory Role of microRNA-17 in Osteogenic Differentiation

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The body maintains bone density by achieving an equilibrium between bone formation and resorption, but failure to maintain this equilibrium can lead to degenerative bone diseases such as osteoporosis, which is characterized by excessive bone resorption and inadequate formation of new bone. Emerging research is showing that microRNAs (miRNAs) are important regulators of osteogenic differentiation and bone formation both in vitro and in vivo. We hypothesized that microRNA-17 (miR-17) is involved in the regulation of osteogenesis in MC3T3-E1 murine pre-osteoblast cells. After overexpressing miR-17 in MC3T3-E1 cells via plasmid transfection, we assessed the cells' osteogenic differentiation capacity compared to plasmid control cells. The differentiation capacity was assessed using alkaline phosphatase staining, and miR-17 cells were found to show lower levels of alkaline phosphatase activity, suggesting that miR-17 overexpression leads to an inhibition of osteogenic differentiation capacity. qRT-PCR was used to assess the levels of several key markers of osteogenic differentiation in the transfected cells. It was found that the markers bone sialoprotein (IBSP) and fibronectin (FN) mRNA are significantly down-regulated in miR-17 transfected cells compared to the GFP control. In addition, miR-17 transfected cells were found to be more elongated than the GFP control cells. Based on previous work with MC3T3-E1 cells, this elongation is also an indicator of differentiation inhibition. We plan to investigate the target that miR-17 acts on to inhibit differentiation by finding potential targets using predictive software, analyzing protein expression via Western Blot analysis, and confirming the target using the Luciferase Reporter Assay. Finally, we plan to use siRNA-mediated knockdown of the target to assess if the phenotypic results are consistent with the miR-17 overexpression data. Elucidating the role of miR-17 in MC3T3-E1 cell differentiation may also prove useful for further studies in determining why breast cancer cells preferentially metastasize to bone tissue at the micro-environmental level.

P27 - Cellular Factor XIIIA Transglutaminase Localizes to Plasma Membrane and Caveolae and Regulates Endocytosis of Fibronectin Matrix in Differentiating Osteoblasts.

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Osteoblast extracellular matrix contains large amounts of fibronectin fibrils that serve as a scaffold for collagen type I deposition. Fibronectin matrix is thus highly important for bone quality and osteoblast differentiation. Fibronectin matrix is constantly renewed and turned over by osteoblast-driven fibronectin internalization via caveolae-mediated cellular endocytosis. Caveolae are small (50–100 nanometer) invaginations of the plasma membrane found in many vertebrate cells and they have been long implicated in vesicular transport of macromolecules. Transglutaminases (TGs) are a family of widely distributed protein crosslinking enzymes that stabilize protein-protein interactions and extracellular matrices. Osteoblasts express Factor XIIIA (FXIIIA) which is found both in cells and secreted to the matrix. While extracellular FXIIIA stabilizes fibronectin matrix by crosslinking it, we hypothesized that cellular FXIIIA regulates fibronectin endocytosis. Our immunofluorescence data shows that FXIIIA colocalizes with caveolin-1, which is a structural and functional protein in the caveolae, in rounded patches specifically in differentiating MC3T3-E1/C14 osteoblasts. TIRF (total internal reflection fluorescence) microscopy results also show co-localization of FXIIIA and caveolin-1, further supporting their presence together on the plasma membrane. Caveolae disrupting agent methyl-β-cyclodextrin (MβCD) abolished FXIIIA patches from the cell surface. The presence of FXIIIA in caveolae was further investigated by preparing caveolae-enriched cellular fractions from osteoblasts by sucrose density gradient ultracentrifugation followed by Western blot analyses. Data shows that FXIIIA co-fractionates with caveolin-1 and that MβCD again disrupts its presence in these fractions. An endocytosis assay involving biotinylation of extracellular and cell surface proteins whose internalization is followed by Western blot analysis, showed that transglutaminase inhibitor, NC9, promotes fibronectin endocytosis and internalization from cell surface. NC9 localizes into caveolae. NC9 did not affect the caveolin-1 levels in caveolae-enriched fractions, however, more of the active form, Tyr14-phosphorylated caveolin-1, was found at the early stage of osteoblasts differentiation upon inhibiting FXIIIA with NC9. It is known that caveolin-1 phosphorylation is mediated by Src-kinases which promote matrix internalization and inhibit osteoblast differentiation and bone formation indicating that FXIIIA blocks this phosphorylation process. In conclusion, the function of cellular FXIIIA is to limit caveolin-1 activation and internalization of fibronectin in osteoblasts. This promotes fibronectin accumulation to the extracellular matrix which is the first step of bone formation.

P28 - Making the Mold: Developmental Histology of Human Primary Lamellar Bone

Corey Maggiano

Modeling accounts for the achievement of diametric size, shape and orientation of long bones. It transpires on the periosteal and endosteal surfaces through uncoupled formation and resorption and contributes to bone strength, determining peak cortical bone mass, total cortical area, and mechanically adaptive morphology. In many bones, asymmetry in modeling growth results in a net linear drift of the diaphysis where bone is resorbed on one surface and formed on the other. These are all important characteristics for understanding bone growth and adaptation in clinical and bioarchaeological contexts. Unfortunately, long bone modeling is only easily experimentally observed in non-human animal models, and fortunately, available modern cadaveric remains are typically from older individuals, leaving many questions regarding the nature of human bone development unasked. My current research program investigates the multidisciplinary space generated between efforts that ensure human health and those that reconstruct relationships among human biology, behavior and past environments. Examining archaeological human bone, using novel techniques in histomophometry can offer insights into the complexity of bone microstructural patterns and regional tissue distributions that inform processes of growth and adaptation to physical activity. Investigations of bone modeling drift also have implications for clinical, archaeological and forensic applications that are just beginning to be more fully understood.

P29 - Characterizing osteoclast differentiation from mouse spleen

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Osteoclasts are bone cells responsible for degradation of bone during physiological processes of remodeling and tooth eruption, as well as in diseases such as rheumatoid arthritis, periodontitis and cancer metastasis to bone. As mediators of pathological bone destruction, osteoclasts represent a suitable pharmacological target for drug development. Osteoclast differentiation protocols from mouse bone marrow are well established. However, in many circumstances, for example in transgenic mice with small or compromised bone marrow compartment, it is desirable to use alternative source of osteoclast precursors.

Spleen is commonly used as a source of monocytes for osteoclastogenesis in co-culture experiments, however it performance in cytokine-induced osteoclastogenesis has not been characterized. Dissociated spleen cells from healthy mice were exposed to known pro-resorptive cytokines, RANKL and MCSF, for up to 12 days and formation of multinucleate osteoclasts expressing osteoclast marker, tartrate-resistant acid phosphatase (TRAP) was assessed. Simultaneously, osteoclasts were generated from bone marrow using established protocols. We have found that osteoclastogenesis can be successfully induced from spleen cultures using similar concentrations of RANKL (50 ng/ml) and MCSF (50 ng/ml). Compared to bone marrow cultures, differentiation from spleen required longer time (9 days for spleen compared to 5 days for bone marrow cultures) and higher plating density of non-adherent cells (75,000/cm2 for spleen compared to 50,000/cm2 for bone marrow). The results were comparable for Balb/c, C57 and FVB mice. Gene expression and functionality assay using calcium phosphate coated plates demonstrated that osteoclasts differentiated from spleen cultures present all main characteristics of osteoclastic cells.

Thus, is study describes a new protocol for osteoclast differentiation, which will be of interest for basic scientists as well as screening new osteoclast-targeting therapies.

P30 - Regulation of Directional Migration of Osteoclasts and Their Precursors by Receptor Activator of Nuclear Factor β Ligand (RANKL)

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Osteoclast is a bone cell responsible for physiological bone resorption, as well as abnormal bone destruction in different disorders such as osteoporosis, rheumatoid arthritis and periodontitis. Reaching the locations that require remodelling is important for the proper execution of osteoclast function and resulting maintenance of bone quality. The goal of this study was to examine and compare the directional migration of osteoclasts and their precursors towards the key osteoclastogenic cytokine RANKL, which is also known as an osteoclast chemoattractant. In addition, we investigated how the presence of resorbable substrates affects directional migration of osteoclasts. Mature osteoclasts generated from RAW 264.7 mouse monocyte cell line or untreated RAW 264.7 cells, were transferred to calcium phosphate coated or uncoated tissue culture plates and either medium or RANKL at different concentrations was applied through a glass micropipette as a local source of a chemoattractant. The cells were incubated for 24h and the difference between the initial and final cell position was analyzed. In all the experiments, 80-95% of osteoclasts were motile over the observation time. When presented with the medium, osteoclasts exhibited equal probability of movement toward or away from the source. However, in the presence of the source of RANKL (50 ng/ml slowly delivered), 73 ± 2% of osteoclasts moved towards the direction of the source. These numbers further increased to 82 ± 3% in the presence of 100 ng/ml RANKL. For untreated RAW 264.7 cells, the maximum migration reached at 50 ng/ml RANKL and significantly decreased with the increase in RANKL to 100 ng/ml. Addition of RANKL decoy receptor osteoprotegerin (OPG) to the culture dish abolished RANKL-induced migration. When osteoclasts were plated on the calcium phosphate substrates, in control conditions 70 of 89 osteoclasts (78%) were motile and 36 of 89 (40%) were resorbing. However, only 20% of motile cells moved in the direction of the micropipette. In the presence of the local source of RANKL (50 ng/ml), the number of resorbing cells were significantly increased to 61 of 109 (56%). While the total numbers of motile cells was not changed (78 of 109, or 71%), the number of cells moving in the direction of RANKL source increased to 69%. Thus, our data demonstrate that RANKL induces directional migration of osteoclasts and their precursors. Moreover, even when osteoclasts are engaged in resorption, they move and respond in changing directionality in the presence of the RANKL source.

P31 - Prostaglandin D2 Induces Apoptosis of Human Osteoclasts Through the Regulation of PI3K/Akt and MAPK/ERK Signaling Pathways

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Prostaglandin D2 (PGD2) is a lipid mediator, which functions by activating two specific receptors: the D-type prostanoid (DP) receptor and chemoattractant receptor homologous molecule expressed on T-helper type 2 cells (CRTH2). Our previous findings have been shown that PGD2 induces osteoclast (OC) apoptosis through the activation of CRTH2-dependent intrinsic apoptotic pathway. The objective of this study is to determine the intracellular signaling mediating PGD2-induced OC apoptosis. Human OCs were generated through the differentiation of human peripheral blood mononuclear cells in the presence of rhRANKL (50 ng/ml) and M-CSF (10 ng/ml) for 21 days, and then treated with PGD2 and its agonists/antagonists. Caspase-3 fluorogenic substrate assay and immunoblotting were performed to determine caspase-3 activity and key proteins involved in phosphatidyli nositol3-kinase (PI3K)/Akt, mitogen-activated protein kinase (MAPK)/extracellular-signal-regulated kinase (ERK) and NF-κB signalling pathways. Human differentiated OCs were pretreated with a specific PI3K inhibitor (LY294002, 1 μM) and a specific inhibitor of MAPK/ERK kinase (MEK)-1/2 (U0126, 1 μM) for 30 min prior to the treatment of PGD2 and its agonists/antagonists for 24 h. We found that 10 nM of both PGD2 and CRTH2 agonist (DK-PGD2) treatments decreased the phosphorylation of Akt at Ser473, whereas the phosphorylation of Akt (Ser473) was augmented by CRTH2 antagonist CAY10471 (10 nM) in OCs. Treatment of OCs with a PI3K inhibitor LY294002 further reduced Akt phosphorylation, but increased caspase-3 activity caused by PGD2. A significant decrease in the phosphorylation of ERK (Tyr204) was also observed in OCs after treatment with 10 nM of PGD2 or CRTH2 agonist (DK-PGD2). Moreover, the treatment of OCs with a specific MEK-1/2 inhibitor (U0126) further reduced ERK phosp horylation at Tyr204, but increased caspase-3 activity in the presence of PGD2 incubation. Interestingly, treatment with DP agonist (BW 245C, 10 nM) or DP antagonist (BW A868C, 10 nM) did not have any effect on either Akt or ERK phosphorylation in OCs. Furthermore, the treatment of OCs with 2 nM and 10 nM of PGD2, DP agonist (BW 245C), CRTH2 agonist (DK- PGD2), DP antagonist (BW A868C), or CRTH2 antagonist (CAY10471) did not alter the phosphorylation of RelA/p65 on Ser536. These results suggest that PGD2 promotes CRTH2-dependent apoptosis in human differentiated OCs by activating the PI3K/Akt and MAPK/ERK, but not NF-κB signaling pathways.

P32 - Finite Element Contact Analysis of Axisymmetric and Non-Axisymmetric Radial Head Hemiarthroplasty

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INTRODUCTION: Treatment of radial head (RH) fractures often requires the use of a radial head hemiarthroplasty. These stiff metallic implants articulate with the native cartilage of the capitellum and ulna and reduce radio-capitellar joint contact to approximately one-third of the natural contact area. The resulting changes in cartilage contact stresses could cause degradation and erosion. The natural RH is elliptical in the transverse plane; however, most current implants are axisymmetric. Recently, more anatomical (non-axisymmetric) geometries have been developed in an effort to reduce damage to the native cartilage. The purpose of this study was to compare axisymmetric and non-axisymmetric RH prosthesis geometries in terms of joint contact area and cartilage stress using a finite element model. Our hypothesis was that the non-axisymmetric RH would have improved articular contact mechanics.

METHODS: A finite element model was developed in ABAQUS with a natural capitellum (extracted from CT data) articulating against a concave RH hemiarthroplasty prosthesis having either an axisymmetric (spherical) or non-axisymmetric (elliptical) contact geometry at various RH orientations. Our model contact areas were within 5% of those obtained experimentally. The axisymmetric RH had a 20.4mm concave radius (Size=24mm). The non-axisymmetric RH had a maximum radius of 20.4mm and a minimum radius of 15.7mm to approximate the natural capitellum. Maximum stress, contact pressure, and contact area were computed for a constant 100N joint load.

RESULTS: It was apparent that the natural capitellum was non-axisymmetric because a circular contact area was not generated when articulating with the axisymmetric RH. The contact area of the non-axisymmetric RH was 22% larger than the axisymmetric RH when all orientations investigated were considered, and the shape of contact was much more elongated compared to the axisymmetric RH. Although the average contact stress was reduced for all rotations, certain RH orientations had higher peak cartilage stress compared with the axisymmetric design.

DISCUSSION: Our results show that a non-axisymmetric RH hemiarthroplasty can increase contact area, reduce average contact stress, and in some positions reduce peak cartilage stress. For some forearm rotations, however, there was not a consistent reduction in peak cartilage stress due to non-conformity with the natural (and also non-axisymmetric) capitellum. Further studies are needed to ascertain the effect of subtle changes in both shape and size of RH prostheses on the load transfer mechanics to cartilage. These studies will help to determine optimal implant design and in-vivo orientation to support long-term cartilage health after radiocapitellar hemiarthroplasty.

P33 - Tension in the Anterior Cruciate Ligament from Multiparametric Magnetic Resonance Imaging

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Introduction: Over the last years, techniques to quantify MRI parameters of biological tissues have been developed to assess their composition and structure. Even fibrous tissues with short transverse relaxation times can now be measured with acceptable examination time.

Objective: The goal of this study is to quantify the tension of the anterior cruciate ligament (ACL) from its MR relaxation properties to help evaluate the outcome of a reconstructive surgery. The hypothesis is that the relaxation properties of the human ligaments are affected by a change in the tension, more specifically in the ACL.

Method: 10 volunteers with healthy knees who gave their informed consent and were approved by the ethics committee of our institution were imaged using a Philips 3T MRI system. The acquisition consisted of T1ρ (T1TFE with 5 different spinlocks, 1, 10, 20, 30, 40 ms), T2 (T1TFE with 5 different echo times, 1, 3, 7, 14, 31 ms) and T2*(mTFE with 8 different echo times, 1.5, 6.5, 11.5, 16.5, 21.5, 26.5, 31.5, 36.5 ms) sequences. A first acquisition was performed with the knee at full extension followed by a second acquisition with the knee at 25 degrees of flexion to ensure a significant change in the tension in the ligament. The mean T1rho, T2 and T2* were calculated on one slice where the ACL was the most present using an active contour algorithm to segment the ligament. A paired Student T-test was performed between the extended and flexed knee.

Results: On the first two subjects, the mean T1rho was equal to 40.34 ± 0.68 in extension and 36.85 ± 1.68 in flexion, the mean T2 was equal to 24.38 ± 3.83 in extension and 24.69 ± 1.08 in flexion and the mean T2* was equal to 18.28 ± 1.78 in extension and 16.15 ± 0.20 in flexion. A difference of 15% ($p=0.1$) between the extended and flexed knee was found for T1rho. However, no differences were found for T2 and T2*.

Conclusion: T1rho seems to be a better candidate than T2 or T2* to evaluate the tension in the ACL. Further development are needed to explore other MR parameters such as T1 or the magnetization transfer (MT). Refined regions of interest in the ligament would be of interest to study the distribution of the stress within the ACL. Positive results could lead to interesting diagnosis and evaluation technique for orthopaedics.

P34 - Computational Modelling to Predict Cartilage Contact Mechanics

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Introduction: An understanding of the contact mechanics of human joint is an important precursor in the study of cartilage wear. Experiment-based techniques for measuring contact area and contact stress are invasive, and unable to provide accurate measurement of contact stress. Finite element (FE) modeling techniques can overcome some of the limitations of experimental methods, and are capable of predicting contact area and pressure. The purpose of this study was to develop a 3D FE joint contact model, and validate the model by comparison against matched experimental data. The sensitivity of model predictions to material properties and cartilage thickness was also investigated. We used the human elbow for this investigation.

Methods: A cylindrical constrained elbow joint loading apparatus was used to measure the cartilage compression across a cadaveric elbow specimen under 80 N of load. The contact area was measured using a casting method. A computer model of the same specimen was created based on computed tomography (CT) images. The same loading conditions were simulated using FE contact analysis in ABAQUS. Cartilage was modelled as a hyperelastic material, and bone was modelled with non-homogenous material properties. The FE model was then used for a parametric study, investigating the stiffness of bone (rigid or deformable), the coefficient of friction between cartilage surfaces (0.01, 0.1, or 0), the stiffness modulus and Poisson's ratio of cartilage (increased or decreased by one standard deviation), and the cartilage thickness distribution (physiological, or uniformly distributed at 0.85, 1.14, or 1.43 mm).

Results: The model-predicted joint compression and contact areas were in good agreement with the experiment-measured values (-4.9% and-3.3%, respectively), and the contact patterns were also in agreement. Assuming osseous structures were rigid decreased contact area and increased contact pressure. Increasing cartilage stiffness caused contact area to decrease, although friction and Poisson's ratio had little effect on the results. Assuming a uniform cartilage thickness greatly increased contact area, and increasing cartilage thickness caused increased contact pressure due to joint over-stuffing.

Conclusion: This study demonstrates the ability of a computational FE model to reliably predict elbow cartilage contact mechanics. The results underline the importance of using accurate material properties and physiological cartilage thickness distributions. This modelling technique should prove useful to assess joint contact mechanics under different loading scenarios and specimen geometries, and to predict the changes in contact mechanics as a result of joint disease such as arthritis and surgical procedures including re-alignment and hemiarthroplasty.

P35 - Development of a Novel Cell Contraction Test for High-Throughput Screening Applications

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Background: Contraction of individual cells plays a major role in promoting crucial body functions such as wound healing by contractile fibroblasts and vessel contraction by smooth muscle cells. The regulation of cell contraction is vital for the tensional homeostasis of healthy tissue; dysregulation is associated with disease states such as fibrosis and chronic hypertension. Our lab has identified an increasing demand of pharmaceutical companies for a functional in vitro test to evaluate compounds that target cell contraction. None of the currently available techniques are efficient, quantitative on large cell populations, or reproducible. Specialized methods such as traction force microscopy and are limited for use in specialized laboratories and incompatible with high-throughput methods.

Objective: To engineer a novel quantitative assay system which tests cell contractility in a high-throughput format that will be suitable for use on a variety of different cell types.

Methods: We build upon an existing principle to quantify cell contraction by detecting visible "wrinkles" in silicone substrates. As improvement over previously used silicone oil substrates, we implemented a two-component silicone elastomer material that is fully polymerized. This polymer was tuned to different stiffnesses to accommodate differently contractile cell types. We further adjusted the polymer surface properties to enhance the visibility of cell-generated wrinkles for automatic image analysis and allow cell attachment. The assay was translated from a single dish to a multi-well assay. Proof-of-principle experiments were performed with contractile primary rat lung fibroblasts between passages 3-6.

Results: To produce optically clear substrates, we increased vacuum times to reduce polymer bubbles and decreased polymer thickness to achieve an ideal focal range for microscopy. Second, with the constant thickness we modulated bulk polymer stiffness and found the maximum stiffness at which contractile cells created wrinkles. With these substrates, cell-generated wrinkles can be achieved for up to one week. We overcame the edge effect in the multi-well plates using a 96-well plate with a detachable bottom that was coated with the polymer before assembly. We used ImageJ to quantify the proportion of wrinkling cells in each image.

Conclusion: Once finished, this novel system will measure cell contractility that has application in both basic research and for time-effective high throughput processing used in industrial drug testing.

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P36 - Low-Frequency High-Magnitude Mechanical Stimulation Inhibits Neuronal Differentiation of PC12 cells.

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Mechanical stimulation can influence stem cell differentiation and may therefore provide improved lineage specification control for clinical applications. Low-frequency cyclical mechanical stimulation (0.01Hz) has recently been shown to suppress adipogenic differentiation of mesenchymal stem cells and modulate osteogenic differentiation of C2C12 myoblasts indicating that effective stimulation frequencies are not limited to those associated with locomotion, circulation, and respiration. We hypothesized that very low-frequency mechanical stimulation (0.001Hz) coupled with high magnitude strain (20%) can also influence neuronal differentiation of PC12 cells. Cells were cultured on either static plates coated with modified silicone (SS culture) or dynamically on modified high-extension silicone rubber (HESR) dishes subjected to the cyclical stimulation (DY culture) with or without addition of neuronal growth factor (NGF). No differences in viability or differentiation potential were observed between PC12 cells cultured on silicone or tissue culture plastic. Neurites (axon-like projections) appeared more rapidly and robustly (multiple projections) in DY culture. However, DY culture resulted in noticeably shorter neurites which strongly correlated with a significant reduction in neurofilament light chain (NF-L) gene expression. MAPK analysis showed that these effects may be mediated by ERK and p38 MAP kinase activity. These data provide insights for a technically simple means for mechanical control of neuronal differentiation.

P37 - Acute Exposure to Whole Body Vibration Significantly Alters Intervertebral Disc Homeostasis.

Presenting author: Priya Patel

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Objective: Degeneration of the intervertebral disc (IVD) is associated with chronic low back pain and current therapies are considered inadequate. Whole-body vibration platforms have been adopted for clinical use in the treatment of back pain, however, there are limited studies investigating the direct effects of vibration on IVD cells. We therefore sought to examine the effects of single exposure to acute vibration on gene expression and protein levels within the IVD.

Methods: Ex vivo and in vivo mouse models were developed to study changes induced by acute vibration using custom-designed platforms. Spinal segments (ex vivo) or mice (in vivo) were subjected to vibration (30 min, 15-45 Hz, 0.3 g) and IVDs were harvested at specific times post-vibration. Gene expression was quantified using real-time PCR and protein levels were examined by quantitative mass spectrometry and immunofluorescence.

Results: In the ex vivo model, acute vibration at 15 Hz induced expression of anabolic genes (aggrecan, biglycan, decorin, type I collagen, Sox9) and suppressed expression of Mmp13, with the most pronounced changes detected 6 h following vibration. In vivo, the effects on anabolic gene expression were even more robust and were accompanied by decreased expression of Adamts4/5 and Mmp3. Moreover, significant increases in the protein levels of type I collagen biglycan, decorin and aggrecan were detected in vivo.

Conclusions: These findings demonstrate dramatic anabolic effects of acute vibration on IVD tissues. The similarity of responses in vivo and ex vivo suggest that most, if not all, of these effects arise through the direct actions of vibration on spinal tissues. Further studies are necessary to determine if this transient effect has a long-term benefit to intervertebral disc health.

P38 - Micromechanics of Intervertebral Disc Degeneration

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Background

The intervertebral disc (IVD) is a complex biphasic tissue with poro-viscoelastic properties and a highly non-linear mechanical behavior. As the composition and structure varies with region, so do the mechanical properties. Previous studies have examined changes in IVD properties related to the severity of degeneration using conventional mechanical and biochemical techniques. Although an evaluation on the regional properties of the IVD is of interest, few studies have addressed the relationship between the ultrastructural properties and micromechanical behavior of the IVD with degeneration. The objective of this study was to establish a novel protocol to characterize IVD microstructural properties with degeneration.

Methodology

Bovine tails of four different ages (12, 18, 24, 30 months) were obtained from the abattoir2-3 hours after slaughter. The first two IVDs of each tail were separated from their adjacent vertebral bodies by removing the bone and retaining the cartilaginous endplates. Sagittal sections (20 μm) were cut from each sample using a cryostat. Consecutive slices were assessed for rheological, chemical and histological properties. Several preliminary conditions were tested (hydration, fixation, glass substrate) in order to optimize our experimental conditions.

A creep test (peak force: 500μN, holding time: 50s) was carried out on Hysitron Triboindenter™ TI 950 using a fluid-cell conical diamond tip (<1μm) on different areas in order to assess the IVD viscosity. The results of the creep test were analyzed using a custom written Matlab code that allows us to investigate the appropriate rheological model for the IVD. A FTIRM analysis (FTS 7000 series' DGILAB' with UMA 600 microscope) was completed on the same areas targeted by nanoindentation. Finally, a spatial map of the micromechanical properties distribution in relation to our histological and chemical evaluation was traced for each section.

Results and discussion

We identified micromechanical properties that reflect IVDs complex mechanical behavior. Hence, a predictive mathematical model can be built on these bases. IVD degeneration is the most common cause of back pain. An understanding on the effects of local tissue material properties is essential for developing finite element models of IVD degeneration, and in evaluating the effectiveness of regenerative strategies.

P39 - Design and Fabrication of a Microfluidic Chamber for Live-Cell Imaging During Pulsatile Fluid Shear.

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Introduction: Mechanotransduction is the process by which cells sense â€“ and respond to â€“ 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. One of the most commonly implicated forms of mechanical stimulation 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 constructed from polydimethylsiloxane (PDMS) using multilayer soft lithography. 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 300 -µm m wide by 500 -µm m tall. A 13.5% saline solution containing 6-µm diameter polystyrene beads was pumped through the chambers. Imaging of the channels under flow was performed using an inverted microscope and high-speed digital camera (300 -1200 frames per second). Flow parameters were calculated by micro-particle imaging velocimetry, using the polystyrene beads as markers. Chambers were disinfected with 70% ethanol for 1 hour and sterilized by treatment with UV overnight. Following sterilization, chambers were pretreated overnight with medium containing 50% fetal bovine serum to enhance cell attachment. MC3T3-E1 osteoblast-like cells were then seeded into the chamber and imaged.

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 shear stresses of up to 3 Pa. Transient pulsatile waveforms have been generated using mechanical impulses and a range of frequencies, from 1-90 Hz, were observed within the channel. In addition, MC3T3-E1 cells adhered and survived within the microfluidic chamber for at least 24 h.

Conclusions: We have developed, fabricated and tested a microfluidic system capable of delivering physiologically relevant fluid shear stresses, under steady flow conditions and transient pulsatile waveforms with components up to 90 Hz. Such stresses can be applied to a chamber capable of hosting live cells, which can be imaged while experiencing controlled flow-induced shear stress. This system will enable future studies involving microscopic imaging of cellular reactions to pulsatile fluid shear.

P40 - Altered Osteocyte Mechanosensitivity in Response to Elevated Extracellular Glucose Levels

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Introduction: Hyperglycemia contributes to resulting in bone loss, and an elevated risk of bone fracture in diabetic patients. The effects of hyperglycemia on osteocyte mechanosensitivity and skeletal pathology are unknown. Osteocytes are bone cells that detect mechanical stimulation, and regulate osteoblasts and osteoclasts via paracrine pathways. This study tests the hypothesis that hyperglycaemia suppress the effects of mechanical loading (fluid shear) on osteocytes. We will examine secretion of anabolic cytokine, PGE2, catabolic cytokine RANKL and osteocyte apoptosis, which has been found to play an important role in initiation of bone remodeling. The cells will be under hyperglycemia conditions with and without fluid shear stress loading.

Methods: MLO-Y4 cells were seeded on rat tail type I collagen (BD Biosciences) coated slides at a density of 3000 cells/cm2 and incubated for 72 hours in one of three media: i) Normglycemic control medium (regular alpha-MEM culture medium containing 5.5mM glucose), ii) osmotic control medium (regular medium supplemented with 20 mM of D-mannitol (Sigma)); and iii) hyperglycaemic medium (regular medium supplemented with 20 mM D-glucose (Sigma)). Slides were placed in parallel plate flow chambers and subjected to 10 dynes/cm2 of oscillating shear stress at 1Hz for 2 hours. Then they are incubated at 37C with fresh media. Media were collected after 24 hours of incubation. Protein Quantification: ELISA (R&D Systems) was used to quantify RANKL in media. PGE2 levels were quantified using EIA assay (Cayman). Total protein was determined using a colorimetric kit (Pierce) and lysis buffer (Cell Signalling Tech) Apoptosis Measurement: Caspase 3/7 level was measured with fluorescent kit (Promega).

Results: Osteocytes in the normglycemic or mannitol-supplemented media showed decreased apoptosis induced by fluid flow, which was abolished in hyperglycaemic medium. In normglycemic and osmotic control media, fluid flow resulted in decreased secretion of RANKL by osteocytes, which was inhibited in hyperglycaemic medium. PGE2 production increased in fluid flow conditions in normglycemic and osmotic control media was suppressed in hyperglycemia media.

Discussion: This study investigated how hyperglycaemia affected protein expression of markers involved in bone remodeling in osteocytes under mechanical loading. Hyperglycemia abolished mechanical loading induced changes in RANKL and PGE2 levels released by MLO-Y4 cells. Osmotic controls did not exhibit the same effect on osteocytes, suggesting hyperglycaemic condition may have direct effects on osteocytes, adversely influencing osteocyte's ability to respond to mechanical loading. This effect may be responsible for the impaired bone structure and properties seen in diabetic patients.

P41 - Elastin-Based Biomaterials: Contributions to Mechanical Properties from Sequence and Structure

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Elastin is the self-assembling extracellular matrix protein that provides elasticity to tissues. A fundamental requirement for forming elastomeric materials is retention of a high degree of conformational disorder even when aggregated. Elastin disorder is strongly related to a high (50%) combined proportion of proline and glycine residues within hydrophobic domains. The majority of elastin hydrophobic domains have an average proline spacing of 4-8 residues. However, the native sequence of hydrophobic domain 30 (D30) is uncharacteristically proline-poor. Here we investigated the contribution of D30 and variants to the self-assembly and material properties of elastin-mimetic biomaterials. Addition of native D30 substantially stabilized the surface of assembled aggregates compared to proline-rich controls, suggesting the potential for interfacial order in mediating droplet growth, rigidity and interactions. Materials were stiffer, consistent with a greater number of contacts between monomers, were less resilient, and displayed less internal resistance to force. Conversely, mechanical properties were restored upon addition of the longer and glycine-rich rat D30, suggesting an important contribution to conformational entropy from domain length and high glycine content. Taken together, we hypothesize structured motifs and cross-linking density play key roles in modulating elastin assembly and mechanics.

P42 - Cartilage Boundary Lubricating Ability of PRG4 Monomers versus Multimers: Effect of Inter- and Intra-Molecular Bonds

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Introduction: Proteoglycan 4 (PRG4), also known as lubricin, is a mucin-like glycoprotein that exists in synovial fluid (SF) and at the surface of articular cartilage. PRG4 exists as monomeric and disulfide-bonded multimeric forms and functions as a critical boundary lubricant necessary for joint health. The ability to form disulfide-bonded multimers is in general a functionally determinant property for many mucins, and may be for PRG4 as well. Indeed, reduction/alkylation (R/A) of PRG4, which breaks both intra- and inter-molecular disulfide-bonds, decreases its cartilage lubricating ability by ~34% and also inhibits its ability to bind to cartilage surfaces. The lubricating properties of non-reduced (NR) PRG4 monomers (PRG4-mono) alone, compared to PRG4 multimers (PRG4-multi), remains to be determined. The objectives of this study were to prepare and characterize NR PRG4-mono and PRG4-multi, and to determine their cartilage boundary lubricating ability.

Methods: PRG4 was prepared by purification from media conditioned by cartilage explants from bovine stifle joints. Enriched NR PRG4-mono and -multi were prepared using size exclusion chromatography with a non-denaturing buffer, containing 0.25% CHAPS. PRG4 samples were then dried and prepared at a physiological concentration of 450 μg/mL in phosphate buffered saline (PBS). Some PRG4-mono was then R/A. Enriched samples were characterized using Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) analysis. Each lubricant was also assessed using an in vitro cartilage-on-cartilage friction test under boundary lubricating conditions. PBS and bovine synovial fluid (SF) served as negative and positive controls, respectively.
Test sequence 1 (n=7): PBS, PRG4-multi, PRG4-mono, SF
Test sequence 2 (n=4): PBS, PRG4-mono (NR), PRG4-mono (R/A), SF

Results: Enriched samples of NR PRG4-mono and –multi were successfully obtained and confirmed by SDS-PAGE. PRG4-mono and -multi functioned as effective friction-reducing cartilage boundary lubricants. <μkinetic,Neq>, the resistance to steady motion, values for PRG4-mono (0.15±0.01) and PRG4-multi (0.09±0.02) were significantly different than both PBS and SF (both $p<0.01$), with PRG4-multi being significantly lower than PRG4-mono ($p=0.02$). PRG4-mono (NR) (0.12±0.01) and PRG4-mono (R/A) (0.11±0.02) were not significantly different from each other ($p=0.974$).

Conclusions: These results demonstrate the cartilage boundary lubricating ability of PRG4 is dependent, in part, upon its disulfide-bonded multimeric structure. The friction coefficients for PRG4-multi are similar to those reported for non-separated PRG4, and values for PRG4-mono are similar to those reported for both R/A and denatured PRG4, at similar concentrations. Higher order protein structure and disulfide bonding, in particular inter-molecular, appear to be necessary for PRG4â€™s cartilage boundary lubricating ability.

P43 - Chitosan Trapping of Anionic Coagulation Factors During Soluble-Microparticle Transition

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Purpose: Chitosan is a polysaccharide used to stabilize blood clot implants for augmented marrow stimulation therapy for cartilage repair. Chitosan contains positively charged glucosamine residues, fluctuates between a soluble state at pH 6 and an insoluble microparticle state at neutral pH, and delays coagulation through mechanisms that remain unclear. We hypothesized that upon mixing acid-soluble chitosan with blood plasma, cationic chitosan chains tether anionic coagulation factors through their acid-rich γ-carboxyglutamic acid (Gla) domain and, during chitosan soluble-microparticle transition, isolates them from soluble factors, hindering the velocity of the coagulation cascade.

Methods: Pull-down assays were carried out with samples consisting in human citrated plasma mixed with isotonic saline or an isotonic chitosan solution pH 4.5 (80% degree of deacetylation, DDA, or 98%DDA). Samples were recalcified and allowed to coagulate at 37ºC in glass tubes. At specific intervals, the reaction was stopped in quench buffer pH 7.2, centrifuged to recover the supernatants and the insoluble pellets were washed 3 times with quench buffer. Proteins adsorbed to chitosan were eluted with 8M urea. Western blot analyses used antibodies directed against the F1.2 prothrombin fragment and alpha-thrombin. Direct binding of chitosan and prothrombin, which contains an anionic Gla domain, and thrombin, was assessed by surface plasmon resonance (SPR). Clot time and clot tensile strength was measured by thromboelastography, using glass beads to reproducibly initiate coagulation.

Results: Recalcified citrated plasma showed reproducible burst coagulation at 7 to 9 minutes, while recalcified chitosan/plasma mixtures initiated coagulation after a 30 minute delay, followed by a highly unique linear increase in clot tensile strength during 120 minutes of coagulation. Plasma-only showed rapid and complete splitting of prothrombin to alpha-thrombin and the F1.2 fragment (which retains the Gla domain) in the soluble phase. In chitosan/plasma mixtures, prothrombin and F1.2 became associated with the insoluble pellet fraction starting at 15 minutes, simultaneous with alpha-thrombin release to the soluble fraction. SPR analyses revealed that prothrombin bound more strongly to non-biodegradable 98%DDA chitosan than biodegradable 80%DDA chitosan. This higher chitosan-prothrombin affinity was paralleled by a lower clot tensile strength.

Conclusion: This study demonstrates the novel finding that chitosan strongly interacts with Gla domain-containing prothrombin and F1.2 fragment, and interferes with fibrin polymerization. Evidence of tethering of prothrombin to chitosan represents a significant step in the elucidation of chitosan-induced coagulation delay and suggests interactions with other Gla-containing coagulation factors.

P44 - Particulate and Protein Network Occlusion Into Calcite Mineral

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Otoconia of the human inner ear are calcium carbonate-containing mineralized structures involved in maintaining balance and in detecting linear acceleration. In addition to their calcitic mineral, otoconia contain abundant proteins including osteopontin (OPN). OPN in mineralized tissues regulates crystal growth, where it can be found in two general forms − as monomers, or as polymers crosslinked by transglutaminase 2. Protein related to mineral can either be surface-bound, or occluded within crystals. To study occlusion into calcite and better understand how otoconia are formed in the inner ear, we have examined calcite crystal growth in the presence of particulates (micro- and nanospheres) and OPN protein monomers and polymers. Calcite crystals were grown in OPN monomer and polymer (crosslinked by transglutaminase 2) solutions, or with added microspheres (0.6-0.86 μm) having different surface chemistries and charge. Polystyrene microspheres were either nonfunctionalized, amine-functionalized, or high- and low-carboxyl-functionalized. Nanometre-sized (14 nm) protein A-gold nanospheres were also used. Scanning electron microscopy revealed that monomer and polymer OPN alter the shape of {104} calcite faces leading to altered crystal morphology with increased steps, kinks and pits. Micro-Raman spectra collected from these crystals revealed a broad peak between 2850 and 3000 cm-1, arising from organic material occluded into the crystals. In the microsphere studies, no association of calcite and nonfunctionalized microspheres were observed, whereas positively charged microspheres showed lower binding to calcite compared to the negatively charged microspheres. Microspheres having a high density of carboxylate groups had a strong interaction with calcite that resulted in the formation of oblate spheroids, similar to the structure observed for human otoconia. Protein A-gold nanospheres showed preferential binding to modified regions of {104} calcite faces. In summary, these data demonstrate particulate and OPN protein occlusion into calcite, providing insight into growth processes for otoconia. Funded by CIHR.

P45 - Effect of Exogenous Fluoride on Ex Vivo Bone and Collagen Material Properties

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Undergraduates (summer students: KN, NL, GRS, MMR), a post-doctoral fellow (MBA), and professors (SO, FV) all contributed in and out of the lab to this project.

Bone is comprised of a compliant collageneous matrix, brittle apatite mineral crystals, and a suite of non-collageneous proteins (NCPs) that together result in a tough composite material. On a molecular scale hierarchical level, the composite material properties of bone depend on the quality of the mineral and collagen components, as well as their interactions at the mineral-collagen interface. The mineral component of bone is measured by "bone mineral density" (BMD). BMD is routinely quantified in clinics by DEXA (dual energy x-ray absorptiometry), and is often used as a proxy for fracture risk. However, BMD does not detect changes in the organic components (collagen and NCP's) of bone. To date, destructive tests such as 3-point bending and microhardness, and the non-destructive mechanical response tissue analyzer (MRTA) can detect changes in bone material properties caused by changes in the organic components.

In this study, the role of the mineral-collagen interface bond on bone material properties was examined. It was assumed that the interfacial bond between bone mineral and collagen is electrostatic, with the slightly positive bone mineral surfaces attracting the electronegative ends of some NCP's, while the other NCP regions interacted with the surrounding collagen. It was hypothesized that the small fluoride ion (F-) would migrate to the positively charged bone mineral surface, and disrupt the interfacial bond between bone mineral and collagen. Partial endocortical F- infusion should not affect the bone mineral, and therefore not affect the BMD, or fragment/denature collagen. However, it might reduce the mineral-collagen interface bond quality within the endocortical tibial volume, which was hypothesized to reduce post-yield material properties and endocortical microhardness.

It was hypothesised that the role of this interfacial bond in bone material properties could be tested with an ex vivo emu tibial model by partial infusion with sodium fluoride solutions, followed by BMD analysis, 3-point bending, collagen biochemical analysis, and microhardness across the tibial cross-section. Emu tibiae were partially endocortically infused with 0, 0.05, 0.1, or 1 M NaF at neutral pH for two weeks. The BMD, elastic modulus, yield stress/strain, ultimate stress, and failure stresses were unchanged by F- treatment. Collagen was not fragmented by F- treatment as shown by SDS-PAGE. However, the observed increased post-yield strain and failure strain, coupled with a decreased endocortical hardness of the F-treated bones, suggest a possible role for mineral-collagen interface strength on bone material properties that is not detected by DEXA.

P46 - Analysis of Human Mesenchymal Stem Cell Interactions to the Hydrophobic/Hydrophilic Surfaces Coated with Competence Stimulating Peptide

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P15 is a collagen biomimetic peptide that promotes osteogenesis, while CSP is an amphiphilic cationic anti-bacterial peptide with potential use in dental bone repair procedures, where bacteria are prevalent. We tested the hypothesis that human mesenchymal stem cells (hMSCs) adhere more to hydrophobic surfaces coated with CSP, or fusion peptide P15-CSP, compared to P15. Primary low-passage hMSCs were trypsinized, allowed to adhere for 1 hour in non-tissue culture-treated 96-well plates, or polystyrene pre-coated with P15, CSP or P15-CSP, washed with PBS and the bound cells quantified. It was found that CSP and P15-CSP, when immobilized by simple adsorption to the plastic, significantly accelerated serum-free adhesion and enhanced seeding efficiency of hMSCs. In contrast, P15 still supported cell adhesion above that of uncoated plastic, although to a lesser extent than CSP. Cell morphology showed extensive cell spreading in these three pre-coated conditions. In addition, when exposed to serum-containing osteogenic medium, hMSCs survived and differentiated in three pre-coated conditions. These results suggest that CSP and P15-CSP peptides can be conveniently used for coating various biocompatible surfaces including implant materials for bone regeneration, and thus may find potential applications whenever a fast and efficient seeding of MSCs is required onto scaffolds in the absence of serum.

P47 - The ED-A Domain in Cellular Fibronectin Provides a Binding Site for the Latent TGF-β1 Binding Protein

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Background: The ED-A splice variant of fibronectin is neo-expressed in fibro-contractive disorders. Secretion of ED-A fibronectin and stiff extracellular matrix (ECM) are prerequisites for the differentiation of fibroblasts into fibrogenic myofibroblasts. The mechanism of how ED-A fibronectin contributes to myofibroblast differentiation and fibrosis is unknown. We hypothesize that ED-A fibronectin acts as an extracellular store for the pro-fibrotic cytokine TGFβ1 by binding to the latent TGFβ1 binding protein (LTBP-1). Objective: To elucidate whether the ED-A domain of fibronectin enhances myofibroblast differentiation by preferentially recruiting latent TGF-β1 to the myofibroblast ECM.

Methods: To provide myofibroblast-suppressing and -permissive conditions, we cultured human dermal fibroblasts on collagen-coated silicone substrates with stiffness of 5 kPa (normal-soft), 50 kPa (fibrotic-stiff), and 3,000 kPa (tissue culture plastic stiff) for 7 days ±TGFβ1. To determine the binding of LTBP-1 to ED-A fibronectin, fibroblasts were treated with two antagonists specific to the ED-A domain (IST-9 and rED-A) and solid phase binding assays were performed with recombinant fibronectin peptides. Using confocal microscopy and Western blotting, we analyzed co-expression and organization of ED-A fibronectin and LTBP-1. Expression of α-smooth muscle actin (α-SMA) was indicative of myofibroblast differentiation.

Results: Without addition of TGFβ1, ED-A fibronectin and LTBP-1 expression were both higher on stiff than on soft substrates; expression of α-SMA was low in both conditions. Addition of TGF-β1 resulted in an expression level increase of all three proteins on stiff substrates. In these conditions, LTBP-1 and ED-A fibronectin always co-localized. Specific blocking the ED-A domain resulted in the loss of LTBP-1 colocalization with ED-A fibronectin in the ECM. Western blotting demonstrated that ED-A antagonist treatment led to translocation of LTBP-1 from the ECM to the medium supernatant. Solid phase binding experiments revealed that the ED-A domain in fibronectin interacts with LTBP-1, with the 11th and 12th flanking domains of ED-A in the fibronectin molecule enhancing this interaction.

Conclusion: ED-A fibronectin serves as a preferential storage site for LTBP-1 in the myofibroblast ECM. Specific inhibition of LTBP-1-ED-A interactions using ED-A antagonists prevents storage of latent TGF-β1 and ultimately interrupts the autocrine loop of local TGF-β1 activation and myofibroblast differentiation.

P48 - Functions of Fibronectin and Heparan Sulfate in Microfibril Assembly and Homeostasis

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Fibrillins constitute the major backbone of multi-functional microfibrils in elastic and non-elastic extracellular matrices. Proper expression, assembly and homeostasis mechanisms are central to the formation and function of these microfibrils. These properties are often compromised in pathological situations such as Marfan syndrome and other fibrillinopathies caused by mutations in fibrillins. In the present study, we report on the role of fibronectin and heparan sulfate proteoglycan in the assembly and homeostasis of fibrillins. We demonstrate through peptide inhibition studies in human dermal fibroblasts, the requirement of a preassembled fibronectin network for fibrillin-1 network assembly and homeostasis in early stages. We observed that fibronectin is present in extracted mature microfibrils from cells and mouse tissues as well as in intermediate molecular weight structures which we hypothesize as being immature microfibrils. Through electron microscopy and immunofluorescence studies, we observed some extracellular fibers co-labeled for both fibronectin and fibrillin-1 while other fibers are labeled individually for either protein. Those results indicate a non-transient mechanism for the involvement of fibronectin in microfibril assembly. In this study, we further characterize the role of heparin/heparan sulfate in microfibril assembly and its interaction with fibrillins. We demonstrate that fibrillin-2 and -3 interact with heparin/heparan sulfate in solid phase binding assay and that fibrillin multimerization increases the avidity for heparin/heparan sulfate. We also show that heparin/heparan sulfate acts as a regulator of fibrillin homo- and heterotypic interactions, which are critical for microfibril assembly. However, heparin/heparan sulfate does not regulate fibrillin interaction with fibronectin. Our results determine the sequence of events leading to microfibril assembly, consolidated in a new model.

P49 - The Role of Fibrillin-1 in Skeletal Muscle Differentiation

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Fibrillins are extracellular proteins that constitute the 10-12 nm diameter microfibrils in elastic and non-elastic connective tissues. Mutations in fibrillin-1 give rise to several heritable connective tissue disorders, including Marfan syndrome (MFS) with clinical symptoms in the musculoskeletal, cardiovascular and ocular systems. Clinical complications in MFS include significant skeletal muscle hypoplasia and hypotonia throughout life. Fibrillin-1 has structural and functional roles in connective tissues, including the regulation of transforming growth factor beta (TGF-β), a growth factor that plays integral roles in cellular differentiation and cellular proliferation. Here, we hypothesize that fibrillin-1 has a central role in the development of skeletal muscle differentiation, and that mutations in fibrillin-1 negatively affect this function. In this project, we use an established mouse myoblast cell line (C2C12 cells) as a model system. C2C12 cells can be differentiated into multinucleated myotubes, recapitulating skeletal myogenesis. Purified recombinant wild-type fibrillin-1 fragments and fibronectin (FN) were exposed either as immobilized or as soluble ligands to C2C12 cells that were induced under low serum conditions for cell cycle exit and fusion of myoblasts to form multinucleated myotubes. Differentiation has been monitored by histological staining (Jenner-Giemsa). The wild-type C-terminal half of fibrillin-1 (rFBN1-C) promoted skeletal muscle differentiation, similar to the effects of FN. In contrast, the N-terminal half of fibrillin-1 (rFBN1-N) caused increased cell clustering and detachment as well as impaired myogenesis. Interestingly, rFBN1-N administered in combination with either rFBN1-C or FN conferred enhanced cell adhesion and myogenic differentiation compared to either protein administered individually. Furthermore, de-glycosylation of fibrillin-1 recombinant fragments conferred pro-adhesive and pro-differentiating properties. These data suggest that there are distinct functional domains in fibrillin-1 and in FN involved in myogenic differentiation. In the long run, this work will ultimately help in finding novel therapeutic strategies for the muscle-related symptoms in MFS.

P50 - Structural and Functional Comparison of Human and Mouse Fibrillin-1 and -2

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Background and Hypothesis: Fibrillin proteins are the major components of microfibrils and are associated with regulation of TGF-beta signaling. Mutations in this protein lead to diseases such as Marfan Syndrome and Weill-Marchesani Syndrome. There are three fibrillin proteins expressed in multicellular eukaryotes, fibrillin-1, fibrillin-2 and fibrillin-3 (Piha-Gossack et al. 2012). Human fibrillin-1 expression overlaps with that of fibrillin-2 and fibrillin-3 in some tissues in early development (Sabatier et al. 2011). Fibrillin-2 and -3 are typically not expressed after birth whereas fibrillin-1 expression persists into adulthood (Quondamatteo et al. 2002). The expression of fibrillin-3 in humans is found to be similar to the temporal expression patterns of fibrillin-2 in human fetal tissues, suggesting a role in early development. However, fibrillin-3 is not expressed in mice as the gene is inactivated due to chromosomal rearrangement (Corson et al., 2004). Therefore, some of the functional aspects associated with human fibrillin-3 are likely compensated by either mouse fibrillin-1 or -2. We hypothesize that human fibrillin-1 and -2 and mouse fibrillin-1 and -2 adopt different functions.

Experimental Procedures: To test this hypothesis, we recombinantly expressed the C-terminal half of mouse fibrillin-1 (mrFbn1-C) and mouse fibrillin-2 (mrFbn2-C) similar to already available constructs for human fibrillin-1 and -2. Recombinant expression was performed in HEK293 cells to ensure proper posttranslational modifications. Monoclonal expression clones were selected. The expressed protein was characterized by SDS-PAGE and Western-blotting. The proteins were purified by immobilized metal affinity chromatography using a fused histidine-tag. Functionally, we tested the interactions of the purified mouse mrFbn1-C and 2-C protein with heparin using solid phase interaction assays. The purified mrFbn1-C and 2-C proteins were used to generate polyclonal antibodies in rabbits, which were then tested for binding affinity with mrFbn1-C and 2-C and cross-reactivity with human fibrillin-1 and -2 by ELISA assays. In addition, immunofluorescence tests were performed using anti-mrFbn1-C and 2-C to stain mouse fibroblast cells (NIH 3T3) and compared with the staining using human fibrillin-1 and -2 antibodies, anti-rF6H and anti-rFBN2-1 respectively.

Results: Mouse fibrillin-1 and -2 bound heparin less strongly than human fibrillin-1 and -2. Antibodies raised against mouse fibrillins demonstrated cross-reactivity to human fibrillin proteins in ELISA tests, whereas no cross-reactivity was detected in immunofluorescence. Similarly, while antibodies raised against human fibrillins were able to detect mouse fibrillin proteins in ELISA tests, no cross-reactivity was observed in immunofluorescence. These data suggest that mouse fibrillins share some structural and functional similarity with human fibrillins.

P51 - Short Fibulins are Highly Susceptible to Proteolysis, Interact with Cells and Heparin, and Form Multimers

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The fibulin family comprises eight extracellular matrix proteins associated with elastic fibers and basement membranes. Elastic fibers provide necessary tissue recoil to organs, including the large arteries and skin, and elastogenesis requires the coordinated effort of many proteins. The short fibulins, fibulin-3, -4, and -5, have been implicated in elastic fiber assembly, stabilization, and function through knockout mouse studies.

Full-length human short fibulins were expressed in human embryonic kidney cells and were purified by chelating chromatography. All three members consistently appear as multiple bands on an SDS-PAGE gel. N-terminal sequencing revealed that all three proteins are readily cleaved within the atypical N-terminal linker region of the first calcium-binding epidermal growth factor domain, resulting in this multiple-band pattern on SDS-PAGE gels. Furthermore, proteolytic susceptibility of the linker region was positively correlated with its length. When matrix metalloproteinases (MMP)-1, -2, -3, -7, -9 and -12 were separately added to each of the short fibulins, a similar banding pattern was produced. Proteolysis of fibulin-3 was almost completely inhibited in cell culture by the addition of 25 μM doxycycline (a broad-spectrum MMP inhibitor).

Strong adhesion of human skin fibroblasts to the short fibulins was measured through crystal violet cell attachment assays. Slightly weaker binding of human lung fibroblasts and human umbilical vein and artery smooth muscle cells was also determined. Although only fibulin-5 has an RGD site, all short fibulins adhere at a similar level to the respective cells and achieve saturated binding with ~25 μg/mL of coated protein. Strong, calcium-dependent binding of fibulin-4 to immobilized heparin was observed in solid phase binding assays, suggesting that this fibulin may bind cell-surface heparan sulfate. Whereas deglycosylation of fibulin-4 had no effect on its ability to bind heparin, it enhanced binding to tropoelastin.

Consistent and reducible fibulin-4 dimers and multimers were observed by SDS-PAGE. Similarly, atomic force microscopy identified ~10-15 nm monomers, ~20-25 nm dimers, and ~30-50 nm multimers in purified fibulin-4 preparations. These three populations were separated by gel-filtration chromatography, and solid phase binding assays established that fibulin-4 multimerization is required for its binding to heparin.

P52 - Impact of Aldosterone and Spironolactone on the Maturation and Organization of the Elastin Network in a Skin Substitute

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Elastic fibers form a complex network that contributes to the elasticity of connective tissues. Alterations in the elastic fiber network are involved in several diseases such as Pseudoxanthoma elasticum (PXE), a genetic disorder characterized by fragmentation and progressive mineralization of dermal connective tissues, primarily the elastic structures. The aim of this work was to develop an in vitro tissue-engineered skin substitute allowing to study maturation and organization of the elastic fiber network. To reconstruct the dermis using the LOEX self-assembly approach, we cultivated dermal fibroblasts in the presence of ascorbic acid, which promotes extracellular matrix assembly and allows the formation of thick sheets of collagenous tissue. Keratinocytes with or without melanocyte were then added in order to reconstruct the epidermis. Since high ascorbic acid concentration decreases tropoelastin mRNA and prevents its attachment on the microfibril network, aldosterone and spironolactone were used with or without ascorbic acid to stimulate the elastogenesis through the IGF1 pathway. Immunohistochemical analysis revealed that elastin staining was weak, whereas fibrillin-2 was strong in the dermis of the skin substitute without melanocyte after 2 months in culture. In contrast, the staining intensity of elastin was improved when we added melanocyte and stronger in the last phase of culture with a decreasing of the fibrillin-2 staining when ascorbic acid, aldosterone and spironolactone were used. Moreover, the staining pattern resembled that of the microfibrillar framework and the elastic fibers present in human skin. We concluded that our skin substitute model is a promising tool to study the maturation and the organisation of the elastin network.

P53 - Comparing Tissue-Engineered Adipose Substitutes to Human Native Fat: A Transcriptome Analysis

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Tissue engineering is a promising way to produce human adipose tissues closely similar to native fat for clinical applications. To engineer those tissues, we took advantage of the endogenous capacity of mesenchymal cells to generate and organize extracellular matrix components upon ascorbic acid stimulation, leading to natural and entirely human substitutes (self-assembly method). Induced adipose-derived stem cells were used to reconstruct functional adipose tissues by this method. In order to validate the relevance of these adipose substitutes, we investigated variations in mRNA expression between our in vitro 3D human model and native subcutaneous lipoaspirated fat. Whole-genome gene expression was performed using the Illumina HumanWG-6 v3 BeadChip. The expression values were log2-transformed and quantile-normalized using the lumi package in R. The Significance Analysis of Microarrays method was used to identify genes differentially expressed (false discovery rate < 5% and fold change > 2.0). Pathway and biofunction analysis was performed using Ingenuity pathway analysis 8.5 (IPA) and Gene set enrichment analysis (GSEA) respectively. Fisher's exact test was used to calculate p value determining the probability that the association between the genes in the dataset and the canonical pathway is explained by chance alone. By assessing the mRNAs that were modulated in reconstructed adipose tissues compared to native adipose tissues, interesting pathways were revealed. As expected, basic biological processes associated with adipocytes were similar such as PPARγ signalling (p value = 8.5E-04), fatty acid metabolism (p value = 0.001) and IGF-1 signalling (p value = 0.001). Interestingly, cell death-related processes were not increased in our in vitro tissues compared to native fat (p value= 0.006). ECM interactions (p-value 1.54-4) seemed to be slightly increased. The engineered tissues displayed an increased association with stemness pathways (p value = 4.9E-04) compared to native fat, but no propensity towards tumorigenicity (p value =2.79E-53). GSEA also revealed ECM associated biofunctions such as: Interaction cell-matrix (p-value 5.95-7). Globally, our investigation demonstrated that our human tissue-engineered adipose substitutes were closely related to native adipose tissue in regard to the major biofunctions and canonical pathways. Such comparative analyses provide helpful information to ensure the safety profile of these tissues before grafting and to further improve their production if deemed necessary.

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P54 - Role of Phosphate Transporter PiT-1 in Chondrocyte Extracellular Matrix Mineralization

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Inorganic phosphate (Pi) – an abundant mineral ion in the human body – is essential for many physiologic processes. Tight regulatory control of Pi levels are vital to maintaining many bodily functions. The combined actions of various Pi regulators in bone, intestine and kidney are essential for overall Pi homeostasis. Such regulation includes the activity of sodium-phosphate (Na-Pi) co-transporters. Among the three classes of Na-Pi transporters, type-III transporters (PiT1 and 2) are ubiquitously expressed and are the sole functional Pi-transporters in osteoblasts where they may be involved in the regulation of bone mineralization. However, the role of PiT1 in cartilage mineralization has not been investigated in any detail. Based on the reported role of these transporters in extracellular matrix (ECM) mineralization in osteoblast cultures, we hypothesized that PiT1 plays an important role in cartilage mineralization. Here, we show that PiT1 transporters are expressed in the chondrocytes of developing cartilage and in ATDC5 chondrogenic cells in culture. PiT1 is the sole Na-Pi transporter expressed in ATDC5 cells cultured in differentiation medium. Moreover, treatment with phosphonoformic acid (FoscarnetTM) – an inhibitor of phosphate transporters – resulted in a dose-dependent decrease in mineralization of ATDC5 cultures. This treatment downregulated osteopontin expression, an indicator of intracellular Pi levels, in differentiated ATDC5 cells, suggesting that Pi transport was indeed impaired upon Foscarnet treatment. Since PiT1 is the sole Pi-transporter expressed in ATDC5 cells, these data suggest that Foscarnet may prevent ECM mineralization by inhibiting intracellular Pi transport through PiT1. In future work, we will validate this by shRNA-mediated specific downregulation of PiT1 in ATDC5 cells. We also will aim to evaluate the effects of PiT1 knockdown on cell proliferation and apoptosis. This study will thus establish the functional role of PiT1 in cell differentiation and ECM mineralization in chondrogenic ATDC5 cultures. Funded by CIHR.

P55 - Ectopic Mineralization of Spinal Tissues in Mice Lacking Equilibrative Nucleoside Transporter 1: Age-Dependent Alterations in the Expression of Genes Regulating Biomineralization

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Background: The equilibrative nucleoside transporter 1 (ENT1) transfers adenosine across plasma membranes. We recently reported that mice lacking ENT1 (ENT1-/-) exhibit progressive ectopic mineralization of spinal tissues resembling diffuse idiopathic skeletal hyperostosis (DISH) in humans (J Bone Miner Res, epub 2012). Pathological calcification of paraspinal tissues was first noted in the cervical-thoracic region at 2 months of age. Lesions extended to the lumbar and caudal regions with advancing age, and eventually involved the paraspinal ligaments, entheses, intervertebral discs and sternocostal articulations.

Objective: Our purpose was to investigate mechanisms underlying ectopic mineralization in ENT1-/- mice. Accordingly, we quantified the expression of genes known to play roles in regulating biomineralization in 2-month-old mice, the age at which lesions were first detectable by micro-CT. Methods: Intervertebral discs (IVD) were isolated by microdissection from female ENT1-/- mice and WT littermate controls at 2 months of age. Tissues were either processed for RNA extraction and real-time RT-PCR, or subjected to enzymatic dissociation for cell isolation and culture.

Results: Compared to tissues from WT mice, IVDs from ENT1-/- mice exhibited greater expression of alkaline phosphatase (Alpl, enzyme responsible for the hydrolysis of PPi to Pi, which promotes mineralization) and ectonucleotide pyrophosphatase/phosphodiesterase 1 (Enpp1, enzyme responsible for the production of PPi, which inhibits mineralization). On the other hand, there was no significant difference in the expression of progressive ankylosis protein (Ank, a putative PPi transporter). These findings are in striking contrast to the pattern of gene expression in IVDs from WT and ENT1-/- mice at 6 months of age (see abstract by Warraich et al.). In these older mice, expression of Alpl, Enpp1 and Ank was significantly less in ENT1-/- mice than in WT mice. Together, these findings suggest that, in older mice, there may be compensatory changes in gene expression as mineralized lesions extend into the IVDs. To further explore underlying mechanisms, an in vitro system was developed for culturing IVD cells from 2-month-old mice. Cultures expressed annulus fibrosus markers over passages 0 to 5. In primary cultures of IVD cells from ENT1-/- mice, we observed greater proliferation and type 1 collagen gene expression than in WT cultures.

Conclusions: It is possible that excessive expression of alkaline phosphatase in IVDs of ENT1-/- mice at 2 months of age leads to the eventual mineralization of these structures seen in older mice. In vitro studies are underway to identify the underlying pathways. Supported by CIHR.

P56 - TGF-ß3 Enhances Link-N Induced Stimulation of Proteoglycan Synthesis by Human Chondrocytes

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Introduction: Osteoarthritis (OA) affects millions of individuals, yet there is currently no treatment that will prevent or repair joint damage. It is well-known that during the progression of OA, the proteoglycan network breaks down and biomechanical properties of healthy cartilage are lost. Previously we have shown the potential of Link-N, a 16 amino acid peptide, in stimulation of proteoglycan production in a whole organ human intervertebral disc culture model, human disc cells cultured in 3-D alginate scaffolds and in vivo rabbit disc degeneration model. With a view to optimizing Link N-induced proteoglycan synthesis by chondrocytes from normal human cartilage, the modulating influences of transforming growth factor beta 1 (TGF-ß1) were investigated.

Materials and Methods: Articular cartilage was isolated from five donors undergoing total hip replacement. Cells were recovered from the cartilage of each femoral head by sequential digestion with Pronase followed by Collagenase. After isolation, the cells were expanded for 7 days under standard cell culture conditions. Normal chondrocytes (PromoCell, Heidelberg, Germany) were expanded in DMEM supplemented with 10% FBS. After 7 days of culture, cells were transferred in 96 well plates (~10.000 cells/well) and exposed to TGF-ß3 (20 ng/ml), Link-N(1 µg/ml), and co-exposed to TGF-ß3+LinkN for 12 days withculture medium changed every third day.The sulfated glycosaminoglycan (GAG, predominantly aggrecan) content of the medium was analyzed using the 1,9-dimethylmethylene blue (DMMB) dye-binding assay. All experiments were performed in triplicates.

Results: The cumulative production of GAG increased with time in culture up to day 12, throughout the culture conditions. At days 9 and 12, the level of GAG synthesis by normal and OA cells supplemented with LinkN was greater than by cells from control. OA cells demonstrated a higher degree of stimulation on day 12 when compared to normal cells.TGF-ß3 enhanced Link N-induced increase in GAG release into the culture medium.

Conclusion: TGF-ß3 enhances the Link N-induced proteoglycan synthesis of human chondrocytes. It is well known that growth factors, such as TGF-β3 can be applied directly for tissue regeneration. Injecting mesenchymal stem cells (MSCs) together with growth factors into articular cartilage can bring added benefits to the repair process, considering that growth factors and MSCs can improve tissue repair individually, and growth factors can stimulate and accelerate the differentiation of MSCs to chondrocytes. Thus, Link N has the potential to be employed together with TGF-ß3 and MSCs in promoting a chondrogenesis-like phenotype and stimulating proteoglycan synthesis of differentiated and resident chondrocytes in articular cartilage repair.

P57 - Endoglin Regulates TGF-b Signaling and Type II Collagen Production in Chondrocytes

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Transforming growth factor-beta (TGF-β) plays a critical role in the maintenance of articular cartilage. Deregulation of its activity results in failure of cartilage repair, leading to chronic degenerative joint disorder, such as osteoarthritis (OA). Our research on cartilage repair focuses on the regulation of TGF-β action by endoglin, a TGF-β co-receptor. Our previous work has shown that chondrocytes express endoglin and that it promotes TGF-β induced ALK1 (Activin Receptor-Like Kinase 1) pathway while it inhibits ALK5 pathway to decrease type II collagen expression. In the current study, we examined whether endoglin is altered in human OA cartilage and whether it regulates TGF-□ signaling and collagen production *in vivo* using an endoglin deficient animal model.

Cartilage biopsies were taken from adult patients with traumatic open joint injury who have no history of OA (N) and from OA patients at hip replacement surgery. These biopsies were analyzed by immunohistochemistry (IHC) and Western blot (WB) to compare levels of endoglin expression. For animal studies, we used an endoglin heterozygous mouse (HET, lacking one endoglin allele) since endoglin null mice are embryonically lethal. Our results show that endoglin is overexpressed in OA human cartilage as compared to N cartilage (n=3). Moreover, HET murine chondrocytes showan increased in basal and TGF-b-induced type II collagen levles as compared to WT counterparts (n=5, $p < 0.05$). Analysis of phospho-Smads (1, 2, 3) and total Smads show that their levels are similar in HET and WT chondrocytes suggesting that endoglin may regulate TGF-□ signaling through non-Smad pathways in these cells.

Altogether, our finding that endoglin expression is upregulated in human OA cartilage suggests that endoglin may play a role in promoting an OA-like phenotype in human chondrocytes. Increased expression of TGF-b-induced type II collagen in endoglin HET mouse chondrocytes suggests that endoglin inhibits TGF-b action and collagen II production in the cartilage in these mice.

Determining mechanism by which endoglin regulates TGF-□ action in chondrocytes to affect type II collagen production may provide a basis for the development of novel therapeutic approaches to improve cartilage repair.

P58 - Effect of Link-N on Proteoglycan Synthesis by Chondrocytes from Normal and Osteoarthritic Human Cartilage in the Presence of IL-1

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Introduction: Osteoarthritis (OA) is a degenerative disease of the articular cartilage that affects millions of people. It is characterized by destruction and eventual loss of articular cartilage from affected joints. OA involves degradation of proteoglycans that make up the pericellular matrix surrounding articular chondrocytes by matrix-degrading enzymes. Previously, we have demonstrated the potential of Link N in the stimulation of proteoglycan production in discs cells and a whole organ human intervertebral disc (IVD) culture model, human IVD cells cultured in 3-D alginate scaffolds and in vivo rabbit disc degeneration model. In this study we examined the effect of Link N on proteoglycan synthesis by chondrocytes from normal and OA human cartilage in the presence of IL-1.

Materials and Methods: Articular cartilage was isolated from five donors undergoing total hip replacement. Cells were recovered from the cartilage of each femoral head by sequential digestion with Pronase followed by Collagenase. After isolation, the cells were expanded for 7 days under standard cell culture conditions. Normal chondrocytes purchased from PromoCell (Heidelberg, Germany), were expanded under the same conditions and used as control. After 7 days, cells were exposed to IL-1β (5 ng/ml), Link N (1 μg/ml), and co-exposed to IL-1β+Link N for 12 day with culture medium changed every three days. The sulfated glycosaminoglycan (GAG, predominantly aggrecan) content of the medium was analyzed using the 1,9-dimethylmethylene blue (DMMB) dye-binding assay.

Results: The cumulative production of GAG increased with time in culture up to day 12 throughout the culture conditions. At days 9 and 12, the level of GAG synthesis by normal and OA cells supplemented with Link N was greater when compared to cells cultured in control medium. Strikingly, a higher degree of stimulation was observed on day 12 in OA versus normal cells. Although there was a tendency towards decreased GAG production from normal chondrocytes in the presence of IL-1ß, decreases in GAG release was only apparent from OA cells on day 6. Link N was effectively able to maintain upregulation of GAG release into the culture medium in normal and OA cells following co-administration of IL-1β+Link N.

Conclusion: Link-N demonstrated an increased capacity to stimulate OA versus normal chondrocytes suggesting a greater potential to stimulate proteoglycan in disease. The beneficial effects of Link N are augmented by its cost effectiveness compared to other non-surgical options in the management of OA.

P59 - Link N Stimulation of Proteoglycan Synthesis by Bovine Intervertebral Disc Cells is Mediated by SMAD1/5 Phosphorylation

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Introduction: We have previously shown that human Link N (DHLSDNYTLDHDRAIH) can stimulate extracellular matrix biosynthesis by intervertebral disc (IVD) cells, both *in vitro* and *in vivo*. Furthermore, it can stimulate chondrogenic differentiation and down-regulate osteogenic differentiation in human mesenchymal stem cells (MSCs). To date, there have been no reports on the effect of bovine Link N (DHHSDNYTVDHDRVIH) on disc cells. The purpose of this study is to compare the effect of bovine Link N (BLN) to that of human Link N (HLN) on bovine IVD cells and determine their mechanisms.

Methods: Coccygeal discs from healthy 22-24 months old steers were obtained 2-3 hours after slaughter. The IVDs were separated from their adjacent vertebral bodies and the cells were isolated from nucleus pulposus (NP) and annulus fibrosus (AF) regions. Subsequently, the NP and AF cells were beaded in 1.2% alginate at a concentration of 35.000 cells/ bead. The beads were then placed in 24 well plates at a density of 9 beads/well and incubated for 18 days with either human or bovine Link-N at a concentration of 1µg/ml. Cell viability was assessed using a live/dead fluorescence assay. The sulfated glycosaminoglycan (GAG) release in the media was analyzed using the 1,9-dimethylmethylene blue (DMMB) dye-binding assay. After 18 days of culture, cells were harvested and used to prepare total RNA. Smad phosphorylation was determined by immunoblotting using specific antibodies.

Results: For both NP and AF cells, the rate of GAG release into the culture media increased with time. This increase was significantly higher for IVD cells incubated with Link N than control cells ($p<0.001$). Furthermore, the levels of GAG release by NP cells treated with BLN or HLN tended to be higher compared to that of AF cells with the same treatment at all time points. Aggrecan message levels were also examined following Link N treatment. BLN and HLN treatments led to a significant increase in aggrecan gene expression when compared to control. Interestingly, a rapid and transient Smad1/5 phosphorylation response was observed in AF cells using HLN.

Conclusions: Although, bovine Link N can stimulate proteoglycan production, synthesis was greater with human Link N under the same concentrations. Furthermore, the increased potency of HLN may be through its activation of Smad1/5.

P60 - Anti-microRNA-378 Enhances Wound Healing Process by Up-regulating Vimentin and Integrin beta-3

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Background: Delayed or impaired wound healing evolves to be a major public health issue worldwide, especially in patients with diabetes mellitus and vascular atherosclerosis. Wound healing is achieved by complex physiological processes: hemostasis, inflammation, re-epithelialization, vascularization and tissue remolding. Until recently, microRNA has emerged as a key regulator of wound healing. Our previous studies revealed that microRNA-378 (miR-378) plays a role in modulating cell migration and differentiation in vitro, and we further demonstrated that the function of miR-378 was subjected to complex regulation in differentiated MC3T3 cells.

Methods: In this study, we developed an anti-miR-378 sponge by inserting multiple tandem microRNA binding sites into vectors with CMV promoter. With highly matched sequence, this homological antisense transcript sufficiently blocks the process of precursor microRNA. CD1 anti-miR-378 transgenic mice were generated by microinjection of transgene fragments into fertilized zygotes. Positive transgenic mice along with control group were subjected to skin biopsy, causing a pair of full-thickness, excisional wound on the back of neck. Wound size was measured everyday thereafter, and tissue samples were collected for immunohistochemistry study. Meanwhile, mouse fibroblast cell line NIH/3T3 was transfected with anti-miR-378 fragments and subjected to migration, differentiation and angiogenesis assay.

Results: Anti-miR-378 sponge could block mature miR-378 generation in vitro and in vivo. Compared to control mice, enhanced wound healing process was shown in anti-miR-378 transgenic mice. In addition, we found vimentin and integrin beta-3, modulators which are important in wound healing process, increased remarkably in transgenic mice samples. Wound scratch and transwell migration tests showed a greater mobility in anti-miR-378 transfected NIH/3T3 cells, which was due to the up-regulation of vimentin and integrin beta-3. They are confirmed as targets of miR-378, thus their expression could be rescued by anti-miR-378. Overexpression of vimentin could also contribute to fibroblasts differentiation and up-regulation of integrin beta-3 by anti-miR-378 is responsible for angiogenesis.

Conclusion: Taken together, we demonstrated that knockdown of miR-378 by endogenous integrated antisense fragments can increase the expression of its targeted proteins, vimentin and integrin beta-3, which enhanced wound healing in vivo and accelerated fibroblasts migration and differentiation in vitro, which adds new knowledge in microRNA studies and might benefit patients suffering from unhealed wound in future.

P61 - Link-N Stimulates Extracellular Matrix Production by Human Intervertebral Disc Cells in the Presence of IL-1

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Introduction: Intervertebral disc degeneration is associated with increased levels of inflammatory cytokines, which induces proteinase production and proteolytic degradation of the extracellular matrix. A repair strategy of the painful degenerated disc requires production of extracellular matrix components and a down regulation of proteinase activity in this inflammatory milieu. These properties are associated with several growth factors such as Link-N. The purpose of the present study was to evaluate the effect of Link N in human IVD cells in the presence of IL-1, a cytokine commonly implicated in disc degeneration.

Materials and Methods: Intervertebral discs were isolated from lumbar spine segments from three donors through Transplant Quebec. Isolated discs were divided into nucleus pulposus (NP) and annulus fibrosus (AF) regions. Single cells were isolated by enzymatic digestion and then beaded in 1.2% alginate. The alginate beads were divided into 2 groups, 1, exposed to IL-1β or 2, simultaneously exposed to IL-1β and Link-N for 24 hrs. RNA was isolated after and gene expression of Aggrecan, MMP-3, MMP-13, ADAMTS-4 and ADAMTS-5 was analyzed by real time PCR and calculated using ΔΔCt method. Beads were also exposed as described above for 48 hrs in the presence of 35SO4 to estimate proteoglycan synthesis.

Results: There was an upregulation in gene expression for aggrecan and a down regulation in gene expression for MMP-3 MMP-13, ADAMTS-5 and ADAMTS-4 in NP cell constructs simultaneously exposed to IL-1β and Link-N when compared to IL-1β exposure alone. Aggrecan expression levels were also upregulated by the presence of Link-N in AF cell constructs. However, while the expression levels of ADAMTS4 and MMP-13 were reduced by Link-N in the AF constructs, there was no decrease in expression levels of MMP-3 and ADAMTS-5. New proteoglycan synthesis estimated by 35SO4 incorporation showed increased levels in constructs exposed to IL-1β and Link-N compared to base line synthesis in un-treated constructs and in constructs treated with IL-1β alone.

Conclusion: Lately focus has shifted from surgical treatments of disc degeneration to medical modalities. One step in this direction is establishing various bioactive peptides as potential regenerative agents. Link-N has shown promise in this direction by stimulating proteoglycan synthesis and also down regulating MMP expression in an inflammatory milieu. Link-N has an effect on proteoglycan synthesis in both NP & AF cells thus indicating that it has the potential to stimulate proteoglycan synthesis across the whole disc, which is important in attempting to regenerate the functional properties of the disc during disc degeneration. These beneficial effects of Link-N are augmented by its cost effectiveness and may establish it as an alternative to surgical treatment of disc degeneration.

P62 - Effect of Intervertech-1 levels on Proteoglycan Synthesis by Intervertebral Disc Cells

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Introduction: Back pain is a common problem affecting a large portion of the population. Intervertebral disc (IVD) degeneration is the single most common cause of back pain. Intevertech 1(IVT-1), currently being patented by Intervertech Inc, represents a novel peptide produced in mammals. The aim of this study was to determine response of nucleus pulposus (NP) and annulus fibrosus (AF) cells to different Intervertech 1 concentrations when cultured in a 3 dimensional alginate scaffold system.

Methods: Cells were isolated from bovine IVD and suspended in 1.2% alginate. Alginate beads were stabilized and exposed to 5nM-400nM of IVT-1 supplemented with 35S-sulfate for 48 hours to determine aggrecan synthesis. Media was collected and dialyzed. Counts per minutes (CPM) were normalized to control and expressed as an arbitrary value.

Results: In both cell types proteoglycan synthesis increased with dosage in culture up to 10 nM, then tended to plateau between 25 nM and 50 nM but increased at 100n M and 200 nM. Maximal response was at 200 nM but declining thereafter. At all time points, the levels of proteoglycan synthesis by AF cells were greater than NP cells.

Conclusions: This study indicates that IVT-1 is able to stimulate proteoglycan synthesis in bovine disc cells cultured in a 3-D system. Promotion of proteoglycan synthesis is the main strategy for disc regeneration therapy and this peptide shows promise in this direction and can be established as a potential therapeutic agent. One major advantage of Intevertech 1 over recombinant growth factors for therapeutic use is the large saving in cost.

P63 - Elucidating the Molecular Mechanisms involved in Targeting V-ATPases to the Plasma Membrane: Discovering Potential Targets for Novel Anti-resorptive Therapeutics

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Background: V-ATPases are ATP driven proton pumps that when targeted to the plasma membrane of osteoclasts, are responsible for acidifying the surface of bone, essential for bone resorption and bone remodeling. Evidence suggests that the 100 kDa V-ATPase 'a' subunit is responsible for targeting the multi-subunit complex to the plasma membrane. The protein sequence of the 'a' subunit contains one putative glycosylation site.

Hypothesis: V-ATPase a4 subunit is glycosylated in vivo and glycosylation of the a4 subunit is important for assembly, folding and/or targeting the rest of the V-ATPase complex to the plasma membrane.

Overall Objective: Elucidate specific features of the a subunit isoforms which govern structure, function and membrane targeting of V-ATPases, in order to facilitate the development of site-directed therapeutics

Significance: To yield important new insights into the structure, function and cell biology of the V-ATPase a subunit that can be exploited for therapeutic targeting.

Methods: Mammalian cells were transfected with C-terminal FLAG tagged version of the V-ATPase 'a' subunit with or without mutations that eliminate the putative glycosylation site (N499D and N499Q). Expression and plasma membrane targeting were assessed by immunofluorescence and immunoblotting. Glycosylation was assessed by immunoblotting whole cell extracts with or without treatment with endoglycosidases PNGase and Endo H enzymes.

Results: Immunoblotting and immunofluorescence evidence demonstrates that the C-terminal FLAG tagged version of the V-ATPase 'a' subunit with or without the glycosylation mutations N499D and N499Q express in mammalian cells. Whole cell extracts were immunoblotted with anti-FLAG resulting in a fuzzy band of about 96 kDa in the wild type suggesting that the 'a' subunit has complex glycosylation. Whole cell extracts from 'a' subunits bearing the N499D and N499Q mutations both resulted in a sharp band of about 93 kDa with reduced intensity relative to the wild type. These results suggest that N499 is the site of glycosylation and that glycosylation is important for proper and efficient protein folding. Immunoblots revealed that the wild type 'a' subunit is Endo-H insensitive but PNGaseF sensitive resulting in a 93 kDa sharp band again providing evidence that the 'a' subunit is glycoprotein.

Conclusions: Our preliminary results suggest that the human V-ATPase 'a' subunit is glycoprotein with N-linked glycosylation at position N499 and this glycosylation may be crucial for the proper folding. Further investigations are required to determine whether the glycolyslation is important to plasma membrane targeting and/or assembly of the V-ATPases.

P64 - E-cadherin's Involvement in Osteoclast Precursor Fusion.

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Fusion between multiple precursor cells is an important commitment step of osteoclast differentiation. Initially observations of individual fusion events were made using long-term imaging strategy. Live-cell movies revealed that RAW 264.7 cells, stimulated with RANKL, use multiple approaches for the generation of multinucleate osteoclasts. Early differentiation, post-48 hours of RANKL treatment, was characterized by simple fusion events between small multinucleate cells with mononuclear cells. However, by 96 hours of RANKL treatment, multinucleate cells fusing with other multinucleate cells were observed, which has not been widely reported. Further observation revealed fusion preferentially occurred at the leading edge of migrating precursor cells or at sites of active pseudopod extension, suggesting that fusion-mediating molecules may localize to these regions. Live immunofluorescent localization of E-cadherin, a potential fusogenic molecule, showed enrichment of E-cadherin at apparent active membrane regions in both RAW and human primary cells. Endogenous E-cadherin was present on the plasma membrane of precursor cells and appeared at sites of cell-cell contact. Elevated endogenous expression levels were strongly associated with early time points during differentiation. Application of functional blocking antibodies against E-cadherin also caused a significant reduction in overall cell fusion. These results suggest an important role for E-cadherin during early stages of osteoclast differentiation, implicate migration as a critical component for successful fusion and demonstrate the utility of live-cell techniques to study the osteoclast fusion event.

P65 - Antagonistic Effects of IL-1α and TGFβ1 on Extracellular Matrix Formation of Reconstructed Dermis by a Self-Assembly Strategy

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Healing is a highly regulated process involving multiple cellular and molecular mechanisms, remodeling the extracellular matrix of injured site to obtain an effective repair of the wound. Disruption of this process can lead to the formation of hypertrophic scars, a fibrotic skin disorder characterized by an excessive collagen deposition and a failure of remodeling of the extracellular matrix. Among the soluble factors involved in this process, cytokines IL-1α and TGFβ1 are hypothesized to play a very important role. Pro-fibrotic effects of TGFβ1 are widely described in the literature, but the anti-fibrotic effect of IL-1α remains a controversial topic according authors. We hypothesized that hypertrophic scars are the result of an imbalance in the production of these two cytokines during the healing process. To evaluate the antagonistic effects of IL-1Î± and TGFβ1, dermis were reconstructed using the self-assembly method developed by our laboratory. Skin fibroblasts were grown in DME containing 10% FCS + ascorbic acid (50 μg/mL) in 12-well plates. The cells were treated with equimolar concentrations of IL-1α or TGFβ1 (0.1176 nM), for 28 days. Then, the culture supernatants were collected and dermal sheet was fixed in paraformaldehyde for 24 hours and was embedded in paraffin. The thickness of the dermis, a key parameter for assessing fibrosis, was measured on histological sections and collagen and MMP secretion quantified using ELISA. The results showed that the reconstructed dermis in the presence of IL-1α were significantly thinner in comparison to controls. By contrast, the dermis treated with TGFβ1 showed a very significant increase of their thicknesses compared to controls. Collagen and MMP secretion modulation can explain these differences. These results support the hypothesis that IL-1α and TGFβ1 can play an antagonist role during the healing process and support the idea that, in parallel with an increase of TGFβ1 secretion, a failure in the production of IL-1α can be at the origin of the formation of hypertrophic scars. Also, these data provide new insights into the pathophysiology of hypertrophic scarring as well as perspectives for development of new treatments.

Key words: extracellular matrix; healing; IL-1α; TGFβ1; fibrosis

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