CCTC 2012

18th Canadian Connective Tissue Conference

June 8 to 10, 2012

University of Toronto, Toronto, Canada

http://connective-tissue-canada.com

Sessions:

- Connective Tissue in Disease - Clinical and Fundamental Aspects
- Stem Cells, Cell Differentiation and Tissue Engineering
- Biomechanics, Mechanobiology, and Biomaterials
- Connective Tissue Remodelling
- The Extracellular Matrix in Connective Tissues
- Bone, Cartilage and Tooth Development
- Cellular Interactions Between Connective Tissue and Other Tissues

Confirmed Speakers:

Paul Janmey (University of Pennsylvania)
Chris McCulloch (University of Toronto)
Mark Pittenger (University of Maryland)

Ben Alman (University of Toronto)
Mike Buschmann (École Polytechnique de Montréal)
Artur De Brum-Fernandes (Université de Sherbrooke)
Jeff Dixon (University of Western Ontario)
Neil Duncan (University of Calgary)
Julie Fradette (University Laval, Quebec)
Lucie Germain (University Laval, Quebec)
Fred Keeley (University of Toronto)
Andras Kapus (University of Toronto)
Andrew Leask (University of Western Ontario)
Michael Raghunath (University of Singapore)
Cari Whyne (Sunnybrook Health Sciences Centre)

Scientific Committee:

Nazish Ahmed
Vanessa Mendes
Andras Kapus
Morrie Manolson
Eli Sone
Tom Waddell
Tom Willett
Lidan You

Conference Chairs:

Boris Hinz (Faculty of Dentistry, U of T)
Craig Simmons (IBBME, U of T)

Contact:

info@connective-tissue-canada.com

Our Sponsors:

MITACS
The Arthritis Society
CANADIAN ARTHRITIS NETWORK | LE RÉSEAU CANADIEN DE L'ARTHRITE
DRI Dental Research Institute UNIVERSITY of TORONTO
Institute of Biomaterials & Biomedical Engineering UNIVERSITY OF TORONTO

Dear Colleagues, dear Friends,

Welcome to the 18th Annual Canadian Connective Tissue Conference (CCTC), taking place at the University of Toronto from June 8 to 10, 2012.

Ahead of you are three days of keynote talks and oral presentations by senior scientists and selected from abstracts. Ample time will be given for the poster sessions and informal discussions.

As in previous years, CCTC 2012 serves to bridge the gaps in our current scientific and clinical understanding of connective tissues in both health and disease through communication of health research evidence between basic scientists, clinicians, and Canadian small medium enterprises.

This year's event is expected to have significant long-term impact by formalizing an inter-disciplinary network for connective tissue researchers. CCTC 2012 will be the birth place of the Canadian Connective Tissue Society by hosting the society's inauguration board, consisting of leading researchers from all over Canada.

We sincerely wish that you will enjoy the program and social activities of the conference, and look forward to meeting you this summer in Toronto!

Sincerely

Boris Hinz
Matrix Dynamics Group, Faculty of Dentistry, University of Toronto

Craig Simmons
Institute of Biomaterials and Biomedical Engineering University of Toronto

Toronto, May, 18, 2012

Programme Overview

Friday, 08.06.2012

05:30 pm	Registration	
06:30 - 08:00 pm	Welcome Reception Medical Science Building Terrace (Kings College Circle Entrance)	Poster Session I 

Saturday, 09.06.2012

08:30 - 10:25 am	**Session 1** Cellular Interactions Between Connective Tissue and Other Tissues MacLeod Auditorium	
10:25 - 11:00 am	Coffee Break Student Commons	Poster Viewing 
11:00 - 12:35 pm	**Session 2** Stem Cells, Cell Differentiation, and Tissue Engineering MacLeod Auditorium	
12:35 - 02:00 pm	Lunch Break Student Commons	Poster Session II 
02:00 - 02:45 pm	**Session 3** Connective Tissue Protein Structure and Function MacLeod Auditorium	
02:45 - 04:00 pm	**Session 4** Bone, Cartilage and Tooth Development MacLeod Auditorium	
04:00 - 05:40 pm	Coffee Break Student Commons	Poster Session III 
07:30 pm	Banquet Dinner at the CN Tower 360° Restaurant	

Sunday, 10.06.2012

08:30 - 09:45 am	**Session 5** Biomechanics, Mechanobiology, and Biomaterials MacLeod Auditorium		
09:45 - 11:10 am	Coffee Break Student Commons		Poster Session IV 
11:10 - 12:25 pm	**Session 6** The Extracellular Matrix in Connective Tissues MacLeod Auditorium		
12:25 - 02:00 pm	Lunch Break Student Commons	Inaugural board meeting of the CCTS (Room MS2290)	Poster Session V 
02:00 - 03:00 pm	**Session 7** Connective Tissue in Disease - Clinical and Fundamental Aspects MacLeod Auditorium		
03:00 - 03:50 pm	**Session 8** Connective Tissue Remodelling MacLeod Auditorium		
03:50 - 04:00 pm	A note from The Canadian Arthritis Trainee Association McLeod Auditorium		
04:00 - 04:30 pm	Closing remarks and awards ceremony McLeod Auditorium		
04:30 - 04:50 pm	CCTS Business meeting (General Assembly) McLeod Auditorium		
05:00 pm	End of Conference McLeod Auditorium		

Contact

CCTC Organization
Matrix Dynamics Group
University of Toronto
Room 234, Fitzgerald Building, 150 College St, Toronto, ON M5S 3E2, Canada
Fax: (416) 978-5956
info@connective-tissue-canada.com
http://connective-tissue-canada.com/

Conference Venue

University of Toronto
Medical Science Building and Terrence Donnelly Centre for Cellular and Biomolecular Research
1 Kings College Circle
Toronto, Ontario, Canada M5S 3E1
Click here for Google Map.

Registration

Medical Science Building, 2nd floor (street level) Student Commons Poster Lounge, enter from King's College Circle (picture).

Poster Sessions

Medical Science (MS) Building, 2nd floor (street level) Student Commons (Room MS 2171)

Sessions

JJR MacLeod Auditorium (room MS 2158) across the Student Commons Poster Lounge

Welcome Reception

Between Medical Science Building and Student Commons Poster Lounge

Canadian Connective Tissue Society (CCTS) Inaugural Board Meeting

Medical Science Building, Room 2290

Dinner Banquet

360° Restaurant at the CN Tower, 301 Front St. Toronto, Ontario

Directions

How to get to the conference venue

1) From Pearson airport

a) Fast, economic and ecological: CCTC supports the TTC (public transport):

Cost: *$3 one way*
Travel time to the CCTC: *~50 min*

- Arriving at **Terminal 1**, exit baggage claim and customs, turn immediately right and head towards the big information sign and booth.
- You can buy TTC tokens at the information booth or have $3.-- change ready.
- Take stairs down one level (Ground Level) and exit the building to the right.
- Go to Bus stop for No. 192 is just across the street (Airport Rocket, http://www3.ttc.ca/Routes/192/Routemap.jsp). Bus goes every 10-15 min.
- Take bus to terminal stop (Kipling Subway Station).
- At Kipling Subway Station, take any subway (Bloor-Danforth line) that is in the station (Kipling is subway terminal and all trains head downtown). Subways go every 5 min; no need to pay again.
- Change at St. George Station and take the Yonge-University-Spadina line South for two stations until Queen's park. Exit to street level Northwest (see Map: „M"), walk 3 min.

- Arriving at **Terminal 3**, buy TTC tokens at the information booth in the terminal (one level up) or have $3.-- change ready
- Exit baggage claim and exit building straight ahead (Arrival Level).
- Cross street and turn left. Go to the end of the bus stop bays for the stop of No. 192 http://www3.ttc.ca/Routes/192/Routemap.jsp). Airport Rocket goes every 10-15 min.
- Take bus to terminal stop (Kipling Subway Station).
- At Kipling Subway Station, take any subway (Bloor-Danforth line) that is in the station (Kipling is subway terminal and all trains head downtown). Subways go every 5 min; no need to pay again.
- Change at St. George Station and take the Yonge-University-Spadina line South for two stations until Queen's park. Exit to street level Northwest walk 3 min.

b) Medium cost: Airport Express Bus Service (stops at all major downtown hotels):

Cost: *$24 one way, $40 return*
Travel time to the CCTC: *50-60 min, depending on traffic*

- Leaves every 30 min from Terminal 1 and Terminal 3.
- Check the Airport Express website for schedules and stops: http://www.torontoairportexpress.com/
- Closest stop to the CCTC is "Metropolitan Hotel", 89 Chestnut Street.

c) The lazy and expensive solution: Cab:

Costs: *$50-60 one way*
Travel time to the CCTC: *50-60 min, depending on traffic*

- Take a cab from Terminal 1 or Terminal 3.

2) From City airport

- Take the Porter shuttle to Union Station
- In Union Station follow the signs to the TTC Subway
- Take the Yonge-University-Spadina line direction Downsview, exit after four stops at Queen's Park.
- Exit to street level Northwest walk 3 min.

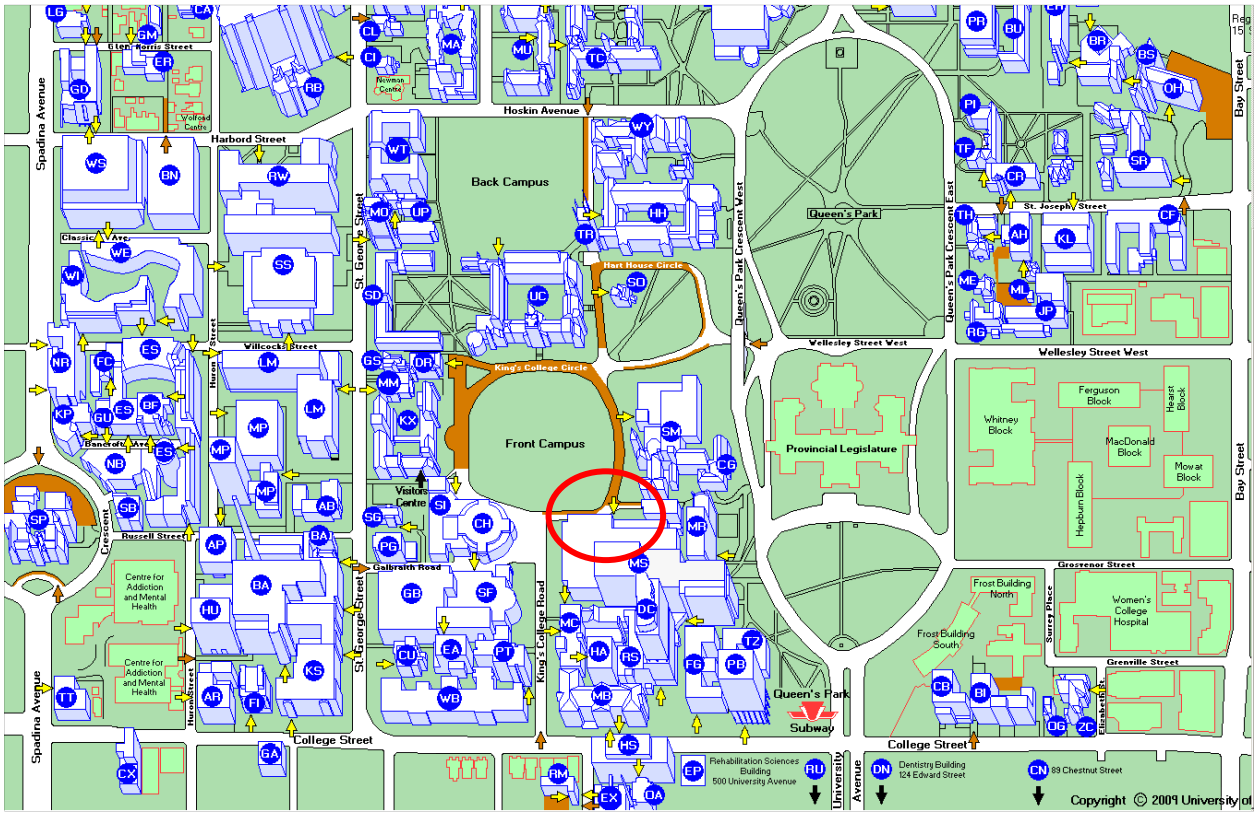
3) By train - arrival Union Station

Take the TTC (public transport):
Cost: *$3 one way*
Travel time to the CCTC: *~10 min*

- For the TTC Subway map, see: http://www.ttc.ca/Subway/interactivemap.jsp
- In Union Station follow the signs to the TTC Subway.
- Take the Yonge-University-Spadina line direction Downsview
- Exit after four stops at Queen's Park and exit to street level Northwest walk 3 min.

4) By car (not recommended)

- Enter "1 Kings College Circle, Toronto" into you GPS.
- Search 45 min for a parking spot; this map may increase your chances: http://www.parking.utoronto.ca/maps.htm.



Accomodation

Accommodation Tips: close to the conference site

89 CHESTNUT RESIDENCE - UNIVERSITY OF TORONTO (Cost effective solution)
www.chestnutresidence.utoronto.ca/
89 Chestnut Street, Toronto, ON M5G 1R1
Phone: (416) 977-0707; Fax: (416) 977-1136

METROPOLITAN HOTEL TORONTO
http://metropolitan.com/toronto, reservations@tor.metropolitan.com
108 Chestnut Street, Toronto, ON M5G 1R3
TEL: (416) 977-5000, FAX: (416) 977-9513, RES: (800) 668-6600

BOND PLACE HOTEL
http://www.bondplace.ca, reservation@bondplace.ca
65 Dundas Street East, Toronto, ON M5B 2G8
Telephone Number: (416) 362-6061, Fax Number: (416) 362 9372
reservation@bondplace.ca

THE STRATHCONA HOTEL
http://thestrathconahotel-px.trvlclick.com/
60 York Street, Toronto, ON M5J 1S8
Phone: (416)363-3321, Fax: (416)363-4679

SHERATON CENTRE TORONTO HOTEL
http://www.sheratontoronto.com/
123 Queen Street West, Toronto, ON M5H 2M9
Phone: (416) 361-1000 Fax: (416) 947-4854, Special Offers Reservations: 866-716-8101

TORONTO MARRIOTT DOWNTOWN EATON CENTRE HOTEL
http://www.marriott.com/hotels/travel/yyzec-toronto-marriott-downtown-eaton-centre-hotel
525 Bay Street, Toronto, Ontario M5G 2L2 Canada
Reservations: 1-800-228-9290 in the USA and Canada.

HILTON GARDEN INN TORONTO CITY CENTRE – OFFICIAL SITE
www.hiltongardeninn.hilton.com
200 Dundas Street East, Toronto, Ontario, Canada M5A 4R6
Tel: 1-(416)-362-7700 ;Fax:1-(416)-362-7706

COMFORT HOTEL DOWNTOWN TORONTO
http://www.choicehotels.ca/en/comfort-hotel-cn228
15 Charles Street East, Toronto M4Y 1S1
Phone 1: (416) 924-1222; Fax: (416) 927-1369

BEST WESTERN PRIMROSE HOTEL DOWNTOWN
http://www.torontoprimrosehotel.com/
111 Carlton Street, Toronto M5B 2G3
Telephone: (416) 977-8000, Reservations: (800) 268-8082

Banquet Venue

360° Restaurant at the CN Tower, 301 Front St. Toronto, Ontario (Link to Google Map). Enter through the main entrance and follow the signs to 360 Restaurant Lounge.

Reservation

All reservations have been made ahead of the meeting on-line and we are now booked out. Please check at the registration desk on site whether returned tickets are available.

Tickets

You must wear your CCTC conference badge and have your meal ticket to enter the 360° Lounge.

How to get there from the conference (see map on back side)

1) Walk (50 minutes)

- CCTC organizers will leave at 6:00pm.
- Walk South on University Avenue until Front St.
- Walk West on Front St. to John St.
- Walk South on John and arrive at the CN Tower.
- CN tower is just next to a large Hot Dog booth.

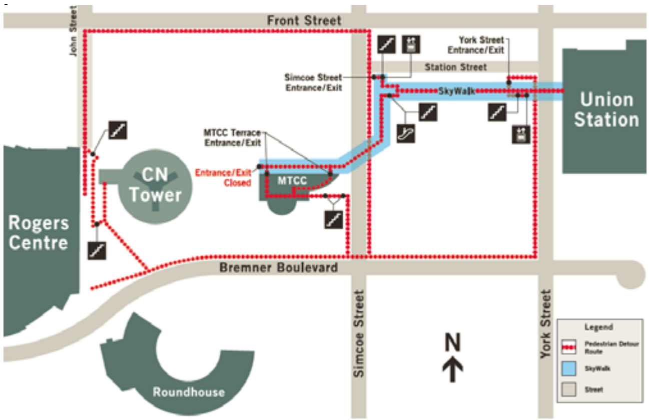
2) TTC (Public Transport) (20 minutes)

Cost: $3 one way, Day Pass $10.50 (For 2 Adults)

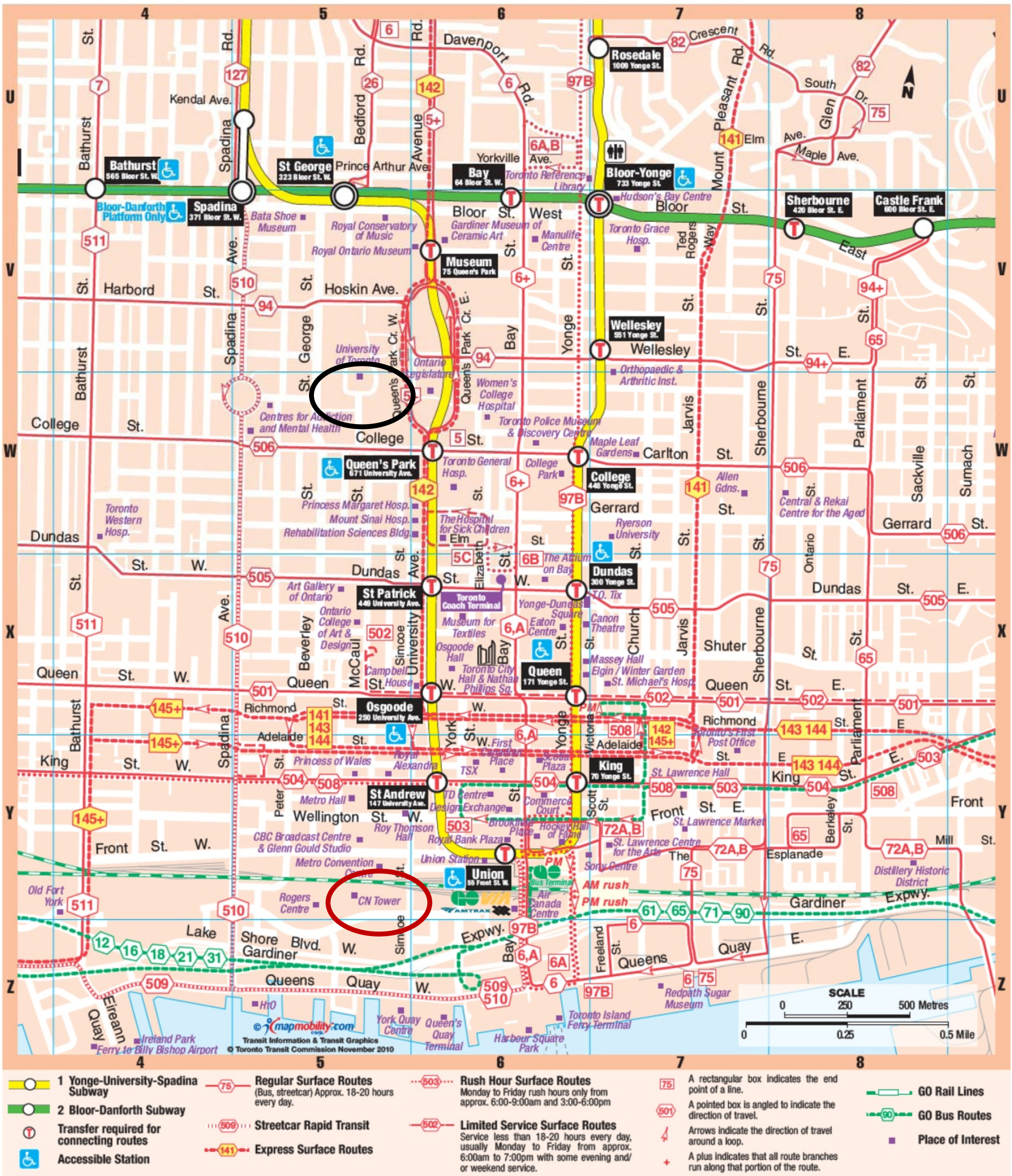
- Enter the subway at the corner of University Avenue and College St.
- Take the yellow line South to Union Station(4 Stops)
- Exit onto Front St and walk West to John St.
- Head South on John and arrive at the CN Tower.
- CN tower is just next to a large Hot Dog booth.

Attire

Semi-formal: No shorts, flip flops, T-shirts



Front Street
John Street
York Street Entrance/Exit
Station Street
Simcoe Street Entrance/Exit
SkyWalk
Union Station
MTCC Terrace Entrance/Exit
CN Tower
Entrance/Exit Closed
MTCC
Rogers Centre
Bremner Boulevard
Simcoe Street
York Street
Roundhouse
N
Legend
Pedestrian Detour Route
SkyWalk
Street



Rosedale
Bathurst
St George
Spadina
Bay
Bloor-Yonge
Sherbourne
Castle Frank
Museum
Wellesley
Queen's Park
College
St Patrick
Dundas
Osgoode
Queen
St Andrew
King
Union
Bloor-Danforth Platform Only
University of Toronto
Royal Ontario Museum
Bata Shoe Museum
Royal Conservatory of Music
Ontario Legislature
Art Gallery of Ontario
Roy Thomson Hall
Metro Convention Centre
Rogers Centre
CN Tower
Union Station
Toronto Island Ferry Terminal
Harbour Square Park
Ireland Park
Ferry to Billy Bishop Airport
Bloor St. West
Bloor St. East
College St.
Dundas St. W.
Queen St. W.
King St. W.
Front St. W.
Gardiner Expwy.
Lake Shore Blvd. W.
Queens Quay W.
Yonge St.
Bay St.
University Ave.
Spadina Ave.
Bathurst St.
Jarvis St.
Parliament St.
Sherbourne St.
SCALE
0 250 500 Metres
0 0.25 0.5 Mile
© mapmobility.com
Transit Information & Transit Graphics
© Toronto Transit Commission November 2010
1 Yonge-University-Spadina Subway
2 Bloor-Danforth Subway
Transfer required for connecting routes
Accessible Station
Regular Surface Routes (Bus, streetcar) Approx. 18-20 hours every day.
Streetcar Rapid Transit
Express Surface Routes
Rush Hour Surface Routes Monday to Friday rush hours only from approx. 6:00-9:00am and 3:00-6:00pm
Limited Service Surface Routes Service less than 18-20 hours every day, usually Monday to Friday from approx. 6:00am to 7:00pm with some evening and/or weekend service.
A rectangular box indicates the end point of a line.
A pointed box is angled to indicate the direction of travel.
Arrows indicate the direction of travel around a loop.
A plus indicates that all route branches run along that portion of the route.
GO Rail Lines
GO Bus Routes
Place of Interest

Local Organizing Committee

Anne Köhler
(Coordinator)

Melissa Chow
(Coordinator)

Vincent Sarrazy
(IT and on-site payments)

Charles Godbout
(AV and speaker support)

Sidharth Chaudhry
(AV and speaker support)

Elena Zimina
(Judges Support)

Franco Klingberg
(Posters, Judges Support)

YongGyun Kwon
(Poster Setup)

Nilesh Talele
(Photography)

Elizabeth Cambridge
(Registration, Support)

Chen Li
(Registration, Support)

Najib Yourish
(Finance Manager)

Marilyn Murphy
(Administrative Manager)

Scientific Committee

Nazish Ahmed (Samuel Lunenfeld Research Institute, U of T)
Vanessa Mendes (Institute of Biomaterials and Biomedical Engineering, U of T)
Andras Kapus (Department of Surgery, St. Michael's Hospital, U of T)
Morrie Manolson (Faculty of Dentistry, U of T)
Eli Sone (Institute of Biomaterials and Biomedical Engineering, U of T)
Tom Waddell (Cellular & Molecular Biology, Toronto General Research Institute, U of T)
Tom Willett (Department of Surgery, Orthopaedic Surgery, U of T)
Lidan You (Department of Mechanical and Industrial Engineering, U of T)

Our Sponsors:

Registration fees

since April 15	
Undergraduate and Graduate students	$130
Post-Docs, Research Associates	$155
Clinical residents and Fellows	$155
Senior Scientists, Professors and PIs	$180

on-site registration (credit card only!)	
Undergraduate and Graduate students	$160
Post-Docs, Research Associates	$185
Clinical residents and Fellows	$185
Senior Scientists, Professors and PIs	$210

Registration fees include attendance to all scientific sessions and poster display, lunch and coffee/tea during the official breaks, attendance to the welcome reception.

Please use our on-line registration form and credit card payment at:
http://connective-tissue-canada.com/registration/

Please note that on-site registration will be by credit card only!

Our Faculty

Keynote Speakers

Paul Janmey (University of Pennsylvania)
Mark Pittenger (University of Maryland)

Invited Speakers

Ben Alman	(University of Toronto)
Mike Buschmann	(École Polytechnique de Montréal)
Artur De Brum-Fernandes	(University de Sherbrooke)
Jeff Dixon	(University of Western Ontario)
Jan Dutz	(University of British Columbia)
Neil Duncan	(University of Calgary)
Julie Fradette	(University Laval, Quebec)
Lucie Germain	(University Laval, Quebec)
Fred Keeley	(University of Toronto)
Andras Kapus	(University of Toronto)
Andrew Leask	(University of Western Ontario)
Michael Raghunath	(University of Singapore)
Cari Whyne	(Sunnybrook Health Sciences Centre)

Banquet Dinner Honorary Speaker

Chris McCulloch (University of Toronto)

Talks from selected abstracts

The scientific committee and session chairs have selected the best 19 abstracts from the Post-doc, graduate and undergraduate categories for short oral communications in a blinded procedure. Every abstract has been reviewed by at least four referees. As in previous years, prizes for the best oral presentations will be awarded during the conference. To have their talk confirmed, all accepted presenters must register on-line ahead of the conference (http://connective-tissue-canada.com/registration/).

Many thanks to the panel members of the oral presentation selection committee: Scientific Committee:

Nazish Ahmed (U of T), *Janet Henderson* (McGill University), *Vanessa Mendes* (U of T), *Fackson Mwale* (McGill University), *Morrie Manolson* (U of T), *Dieter Reinhardt* (McGill University), *Eli Sone* (U of T), *Tom Willett* (U of T), *Lidan You* (Department of Mechanical and Industrial Engineering, U of T).

Keynote Speakers

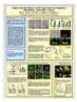
Dr. Paul Janmey received an A.B. degree from Oberlin College in 1976 and a Ph.D. in Physical Chemistry from the University of Wisconsin in 1982, working in the lab of J.D. Ferry on fibrin polymerization. A post-doctoral fellowship in the Hematology Unit of Massachusetts General Hospital motivated application of methods of polymer physics to the cytoskeleton. Since then his lab has studied the viscoelastic properties of biopolymer networks and the regulation of cytoskeletal and extracellular matrix assembly. Current work is focused on the response of cells to the viscoelastic properties of their environment and on developing new soft biocompatible materials for tissue engineering and wound healing.

Dr. Mark Pittenger is a cell and molecular biologist. He received his undergraduate training at Worcester Polytechnic Institute, Massachusetts, worked as a research associate in the Dept. of Biochemistry and Biophysics at U Penn. He entered Johns Hopkins University School of Medicine for his PhD training in the Dept. of Biological Chemistry to study tubulin expression with Dr. Don Cleveland and also studied non-muscle actin isoforms with Dr. Susan Craig. Dr. Pittenger performed post-doctoral studies on gene expression in the Dept. of Genetics at Yale University School of Medicine. He then moved to Cold Spring Harbor Laboratory to work with Dr. David Helfman on protein isoforms, expression systems, and regulation of cancer cells. In 1994, Dr. Pittenger joined start-up stem cell company Osiris Therapeutics Inc. to investigate the cell biology and gene expression of human mesenchymal stem cells. His group identified many characteristics of the hMSCs, and demonstrated clonally-derived hMSCs could differentiate to at least 3 different lineages. As early as 1998, they delivered MSCs into hearts with the goal of improving cardiac repair. With a cardiac surgical team at Johns Hopkins School of Medicine, they showed that MSC treatment could improve the remodeling that occurs after infarction in large animal models. Dr. Pittenger's team also made contributions towards understanding the interaction of MSCs with the different immune cells and identifying mechanisms by which allogeneic MSC reduce inflammation and allo-transplantation can be facilitated. Dr. Pittenger continues to investigate MSC biology, tissue regeneration, and clinical applications of MSCs, particularly in the heart.

Dr. Chris McCulloch is the Canada Research Chair in Matrix Dynamics at the Faculty of Dentistry, University of Toronto and is the Director of the Matrix Dynamics Group at that institution. His research focusses on how cell-matrix adhesions mediate the mechanical and chemical signals that enable matrix remodeling in cardiac and periodontal tissues. His laboratory is funded by the CIHR and by the Heart and Stroke Foundation. Notably, Chris has investigated and capitalized on new funding models of applied research through the SRED program. This program will be discussed at the Saturday night dinner in the context of investigator-driven companies and the impetus for finding new research support systems in Canada.

Poster Sessions

Posters will be displayed throughout the entire conference and presented in five poster sessions. Ample time will be given during conference breaks to view and informally discuss the posters. The best poster presentations will be awarded during the conference. Please see below for poster printing instructions.

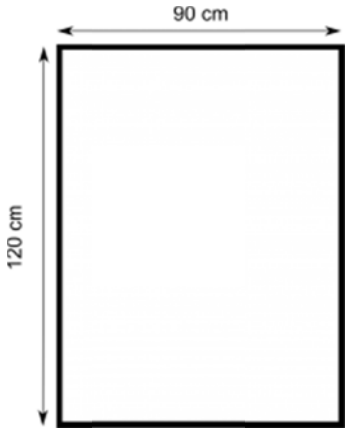


Poster Sessions Summary

Friday	06:30 -	Posters Setup	
Friday	06:30 - 08:00 pm	Poster Session I	(all numbers)
Saturday	12:35 - 02:00 pm	Poster Session II	(viewing all poster numbers)
Saturday	04:00 - 05:40 pm	Poster session III	(presenting even poster numbers)
Sunday	09:45 - 11:10 am	Poster session IV	(presenting odd poster numbers)
Sunday	12:25 - 02:00 pm	Poster session V	(all poster numbers) tear-down by

Poster Printing Instructions

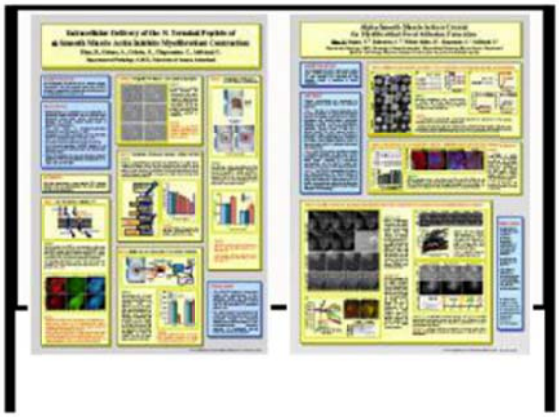
To avoid any problems during the poster setup, please follow these instructions carefully.



Poster dimensions are 90 cm wide and 120 cm high (vertical). Your poster width **must not** exceed 90 cm.

Since two posters will be displayed on the same panel there is no possibility to display posters wider than 90 cm.

We are afraid that wider posters will need to be rejected since they will compromise the display of posters on the same board.



Join the Canoe Event to raise money for The Arthritis Society

The Arthritis Society and The Canadian Arthritis Trainee Association (CATA) are sponsors of the CCTC 2102, Why not returning some of the favor and raise money the fun way by going for a free sunset canoe ride in the Toronto harbor Sunday night, June 10th.

A message from Morris Manolson:

"Dear CCTC participant, as part of the CCTC meeting this year, we are forming a team to fundraise for the Arthritis Society. If our CCTC team raises over $2,500 in donations, the active fundraisers will get to go on a free sunset canoe ride on the Toronto Harbor, courtesy of the Harbourfront Canoe & Kayak Centre.

I strongly encourage all students and supervisors who have ever received any type of funding from The Arthritis Society and/or the Canadian Arthritis Network to participate in this fund raising event. This is our opportunity, and I would argue, our responsibility to give back to the Society which has helped fund our research and our trainees for so many years.

The Arthritis Society is having their annual 'Walk for Arthritis" on the morning of June 10th, when everyone at CCTC will unfortunately be attending the first session of talks. So as an incentive to fundraise, the Harbourfront Canoe & Kayak Centre has very graciously agreed to give the CCTC team members a sunset tour of the Toronto Harbor in a voyager canoe that night! No canoeing experience necessary, and if you have not been in the Toronto Harbor at sunset, you are missing a great experience and the best view of the Toronto skyline!!

Please help me give back to the society which has funded our research and trainees for so many years. Click on the link below, type in CCTC for the "Team Name" (full name of the team is CCTC-Canoe Kayak Centre-CATA), join the team and start raising money:

https://arthritis.akaraisin.com/Common/Participant/Search.aspx?seid=4761&mid=23

Maybe a blurb about what CATA is: The CATA is a group that brings trainees affiliated with arthritis-related research together to create a strong Canadian student-based network that will contribute and actively participate to the field of arthritis research.

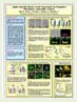
Looking forward to canoeing with all CCTC team members on Sunday night!

Morris F. Manolson
Faculty of Dentistry University of Toronto
email: m.manolson@utoronto.ca
Office: 416-979-4900 ext 1-4392#, FAX: 416-979-4936
124 Edwards St., Toronto, Ontario, M5G 1G6
http://www.utoronto.ca/bonelab/home.htm"

Friday

Friday, 08.06.2012

05:30 pm	Registration opens (until 08:00 pm)
06:00 pm	Poster mounting (all posters on display all days)

06:30 - 08:00 pm		**Poster Session I (all poster numbers)**

06:30 pm	**Welcome Reception**

Saturday

Saturday, 09.06.2012

08:00 am	Registration opens (until 11:00)
08:00 am	Mounting of late posters

Session 1: Cellular Interactions Between Connective Tissue and Other Tissues

Co-chairs: ***Nazish Ahmed (University of Toronto), Dirk Hubmacher (McGill University)***

08:30 - 09:00 am	Opening Remarks (Boris Hinz, Craig Simmons, Conference Chairs)	**08:30 - 10:25 am**
09:00 - 09:20 am	**Andras Kapus** (University of Toronto) *"Epithelial-myofibroblast transition: the concept of topical susceptibility to TGFβ"*	
09:20 - 09:35 am	**Carole L. Galligan** (University Health Network, Toronto) *"Activated fibrocytes contribute to inflammation and tissue remodelling in rheumatoid arthritis"*	
09:35 - 09:55 am	**Lucie Germain** (University Laval, Quebec) *"Exploiting the cross-talk between keratinocytes and fibroblasts to improve tissue-engineered skin substitutes"*	
09:55 - 10:05 am	**Wing-Yee Cheung** (University of Toronto) *"Effect of osteocyte apoptosis on osteoclast precursor recruitment"*	
10:05 - 10:25 am	**Jan Dutz** (University of British Columbia) *"Morphea - a sclerotic skin condition associated with type 1 interferon release"*	
10:25 - 11:00 am	***Coffee Break, poster viewing (sponsored by The Canadian Arthritis Trainee Association, CATA)***	

Session 2: Stem Cells, Cell Differentiation, and Tissue Engineering

Co-chairs: Eli Sone (University of Toronto), Vanessa Mendes (University of Toronto)

11:00 - 11:40 am	**Keynote lecture: Mark Pittenger** (University of Maryland) *"Mesenchymal Stem Cells: A Paradigm for Cellular Therapy and Tissue Engineering"*	11:00 am - 12:35 pm
11:40 - 11:50 am	**Nilesh Talele** (University of Toronto) *"Culture on soft substrates suppresses the fibrotic fate of mesenchymal stem cells"*	
11:50 - 12:05 am	**Irina Voronov** (University of Toronto) *"Elucidating the mechanism of impaired in vitro osteoclastogenesis in cells from +/R740S osteopetrotic mice"*	
12:05 - 12:25 am	**Julie Fradette** (University Laval, Quebec) *"Expanding" tissue engineering applications through the use of human adipose-derived stem cells"*	
12:25 - 12:35 pm	**Nancy Karam** (Sainte-Justine University Hospital, Montreal) *"Overexpression of transcription factor Pitx1 inhibits osteoblast differentiation and bone mineralization"*	
12:35 - 02:00 pm	***Lunch, Poster viewing (Poster Session II)***	

12:35 - 02:00 pm  **Poster Session II (viewing all poster numbers)**

Session 3: Connective Tissue Protein Structure and Function

Co-chairs: Tom Willett (Mt. Sinai Hospital, Toronto), Lisa Muiznieks (Univ. of Toronto)

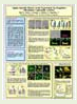
02:00 - 02:20 pm	**Fred Keeley** (University of Toronto) *"Rules of disorder: Why elastin is elastic"*	02:00 - 02:45 pm
02:20 - 02:30 pm	**Betty Hoac** (McGill University, Montreal) *"Proteolytic processing of osteopontin by PHEX and accumulation of osteopontin fragments in Hyp mouse bone, the murine model of X-linked hypophosphatemia"*	
02:30 - 02:45 pm	**Eric Bergeron** (McGill University, Montreal) *"Fibrillin assembly is dependent on the integrin-binding RGD motif, but not the free cysteine in the first hybrid domain"*	

Saturday

Session 4: Bone, Cartilage and Tooth Development

Co-chairs: Morrie Manolson (University of Toronto), Kristen Fay Gorman (Univ. of Vermont)

02:45 - 03:05 pm	**Mike Buschmann** (École Polytechnique de Montréal) *"Polymeric Nanoparticles for Novel Therapeutic Applications"*	02:45 - 04:00 pm
03:05 - 03:20 pm	**Nazish Ahmed** (University of Toronto) *"Passaged chondrocytes acquire characteristics of bone marrow stromal cells"*	
03:20 - 03:40 am	**Jeff Dixon** (University of Western Ontario) *"The network of purinergic ligands and receptors in bone: Emerging roles in development, remodeling and mechanotransduction"*	
03:40 - 03:50 pm	**Sharifa Alebrahim** (McGill University, Montreal) *"Role of sphingomyelin phosphodiesterase 3 in tooth mineralization"*	
03:50 - 04:00 pm	**Noelle Ochotny** (University of Toronto) *"The V-ATPase a3 subunit mutation R740S results in osteoclast apoptosis possibly due to defective autophagy"*	
04:00 - 05:40 pm	***Coffee Break, poster session III***	

04:00 - 5:40 pm  **Poster session III (even poster numbers)**

Banquet Dinner (CN Tower 360° Restaurant)

07:30 pm **Honorary Speaker: Chris McCulloch (University of Toronto)**

"Braving the New World of Research Funding"

Sunday, 10.06.2012

Session 5: Biomechanics, Mechanobiology, and Biomaterials

Co-chairs: Lidan You (University of Toronto), Fackson Mwale (McGill University)

08:30 - 09:45 am

08:30 - 08:50 am	**Neil Duncan** (University of Calgary) *"Mechanics of Stem Cell Differentiation in Fracture Repair"*
08:50 - 09:05 am	**Derek H. Rosenzweig** (McGill University, Montreal) *"Cartilage mechanical injury induces chondrocyte apoptosis via MAP kinase signalling"*
09:05 - 09:15 am	**Wen Li Kelly Chen** (University of Toronto) *"Cdc42 is a convergent regulator of matrix mechanical and biochemical signaling for mesenchymal stem cell osteogenic differentiation"*
09:15 - 09:35 am	**Ben Alman** (University of Toronto) *"Developmentally Important Signalling Pathways in Osteoarthritis"*
09:35 - 09:45 am	**P. C. Dave P. Dingal** (University of Pennsylvania) *"Fibrillar collagen is equivalent to stiff ECM in driving marrow stromal cell differentiation into ECM-deficient, myofibroblastic-like phenotype"*
09:45 - 11:10 am	***Coffee Break, Poster session IV***

09:45 - 11:10 am  **Poster session IV (odd poster numbers)**

Session 6: The Extracellular Matrix in Connective Tissues

Co-chairs: Dieter Reinhardt (McGill University), Elena Zimina (University of Toronto)

11:10 - 12:25 am

11:10 - 11:50 am	**Keynote Lecture: Paul Janmey** (University of Pennsylvania) *"Mechanosensing through multiple types of transmembrane receptors"*
11:50 - 12:05 am	**Vincent Sarrazy** (University of Toronto) *"TGF-ß1 is activated by integrin-mediated contraction of human cardiac fibroblasts"*
12:05 - 12:15 pm	**Alexander J. Lausch** (University of Toronto) *"A physiologically relevant model of collagen mineralization"*
12:15 - 12:25 pm	**Vamsee Dhar Myneni** (McGill University, Montreal) *"Transglutaminase enzymes regulate adipocyte differentiation via modulating fibronectin synthesis and actin cytoskeleton"*
12:25 - 2:00 pm	***Lunch, Poster Session V***
12:25 - 02:00 pm	Board meeting CCTS, Lunch provided, Red Room

Sunday

Sunday

12:25 - 02:00 pm		**Poster session V (all numbers) tear-down by 2 pm**

Session 7: Connective Tissue in Disease - Clinical and Fundamental Aspects

Co-chairs: ***Tom Waddell (University of Toronto), Helen Langevin (McGill University)***

02:00 - 03:00 pm

02:00 - 02:20 pm	**Artur J De Brum-Fernandes** (Université de Sherbrooke) *"PGD2 and its analogues as potential anabolic agents for bone"*
02:20 - 02:30 pm	**Sumeeta Warraich** (University of Western Ontario) *"Loss of Equilibrative Nucleoside Transporter 1 (ENT1) in Mice Leads to Progressive Ectopic Mineralization of Spinal Tissues Resembling Diffuse Idiopathic Skeletal Hyperostosis (DISH) in Humans"*
02:30 - 02:40 pm	**Bashar Alkhatib** (McGill University, Montreal) *"Chondroadherin Fragmentation as a Biochemical Marker for Early Stage Disc Degeneration"*
02:40 - 03:00 pm	**Cari Whyne** (Sunnybrook Health Sciences Centre) *"Spinal Metastases: Structural Integrity and Response to Treatment"*

Session 8: Connective Tissue Remodelling

Co-chairs: Janet Henderson (McGill University), Jean-Francois Lavoie (Univ. of Toronto)

03:00 - 03:50 pm

03:00 - 03:20 pm	**Michael Raghunath** (Nationaly University of Singapore) *"Growth factor ambush: How short can TGFβ pulses get and how long do they last (in vitro)?"*
03:20 - 03:30 pm	**Michael H. Wang** (McGill University, Montreal) *"The Effect of Mast Cell Deficiency on Bone Tissue Regeneration"*
03:30 - 03:50 pm	**Andrew Leask:** (University of Western Ontario) *"CCN2: a master regulator of tissue repair and fibrosis"*

03:50 - 04:00 pm	A note from The Canadian Arthritis Trainee Association
04:00 - 04:30 pm	Closing remarks and award ceremony: Craig Simmons, Boris Hinz
04:30 - 04:50 pm	CCTS Business meeting: Jeff Dixon, Boris Hinz
05:00 pm	End of Conference

Abstracts Oral Presentations

(by order of appearance in the program)

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Abstract Number: O01

Invited Speaker

EPITHELIAL-MYOFIBROBLAST TRANSITION: THE CONCEPT OF TOPICAL SUSCEPTIBILITY TO TGFβ

Andras Kapus

Keenan Research Centre in the Li Ka Shing Knwledge Institute, St. Michael's Hospital and Dept Surgery, University of Toronto

Epithelial-myofibroblast transition (EMyT), the most robust form of epithelial-mesenchymal transition characterized by α-smooth muscle actin (SMA) expression, is a significant contributor to fibrogenesis. While TGFβ is indispensable for EMyT, it is usually not sufficient, as intact epithelia are often resistant to the myogenic (SMA-inducing) effect of this cytokine. The other prerequisite is the uncoupling/injury of the intracellular contacts (two-hit paradigm). These inputs synergize to activate myocardin-related transcription factor (MRTF) a major driver of the SMA gene. These observations raised the idea that intact and contact-deprived areas of the same epithelial layer might exhibit differential susceptibility to TGFβ. To test this hypothesis we developed a model, wherein contact formation was prevented by a surgical tape ("wound') in the middle of an otherwise confluent monolayer of kidney tubular cells, which was then left untreated or exposed to TGFβ. Subsequently 2-mm cellular strips adjacent to the "wound" or in the intact area were removed and analyzed by qPCR and western blots. TGFβ provoked SMA expression exclusively in the wound and exerted dramatically different effects at the two loci both in terms of transcription and stability of key myogenic regulators (Smad3, β-catenin, MRTF). Moreover, contact disassembly induced the lengthening of the primary cilium, while TGFβ triggered complete deciliation of the cells exclusively at the wound. Chemical deciliation before EMyT-induction profoundly inhibited TGFβ-induced myogenic program and altered the stability of myogenic regulators. Taken together, cell contacts and the primary cilium are key determinants of topical sensitivity to TGFβ, predisposing injured areas to EMyT and fibrogenesis.

Abstract Number: O02

Research Associate

ACTIVATED FIBROCYTES CONTRIBUTE TO INFLAMMATION AND TISSUE REMODELLING IN RHEUMATOID ARTHRITIS

Carole L. Galligan[*#] **and Eleanor N. Fish**[*#]

[*] Department of Immunology, University of Toronto, [#] Toronto General Research Institute, University Health Network, Toronto, Ontario, Canada

Rheumatoid arthritis (RA) is a common, systemic autoimmune disease resulting in joint inflammation. Fibroblast-like synovial (FLS) cells in affected joints are responsible for pannus formation and cytokine/chemokine production, resulting in the recruitment of leukocytes and bone and cartilage destruction. We have identified a multipotent stem cell population of activated fibrocytes in the peripheral blood of individuals with RA that may have a role in the pathogenesis of RA. We characterized the contribution of circulating fibrocytes to the pathogenesis of RA in mouse models of disease. Activated fibrocytes were isolated from mice with collagen-induced arthritis (CIA) and transferred intravenously (iv) into recipient mice induced to develop collagen-antibody induced arthritis (CAIA). The activation status of fibrocytes was determined using multidimensional phosphoflow cytometric analysis of the intracellular signaling effectors, STAT5, STAT1, AKT and JNK. Fibrocyte trafficking and matrix metalloproteinase (MMP) activity were assessed in real time using fluorescence molecular tomography (FMT), specifically labeling fibrocytes with CellVue Maroon and measuring MMP activity using MMPSense 680. Circulating numbers of fibrocytes are increased early during onset of CAIA, concomitant with their activation, as measured by intracellular phosphorylation of STAT5. Adoptive transfer of activated fibrocytes augmented disease scores and increased MHCII expression and PBMC phosphoactivation profiles in recipient mice with CAIA. Notably, adoptively transferred fluorescent labeled activated fibrocytes rapidly migrated into affected joints in recipient mice with CAIA and this was associated with augmented neutrophil recruitment into affected joints and MMP activation. Therefore, circulating activated fibrocytes migrate to joints and influence the onset of disease processes in arthritis.

Abstract Number: O03

Invited Speaker

EXPLOITING THE CROSS-TALK BETWEEN KERATINOCYTES AND FIBROBLASTS TO IMPROVE TISSUE-ENGINEERED SKIN SUBSTITUTES

Lucie Germain

Laval University, LOEX

Complex tissue-engineered skin substitute (TES) reconstructed with both epidermal and dermal layers are developed in vitro for experimental and clinical applications. While specific properties pertaining either to the epidermis or to the dermis are expected, the cross-talk between keratinocytes and fibroblasts is essential to achieve an adequate functionality. Tissue-engineered skin (TES) produced by the self-assembly approach will be used as an example to show how the interactions between keratinocytes and fibroblasts modifies these specific properties. TES is obtained by culturing keratinocytes on tissue-engineered dermis (TED) comprised of fibroblasts and the endogenous extracellular matrix they synthetized and organized in vitro. In contrast to other models, epithelial stem cells are preserved in the basal layer of these TES. This is crucial to ensure the long-term integration of such substitutes after grafting. The persistence of stem cells is attributed to the well-organized basement membrane at the dermo-epidermal junction, for which cross-talk is necessary. The dermis is responsible for the mechanical properties of the skin. TES presents adequate resistance and elasticity. Contraction was monitored over a one week period after TES detachment from their anchoring substrate and transfer on a soft gel reproducing the mechanical properties of connective tissue. TED contraction was much greater than TES contraction indicating that keratinocytes influences the structural stability of the engineered tissue. The maturation of the epidermis induced by culturing at the air-liquid interface ensures minimal contraction of the TES. TES structural stability is essential since the main sequalae of patients surviving major burn injuries result from contractures and hypertrophic scars. The potential role of several factors such as extracellular matrix proteins, matrix metalloproteinases and growth factors in the epithelial cells-fibroblasts interactions will be discussed.

Abstract Number: O04

Graduate Research

EFFECT OF OSTEOCYTE APOPTOSIS ON OSTEOCLAST PRECURSOR RECRUITMENT

Wing-Yee Cheung[*#], **Craig A. Simmons**[#,$,], **Lidan You**[#,$]

[#] Institute of Biomaterials and Biomedical Engineering, University of Toronto, [$] Department of Mechanical and Industrial Engineering, University of Toronto

Osteocyte apoptosis precedes osteoclast resorption in vivo. However, the specific mechanism by which osteoclast precursors (OCPs) are initially delivered to a remodeling site is not well understood. We hypothesize that apoptotic osteocytes secrete cytokines that: 1) promote angiogenesis such that OCPs can be delivered closer to the remodeling site; and 2) activate the endothelium to promote OCP adhesion and transmigration across the endothelium and into the remodeling site directly.

In our in vitro study, we found that osteocyte populations containing apoptotic cells promote angiogenesis (endothelial cell proliferation, migration, and tubule formation, $p<0.05$ respectively) in a VEGF-mediated manner. Furthermore, we found that osteocyte populations containing apoptotic cells secrete elevated levels of IL-6 and sIL-6R ($p<0.05$). IL-6 and sIL-6R activated the endothelium to express ICAM-1 ($p<0.05$), which assisted in OCP adhesion ($p<0.05$).

To verify our observations in vivo, we are using an ovariectomized (OVX) mouse model that allows us to observe the effect of apoptotic osteocytes in OCP recruitment. OVX or sham surgeries were performed on 17-week-old virgin female C57/Bl6 mice (n=10/condition). Mice were sacrificed 7 days after surgery. Femurs were collected, embedded, sectioned and stained. Cleaved caspase-3, CD31 and RANK receptor antibodies were used to stain for apoptotic cells, endothelial cells and OCPs, respectively. In on-going work, spatial correlations between apoptotic osteocytes, newly formed blood vessels and OCPs are being determined in OVX and sham conditions.

Data from this study will provide new insight on the interactions between apoptotic osteocytes, endothelial cells and OCPs at the initial onset of bone remodeling.

Abstract Number: O05

Invited Speaker

MORPHEA - A SCLEROTIC SKIN CONDITION ASSOCIATED WITH TYPE 1 INTERFERON RELEASE

Jan Peter Dutz

Professor, Department of Dermatology and Skin Science, University of British Columbia

As type 1 interferons (IFN) have been implicated in the pathogenesis of systemic sclerosis, we proposed that type 1 IFN promote localized inflammation and fibrosis in morphea. To investigate the expression of the type 1 IFN-inducible protein myxovirus A (MxA) and the presence of plasmacytoid dendritic cells (pDC) in lesions of morphea, lesional skin of 10 patients with morphea was examined by immunohistochemistry for the presence of the type 1 IFN-inducible protein, myxovirus A (MxA), and the pDC markers, CD123 and BDCA-2, and was compared with lesional skin of cutaneous lupus erythematosus, lichen planus and keloid. Lesional and non-lesional morphea skin was compared. MxA was expressed in the epidermis as well as the reticular dermis and subcutis in morphea. pDCs were abundant around vessels and between fibrous bundles. Non-lesional biopsies demonstrated little or no expression of MxA and pDC markers. Keloid showed minimal expression of MxA and pDC markers. We demonstrate the expression of type 1 IFN-related protein MxA and plasmacytoid DCs in lesional but not in non-lesional biopsies of morphea. These findings suggest a potential role for type 1 interferons in the pathogenesis of morphea. The role of inflammation in morphea will be reviewed.

Abstract Number: O06

Keynote Speaker

MESENCHYMAL STEM CELLS: A PARADIGM FOR CELLULAR THERAPY AND TISSUE ENGINEERING

Mark F. Pittenger

University of Maryland School of Medicine

Mesenchymal Stem Cells (MSCs) are being studied by an ever increasing number of laboratories, leading to a rich and complex field. Many reports have been published about mesenchymal cell biology, control of gene expression, cellular differentiation, responses to tissue damage and the initiation of repair. As models of stem cells, MSCs can teach us about control of proliferation and differentiation signals, and the genes that regulate mesenchymal decisions. As building blocks for tissue engineering, they can help us design strategies for creating complex tissues. As players in the body's immune system, MSCs are a useful tool to help dissect complex innate and adaptive responses, and understand whether MSCs can be used successfully for allogeneic as well as autologous transplantation. There is a complexity to MSC biology that can confound experts as well as researchers new to the field. While the field of MSC research and applications is expanding rapidly, it is useful to examine the characteristics of MSCs, their interactions with other cell types, biomaterials and the use of MSC's for tissue repair and regeneration. As always, there remain some unanswered questions about MSCs, but their properties insure there will be continued interest in these cells even when iPS or ES cells become more readily available and better understood.

Abstract Number: O07

Graduate Research

CULTURE ON SOFT SUBSTRATES SUPPRESSES THE FIBROTIC FATE OF MESENCHYMAL STEM CELLS

N. TALELE[$], J. FRADETTE[^], J. DAVIES[*], B. HINZ[$]

[$] Laboratory of Tissue Repair and Regeneration, Matrix Dynamics Group, Faculty of Dentistry, University of Toronto, Toronto, Canada, ^ Laboratoire d'Organogénèse Expérimentale, Centre de recherche FRSQ du CHA Universitaire de Québec, Université Laval, Québec, Canada, [*] Institute for Biomaterials and Biomedical Engineering, University of Toronto, Toronto, Canada

Mesenchymal stem cells (MSCs) used for the treatment of fibrocontractive diseases, bear the risk of differentiating into fibrotic myofibroblasts (MFs) that worsen the disease. One main stimulating factor for MF differentiation is the stiffness of the fibrotic tissue.

We hypothesize that mechanically triggered MF differentiation reduces the regenerative potential of MSCs.

First, to identify a suitable MSC source, fibrogenesis was compared between MSCs from adipose tissue (hAMSCs), bone marrow (hBMSCs) and umbilical cord (hUMSCs). Second, rat MSCs were directly isolated on normal-soft versus fibrosis-stiff substrates and then sub-cultured on a range of differently stiff substrates for assessing their profibrogenic potential. Third, we tested whether MF differentiation affects the multipotency of MSCs. Sorted MF-MSC and non-MF-MSC populations were subjected to stiff and soft substrates. We then compared both populations for the presence of MSC surface markers by immunofluorescence microscopy, flow cytometry and their potential to undergo induced tri-lineage differentiation.

Growth of hAMSCs and hBMSCs on soft substrates resulted in decreased expression of the MF marker α-SMA, in contrast to high levels of fibrotic marker on stiff substrates. Direct isolation of rBMSCs on soft substrates suppressed fibrotic development that was stimulated by sub-culture on stiff but not on soft substrates. Similarly, purified hBMSC-MF populations lost fibrotic characteristics upon soft substrate culture. Non-MF-MSCs and MF-MSCs both expressed MSC surface markers. However, MF-MSCs exhibited different tri-lineage potential compared with non-MF-MSCs.

Our approach of MSC isolation and subculture on tissue-compliant substrates eliminates the culture plastic effect of inducing spontaneous MF differentiation.

Abstract Number: O08

Post-doctoral Research

ELUCIDATING THE MECHANISM OF IMPAIRED IN VITRO OSTEOCLASTOGENESIS IN CELLS FROM +/R740S OSTEOPETROTIC MICE

Voronov I. [#], **Ochotny N.** [#], **Jaumouillé V.** [$], **Owen C.** [*], **Aubin J.E.** [#%], **Manolson M.F.** [#]

[#] Faculty of Dentistry, University of Toronto, [$] Cell Biology Program, Research Institute, Hospital for Sick Children, [*] Centre for Modeling Human Disease, Samuel Lunenfeld Research Institute, Mt Sinai Hospital, [%] Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada

Vacuolar H+-ATPase (V-ATPase) is a multimeric enzyme at the ruffled border of osteoclasts responsible for acidification of the resorption lacunae. V-ATPases containing the a3 subunit are highly enriched in osteoclasts and are located at the ruffled border in the resorbing cells or in the lysosomes in non-resorbing osteoclasts. Heterozygous mice with an R740S mutation in a3 (+/R740S) have defective V-ATPase activity resulting in mild osteopetrosis. Lysosomal pH in +/R740S osteoclasts is significantly higher compared to +/+ cells. Furthermore, unlike other models of osteopetrosis, in vitro osteoclastogenesis in +/R740S cells is impaired. These results suggest that V-ATPase-dependent lysosomal acidification plays a role in osteoclast differentiation.

Gene expression analysis of +/R740S and +/+ bone marrow-derived osteoclasts showed that mRNA levels of key osteoclast markers, e.g. TRAP, OSCAR, NFATc1, were significantly decreased in +/R740S cells. Immunoblotting confirmed these results. This diminution of gene and protein expression was due to decreased NFATc1 activation in +/R740S osteoclasts compared to controls, as measured by nuclear translocation using immunofluorescence. The molecular mechanism of NFATc1 inhibition is still not known, however, we do know that it is not due to increased levels of calcineurin A, the phosphatase responsible for de-phosphorylation of NFATc1, or DYRK1A, one of the kinases responsible for phosphorylating NFATc1.

Our results suggest that lysosomal pH plays an important role in transcription factor activation. Future experiments will determine the mechanism of impaired NFATc1 activation and will help to elucidate precise roles of the V-ATPase a3 subunit in osteoclastogenesis.

Abstract Number: O09

Invited Speaker

«EXPANDING» TISSUE ENGINEERING APPLICATIONS THROUGH THE USE OF HUMAN ADIPOSE-DERIVED STEM CELLS

Julie Fradette

LOEX, Université Laval, Department of Surgery

Regenerative medicine and tissue engineering applications have greatly expanded with the discovery of postnatal mesenchymal stem cells featuring great plasticity. Such stem cells can be harvested from adipose tissue which represents an almost ideal cell source. My team is using these adipose-derived stem/stromal cells (ASCs) as building blocks for the production of various human tissues, including adipose tissue itself and connective tissues serving as stromal compartments for skin and bladder reconstruction. These unique tissues feature a rich extracellular matrix produced by the mesenchymal cells, recreating a very physiological 3D environment without exogenous biomaterials. In order to engineer functional adipose tissues in vitro, a differentiation step has been added during tissue formation. The resulting tissues secreted important levels of cytokines and growth factors such as leptin, Ang-1, HGF, and VEGF. When engineered for the purpose of becoming autologous substitutes, these tissues can further be enhanced by adding a preformed capillary network in vitro, which then stimulates vascularization by the host, as determined by confocal microscopy analyses at various time-points. Magnetic resonance imaging of nude mice grafted with engineered human tissues allowed non-invasive evaluation of volume retention over time. The different types of human tissues that we are engineering are allowing us to study interactions between adipocytes and endothelial cells as well as gene profiling of extracellular matrix variations and remodeling associated with adipogenic differentiation in a tissular context. Finally, novel data reporting on bone tissue engineering using ASCs will be presented. Supported by CIHR and NSERC.

Abstract Number: O10

Graduate Research

OVEREXPRESSION OF TRANSCRIPTION FACTOR PITX1 INHIBITS OSTEOBLAST DIFFRENTIATION AND BONE MINERALIZATION

Nancy Karam[#,$],M.Sc., Benoit St-Jacques, Ph.D[$], Jean Francois Lavoie[#, $]Ph.D, Alain Moreau[#, %, $]Ph.D.

[#]Viscogliosi Laboratory in Molecular Genetics of Musculoskeletal Diseases, Sainte-Justine University Hospital Research Centre, Montréal, Qc, CANADA; [%]Department of Stomatology, Faculty of Dentistry, Université de Montréal; [$]Department of Biochemistry, Faculty of Medicine, Université de Montréal;

Ageing Pitx1+/- heterozygous mice develops OA-like lesions in cartilage associated with a drastic increase in cortical and trabecular bone formation.

Conversely, expression analysis of osteoblasts derived from monozygotic twins discordant for osteoporosis reported a 8.6 fold up-regulation of Pitx1 expression in osteoblasts from osteoporotic twins when compared to healthy ones.

Collectively, these data prompted us to investigate the role of this transcription factor in osteoblast differentiation and bone mineralization. Transgenic mCol1a1-Pitx1 mice over expressing Pitx1 specifically in bone tissue under the type-I collagen promoter were generated and phenotypically characterized.

MicroCT analysis showed a 30% decrease in the cortical thickness of transgenic mice, a 15 to 30% decrease in the trabecular thickness and a 16% to 50% reduction in the trabecular separation and connectivity, with a shift of the trabecular micro-architecture from rode-like to a plate-like forms when compared to the wild-type ones. Biomechanical assessment of transgenic femurs using the tri-bending test points revealed a reduction of 25% to 58% in bone strength. Histomorphometric quantification demonstrated a decreased bone formation rate compared with wild-type littermate. At the biochemical level, RANKL/OPG ratio decreased by 25 to 50% in transgenic mice when compared to wild-type ones suggesting a compensatory mechanism to alleviate the effects of PITX1 by reducing osteoclastogenesis. Differentiation assays in vitro showed that overexpression of PITX1 hardly abrogates the maturation of mCol1a1-Pitx1 osteoblasts when compared with wild- type ones.

Collectively, these data define an unrecognized and crucial role for PITX1 in the regulation of osteoblast differentiation and bone mineralization

Abstract Number: O11

Invited Speaker

RULES OF DISORDER: WHY ELASTIN IS ELASTIC

Fred W Keeley

Molecular Structure and Function Program, Research Institute, Hospital for Sick Children

Elastin is the connective tissue protein responsible for the properties of extensibility and elastic recoil in large arteries, lung parencyma and other tissues. Because elastin, once laid down in its polymeric form in the extracellular matrix, does not turnover at any appreciable rate the protein must have remarkable durability to withstand a lifetime of extension and recoil without mechanical failure. The structure of elastin appears to be 'precariously' balanced between the extensive conformational disorder required of an entropic elastomer and the hydrophobic collapse that might be expected of a protein in which over 80% of the amino acids residues are non-polar in character. At the same time, interactions between hydrophobic domains are clearly important for the ability of elastin monomers to self-organize into a network structure. We will describe how, together with our colleagues, our laboratory uses biochemical, bioinformatic and molecular modeling approaches to understand how this balance and the unusual physical properties of elastin arise from sequence and domain arrangements in the protein.

Abstract Number: O12

Graduate Research

PROTEOLYTIC PROCESSING OF OSTEOPONTIN BY PHEX AND ACCUMULATION OF OSTEOPONTIN FRAGMENTS IN HYP MOUSE BONE, THE MURINE MODEL OF X-LINKED HYPOPHOSPHATEMIA

Hoac B[#*], Barros NMT[$§*], Neves RL[$§], Addison WN[%], Assis DM[$], Murshed M[#†], Carmona AK[$], and McKee MD[#‡]

[#] Faculty of Dentistry, McGill University, Montreal, QC, Canada, [$] Departamento de Biofísica, Universidade Federal de São Paulo, São Paulo, SP, Brazil, [§] Departamento de Ciências Exatas e da Terra, Universidade Federal de São Paulo, Diadema, SP, Brazil, [%] Department of Oral Medicine, Infection and Immunity, Harvard School of Dental Medicine, Boston, MA, USA, † Department of Medicine, Faculty of Medicine, McGill University, Montreal, QC, Canada, [‡] Department of Anatomy and Cell Biology, Faculty of Medicine, McGill University, Montreal, QC, Canada, [*] contributed equally to the work

X-linked hypophosphatemia (XLH/HYP) is caused by mutations in the zinc-metallopeptidase PHEX gene (phosphate-regulating gene with homologies to endopeptidase on the X chromosome). PHEX is expressed by mineralized tissue cells, and inactivating mutations in PHEX lead to distal renal effects and accumulation of mineralization-inhibiting, ASARM-containing (acidic serine- and aspartate-rich motif) peptides derived from the mineral-binding matrix proteins of the SIBLING family (small, integrin-binding ligand N-linked glycoproteins). While the latter observation suggests a local, direct matrix effect for PHEX, its physiologically relevant substrate proteins have not been identified. Here, we investigated two SIBLING proteins containing the ASARM motif – OPN (osteopontin) and BSP (bone sialoprotein) – as potential substrates for PHEX. Using cleavage assays, gel electrophoresis and mass spectrometry, we report that OPN is a full-length protein substrate for PHEX. Degradation of OPN was essentially complete, including hydrolysis of ASARM, resulting in only small residual fragments. Immunoblotting of Hyp (the murine homolog of human XLH/HYP) mouse bone extracts lacking PHEX activity clearly showed accumulation of an ~35 kDa OPN fragment not present in wild type mouse bone. Immunohistochemistry and immunogold labeling for OPN in Hyp bone likewise showed accumulation of OPN and/or its fragments as compared to wild type bone. Incubation of Hyp mouse bone extracts with PHEX resulted in the complete degradation of these fragments. In conclusion, these results identify full-length OPN and its fragments as novel, physiologically relevant substrates for PHEX, suggesting that accumulation of mineralization-inhibiting OPN fragments may contribute to the osteomalacia characteristic of XLH/HYP. Supported by CIHR, FRQ-S and FAPESP.

Abstract Number: O13

Post-doctoral Research

FIBRILLIN ASSEMBLY IS DEPENDENT ON THE INTEGRIN-BINDING RGD MOTIF, BUT NOT THE FREE CYSTEINE IN THE FIRST HYBRID DOMAIN

Dirk Hubmacher[#,§,¶], Eric Bergeron[#,¶], and Dieter P. Reinhardt[#,*]

[#]Department of Anatomy and Cell Biology, Faculty of Medicine, McGill University, Montreal, QC, Canada, [§]Present address: Lerner Research Institute, Cleveland Clinic, Cleveland, OH, USA, [*]Faculty of Dentistry, Division of Biomedical Sciences, McGill University, Montreal, QC, Canada, [¶]These authors contributed equally

Fibrillins (FBNs) are major structural components of extracellular microfibrils. Marfan syndrome is the most common disorder associated with mutations in FBN-1 affecting the skeletal, ocular and cardiovascular systems. Some key functional sites have been suggested for critical roles in FBN assembly and functionality. Since FBNs undergo disulfide-bond mediated multimerization in the microfibril assembly process, the unpaired Cys204 in FBN 1 is a candidate for intermolecular disulfide-bond cross-links. Furthermore, FBN-1 contains one RGD site which interacts with integrins and regulates cell attachment. Mutations close to this site cause stiff skin syndrome. In order to determine assembly properties of FBN-1 and to analyze the role of key functional sites, we have produced a recombinant wild-type full length FBN-1 (FBN-1-WT), a RGD-RGA mutant (FBN-1-RGA) and a Cys204-Ser204 mutant (FBN-1-Ser). These full length constructs were produced and secreted by epithelial-like HEK293 cells, but they did not assemble into typical microfibril networks. The FBN-1-WT and the FBN-1-Ser mutants deposited as punctae in the extracellular matrix, while the FBN-1-RGA mutant did not show this dotted deposition pattern. Co-culture of the recombinant HEK293 cells with mouse NIH3T3 fibroblasts triggered microfibril assembly of the secreted FBN-1-WT and FBN-1-Ser mutants, a process that required the presence of fibronectin. The FBN-1-RGA mutant, however, did not assemble into microfibrils. These results demonstrate a critical role of the RGD site in FBN-1 in the assembly of microfibrils whereas the surface-exposed Cys204 does not appear to have a role. This mechanism has important implications for the differential pathogenesis of Marfan syndrome and stiff skin syndrome.

Abstract Number: O14

Invited Speaker

POLYMERIC NANOPARTICLES FOR NOVEL THERAPEUTIC APPLICATIONS

Michael D. Buschmann

Ecole Polytechnique

Therapeutic delivery of nucleic acids using non-viral systems provides greater safety than viral delivery but generally suffer from low levels of transgene expression. Amongst non-viral systems, the use of phospholipids and cationic polymers are the most advanced. We have developed nanoparticles that are complexes of the natural cationic polymer chitosan with plasmid DNA (pDNA) or with small interfering RNA (siRNA) and have achieved high levels of transgene expression from pDNA and specific gene silencing using siRNA. A series of in vitro cell transfection studies revealed the importance of preparing specific chitosans, that are polymers of glucosamine and N-acetylated glucosamine, to obtain an optimal level of transgene expression. Live intracellular confocal imaging has provided mechanistic insight into the transfection process while physicochemical and nano-imaging have further allowed the establishment of structure function relationships. The identified chitosans were then tested in vivo and shown to express the growth factors FGF-2 and PDGF-BB via subcutaneous and intramuscular administration. By tailoring the type of chitosan used in these latter studies we were able to either stimulate or abrogate the generation of neutralizing antibodies to the transgene product. An important recent study has further shown significant therapeutic potential of these systems in the delivery of glucagon-like peptide 1 in a small animal diabetes model.

Abstract Number: O15

Post-doctoral Research

PASSAGED CHONDROCYTES ACQUIRE CHARACTERISTICS OF BONE MARROW STROMAL CELLS

Ahmed N[#], Taylor D W[#] and Kandel R A[$]

[#]Samuel Lunenfeld Research Institute, Mount Sinai Hospital, 25 Orde Street, Toronto, ON, Canada M5T 3H7, [$]Department of Pathology and Lab Medicine, Mount Sinai Hospital, 600 University Avenue, Toronto, ON, Canada M5G 1X5

One of the factors preventing regenerative therapies for degenerative cartilage diseases is a suitable source of cells. Chondrocytes are the only cell type of cartilage but in vitro culture results in loss of phenotype and ability to generate cartilaginous tissue. In this study we show that monolayer cultured articular chondrocytes (AC) under go a phenotype switch that is similar to that of marrow stromal cells (MSCs).

To promote cell expansion we cultured bovine AC in monolayer at 2000/cm2 cell density and passaged them twice. Under the phase contrast microscope the passaged cells (P2) appeared similar in morphology to MSCs the cells were bigger, more spread and spindle shaped. Higher type I to type II collagen gene expression ratio is one of the defining features of MSCs which was seen in the P2 as well. FACS analysis showed that the P2 have acquired an antigen profile very similar to MSCs with nearly 100% of CD44+ and 40% CD105+ cells. P2 failed to respond to the chondrocytes culture condition where unlike the primary chondrocytes P2 did not deposit extracellular matrix (ECM). However, when cultured in conditions similar to chondrogenic differentiation conditions suitable for MSCs the P2 responded very well and after 3 weeks of culture accumulated significant amounts of proteoglycan rich ECM that stained positive for type II collagen. Further studies on trans-differentiation capacity of these cells towards adipocytes and osteoblasts are undergoing. We show that upon monolayer culture chondrocytes acquire MSCs-like cell surface receptor profile and phenotype.

Abstract Number: O16

Invited Speaker

THE NETWORK OF PURINERGIC LIGANDS AND RECEPTORS IN BONE: EMERGING ROLES IN DEVELOPMENT, REMODELING AND MECHANOTRANSDUCTION

Jeff Dixon

Department of Physiology and Pharmacology, Schulich School of Medicine & Dentistry

ATP and other nucleotides are released from cells in response to mechanical stimuli. Studies by us and others have shown that adenosine and uridine nucleotides interact with osteoblasts and osteoclasts through multiple subtypes of cell-surface P2 purinergic receptors. P2X receptors are ligand-gated cation channels, whereas P2Y are G protein-coupled receptors. Moreover, the extracellular metabolism of ATP gives rise to pyrophosphate (inhibiting mineralization), phosphate (promoting mineralization) and adenosine. In turn, adenosine acts through the P1 family of G protein-coupled purinergic receptors and is cleared from the extracellular fluid by specific transporters. Mice with loss of P2X7 receptor function exhibit a unique skeletal phenotype – diminished bone formation, excessive resorption, and impaired response to mechanical loading. Our studies have revealed that activation of P2X7 receptors on osteoclasts leads to cell death. In contrast, activation of P2X7 receptors on osteoblasts stimulates osteogenesis. Moreover, the presence of multiple P2 receptor subtypes – with different affinities for ATP – increases the range of ATP concentrations for which dose-dependent responses are possible. This provides a novel mechanism by which osteoblasts may transduce differences in the intensity of mechanical stimuli over a wide dynamic range. The P2X7 receptor itself is a potential target for the development of drugs with combined anabolic and antiresorptive effects. Understanding the roles of other P2 and P1 receptors in bone is emerging. This network of purinergic ligands and receptors provides a potential mechanism for encoding spatial and temporal information that permits bone cells to coordinate their activity during development, remodeling and response to mechanical stimuli.

Abstract Number: O17

Graduate Research

ROLE OF SPHINGOMYELIN PHOSPHODIESTERASE 3 IN TOOTH MINERALIZATION

ZOHREH KHAVANDGAR[#], SHARIFA ALEBRAHIM[#] and MONZUR MURSHED[#,$]

[#] Faculty of Dentistry, McGill University, Montreal, Canada, [$] Department of Medicine, McGill University, Montreal, Canada

Sphingomyelin phosphodiesterase 3 (Smpd3) encodes a membrane-bound enzyme, which cleaves sphingomyelin to generate several bioactive lipid metabolites. A recessive mutation called fragilitas ossium (fro) in the Smpd3 gene leads to impaired mineralization of bone and tooth extracellular matrix in fro/fro mice. These phenotypic abnormalities are similar to the skeletal pathology of patients with some forms of osteogenesis/dentinogenesis imperfecta.

Our objectives are to 1) Characterize the tooth mineralization defects in fro/fro mice and 2) Determine whether SMPD3 regulates tooth mineralization locally.

We collected mandibles from both +/fro and fro/fro mice at P1, P3, P7, and P14 and examined the gross morphology of the teeth by X-Ray and micro-CT analysis. Undecalcified tissue sections were used for histological analysis. The expression of SMPD3 and other tooth cell markers were analyzed by immunohistochemistry. Finally, we used X-Ray and histological techniques to examine the tooth morphology and mineralization in a rescued model - fro/fro;Col1a1-Smpd3 mice, in which Smpd3 expression was restored in odontoblasts, the cell type involved in dentin formation.

We observed a delayed mantle dentin mineralization and consequently, delay in enamel matrix formation in fro/fro mice. These tooth abnormalities progressively improved with time. Our immunohistochemistry data showed that in tooth, SMPD3 is expressed by odontoblasts only. SMPD3-deficiency however did not affect the differentiation and migration of these cells. Restoration of SMPD3 expression in the odontoblasts corrected the tooth mineralization defects in fro/fro;Col1a1-Smpd3 mice.

SMPD3 expression in odontoblasts is required for tooth mineralization.

Abstract Number: O18

Graduate Research

THE V-ATPASE a3 SUBUNIT MUTATION R740S RESULTS IN OSTEOCLAST APOPTOSIS POSSIBLY DUE TO DEFECTIVE AUTOPHAGY

N. Ochotny[#], A.M. Flenniken[$], C. Owen[$], I.Voronov1, J.E. Aubin[#%], and M.F. Manolson1[#]

[#]Faculty of Dentistry, University of Toronto, , [$] Centre for Modeling Human Disease, Samuel Lunenfeld Research Institute, Mt Sinai Hospital, Toronto, Ontario, [%] Department of Molecular and Medical Genetics, University of Toronto

Mutations in vacuolar-type H+-ATPases (V-ATPases) disrupt bone resorption, causing osteopetrosis in mice and humans. V-ATPases are proton pumps composed of 14 subunits including the 100 kDa 'a' subunit. Of the 4 'a' isoforms a3 is enriched in osteoclasts where it is essential for bone resorption. Mouse models for osteopetrosis that lack a3, Tcirg1-/- and oc/oc have osteoclasts that are normal in appearance and number. A mouse genome-wide ethylnitrosourea (ENU) mutagenesis screen for dominant mutations affecting bone mineral density (BMD) identified a mouse with a high BMD/osteopetrotic phenotype carrying a dominant V-ATPase a3 missense mutation replacing arginine 740 with serine (R740S). The R740 residue is perfectly conserved in human and mouse 'a' isoforms. At birth, transmission electron microscopy reveals that Tcirg1R740S/R740S pups have abnormal looking osteoclasts with poorly defined ruffled borders. TUNEL staining of long bone sections from 2 and 4 day old Tcirg1R740S/R740S mice shows increased apoptosis of osteoclasts compared to WT. TRAP staining shows more osteoclasts in Tcirg1R740S/R740S long bone compared to WT. There appears to be no bone resorption in Tcirg1R740S/R740S mice (no tooth eruption, no marrow cavities). Generated osteoclasts in vitro are from RANKL-MCSF treated spleen cells of 4 day old Tcirg1R740S/R740S and WT mice. The Tcirg1R740S/R740S osteoclast membranes hydrolyze ATP at similar rates to WT indicating uncoupled ATP hydrolysis from proton transport. Immunoblots of Tcirg1R740S/R740S osteoclasts show similar a3 expression but decreased expression of the autophagosome marker, LC3II. Decreased LC3II expression suggests that the increased apoptosis observed for Tcirg1R740S/R740S osteoclasts is due to defective autophagy.

Oral Presentations – Banquet Talk

Abstract Number: O19

Banquet Speaker

BRAVING THE NEW WORLD OF RESEARCH FUNDING

Chris McCulloch

University of Toronto

Funding is an absolute requirement for research productivity but the vagaries of finding financial support are challenging and can be deeply frustrating. The Canadian government, in considering its mandate for advancing the health and prosperity of Canadians, is now encouraging the linkage of funding for biomedical research with the development of new products and services that have commercial promise. In thinking of future research funding possibilities two approaches will be considered here. In the first approach, identifying important health problems that have potential for advancement of new diagnostic and therapeutic strategies are likely to generate new funding opportunities, particularly when conducted in collaborative groups. In the second approach, the Scientific Research and Experimental Development (SRED) program will be reviewed as an alternative funding model that provides new possibilities but that also brings with it special requirements for success.

Abstract Number: O20

Invited Speaker

MECHANICS OF STEM CELL DIFFERENTIATION IN FRACTURE REPAIR

Neil Duncan

University of Calgary

Biomechanical stimuli have been shown to modulate stem cell differentiation and fracture healing progression. Several mechanoregulatory algorithms based on multi-scale mechanics and fluid flows have been developed to predict differentiation pathways into musculoskeletal tissues. The goal of this research is to understand the role of mechanics in regulating differentiation pathways of stem cell seeded gel constructs for the repair of bone fractures. Stem cell seeded collagen gels are subjected to one-dimensional confined-compression experiments and correlated with gene expression and intercelullar communication studies. The cell-gel constructs are also implanted into an ex vivo burr hole fracture model in a mouse tibia subjected to loading. Computational models based on differentiation algorithms are used to predict the differentiation pathways in these experiments. These numerical predictions of tissue formation and stem cell differentiation will be correlated with the tissue culture investigations examining the biosynthetic activity to develop a greater understanding of the role of mechanical factors in the differentiation of stem cells into musculoskeletal tissues.

Abstract Number: O21

Post-doctoral Research

CARTILAGE MECHANICAL INJURY INDUCES CHONDROCYTE APOPTOSIS VIA MAP KINASE SIGNALLING

Derek H. Rosenzweig and Thomas M. Quinn

Soft Tissue Biophysics Laboratory, Department of Chemical Engineerng, McGill University, Montreal, QC, Canada

Previous studies indicated depth- and time-dependences of chondrocyte biological responses to cartilage injury, including activation of mitogen activated protein (MAP) kinase pathways, which may be involved in the progression to post-traumatic osteoarthritis. We examined changes in cartilage-specific and apoptotic gene expression post-injury for up to 15 days, and to identify pathways mediating apoptosis, the spatial and temporal distributions of activated MAP kinases and cleaved Caspase-3 were examined. Furthermore, cell viability and Caspase-3 activity in the presence of specific MAP kinase inhibitors was monitored. Injury caused an acute decrease in viability post-injury, which was associated with decreases in cartilage-specific and anti-apoptotic gene expression and a transient increase in pro-apoptotic gene expression. MAP kinase activity was different for ERK, JNK and p38, exhibiting distinct depth- and time-dependences over the 15 days following injury. Cleaved Caspase-3 activity was significantly increased in middle and deep tissue zones up to 6 days post-injury. Inhibition of MAPKs resulted in increased viability post-injury, with distinct depth-dependences of these effects for ERK and p38. Rapid activation of MAPK signalling in cartilage explants post-injury correlated with changes in chonodrocyte gene expression and apoptotic activity. Moreover, blockade of MAPK signalling within injured explants altered caspase-3 activity in chondrocytes, where p38 inhibition initially enhanced apoptosis and both p38 and ERK inhibition later suppressed apoptosis as a function of tissue depth. These data indicate time and tissue-depth dependencies of MAP kinase signalling which directly correlate with cell death, thus yielding improved mechanistic insights for diagnosis and treatment of cartilage injuries.

Abstract Number: O22

Graduate Research

Cdc42 IS A CONVERGENT REGULATOR OF MATRIX MECHANICAL AND BIOCHEMICAL SIGNALING FOR MESENCHYMAL STEM CELL OSTEOGENIC DIFFERENTIATION

Wen Li Kelly Chen[#], David A. Romero[$], Cristina H. Amon[$] and Craig A. Simmons[#,$]

[#] Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, Canada, [$] Department of Mechanical & Industrial Engineering, University of Toronto, Toronto, Canada

Biochemical and mechanical properties of the extracellular matrix (ECM) are known to independently influence stem cell function. Given the complexity of cellular responses, an improved understanding of the integrated effect of multiple signals, rather than their individual actions, is necessary.

To this end, we developed a matrix array screening platform to study human bone marrow stem cell (hMSC) osteogenic differentiation under various combinations of ECM-derived signals. Permutations of collagen-I, fibronectin, and laminin at different concentrations (0-200 μg/mL) were patterned onto polyacrylamide substrates of various elasticities (11- 144 kPa). hMSCs were cultured on matrix arrays for 9 days in differentiation media with or without chemical inhibitors to specific signalling targets. The extent of osteogenic differentiation (alkaline phosphatase (ALP)/DAPI fluorescence) was modeled as a function of matrix inputs using Bayesian statistical methods.

The response surfaces revealed a biphasic relationship between osteogenesis and substrate stiffness, with the exact location and amplitude of the optimum contingent on matrix composition. Stiffness-dependent osteogenesis was largely mediated by Cdc42 activity and was independent of ROCK signalling. Corresponding biochemical analysis confirmed that activated Cdc42 level was biphasically modulated by substrate stiffness. The osteogenic effect of laminin was also mediated via Cdc42 signaling, as competitive inhibition of laminin receptor binding significantly reduced Cdc42 activity and ALP expression.

Together, these data demonstrate that matrix-dependent MSC osteogenesis is multifactorial and suggest Cdc42 as a convergent regulator of this process. The combination of unbiased exploratory techniques, using microtechnologies and statistical modeling, to generate non-intuitive hypothesis can accelerate novel mechanistic discoveries.

Abstract Number: O23

Invited Speaker

DEVELOPMENTALLY IMPORTANT SIGNALLING PATHWAYS IN OSTEOARTHRITIS

Benjamin Alman

The Hospital for Sick Children and the University of Toronto

Osteoarthritis is associated with articular cartilage chondrocytes undergoing phenotypic changes that are reminiscent of their end-stage differentiation in the growth plate. We examine human osteoarthritic samples and mice in which osteoarthritis was surgically induced and find that Hh signaling is activated in osteoarthritis. Using several genetically modified mice, we found that higher levels of Hh signaling in chondrocytes cause a more severe osteoarthritic phenotype. Furthermore, we show in mice and in human cartilage explants that pharmacological or genetic inhibition of Hh signaling reduces the severity of osteoarthritis. Together, these findings raise the possibility that Hh blockade can be used as a therapeutic approach to inhibit articular cartilage degeneration in osteoarthritis.

Abstract Number: O24

Graduate Research

FIBRILLAR COLLAGEN IS EQUIVALENT TO STIFF MATRIX IN DRIVING MARROW STROMAL CELL DIFFERENTIATION INTO MATRIX-DEFICIENT, MYOFIBROBLASTIC-LIKE PHENOTYPE

P. C. Dave P. Dingal[*], Matthew Raab[#], Jae-Won Shin[$], Amnon Buxboim[%] and Dennis Discher[*#%]

[*]Chemical and Biomolecular Engineering, [#]Bioengineering, [$]Pharmacology Graduate Group, [%]Physics and Astronomy, University of Pennsylvania

Scars tend to be stiffer than normal tissue, which has prompted the use of stiff matrices as models of scars, but scars are also rich in fibrillar collagen-I. Here, we introduce a soft matrix embedded with distinctly fibrillar collagen type-I, and show this is sufficient to drive bone marrow stromal cells (MSCs) into a contractile, myofibroblastic-like phenotype – 'myo-MSCs'. These cells have been reported to minimize scarring in a unique wound healing response, exemplified by their application to myocardial infarcts. Transcriptome analysis in response to matrix rigidity points to an upregulation of genes that participate in the cellular contractile machinery, notably α-smooth muscle actin (SMA), but a decreased expression of matrix protein genes for collagens type I, VI, and tenascin-C; TGFβ1 and TGFβRII, implicated in progressive fibrosis, are also downregulated. MSCs cultured on the embedded-fiber, soft matrix exhibit many similarities to cells on rigid substrates. Phosphorylation at serine-1943 of non-muscle myosin IIA, which deactivates stress fiber assembly, is decreased on both fibrosis-like and rigid substrates but almost twice higher on a soft substrate. Interestingly, these 'myo-MSCs' hint that, unlike myofibroblasts, they do not become hyper-contractile. This supports the notion that MSC engraftment into wounded tissues suppresses fibrosis, highlighting the promise of these cells in restoring normal tissue function.

Abstract Number: O25

Keynote Speaker

MECHANOSENSING THROUGH MULTIPLE TYPES OF TRANSMEMBRANE RECEPTORS

Paul Janmey

University of Pennsylvania

Physical properties of tissues and cells provide signals that are just as strong and specific as those provided by chemical stimuli. Tissue stiffness is tightly regulated under normal conditions and often changes in disease. In vitro, cell stiffness is not a constant, but depends on the physical properties of the extracellular environment as well as on the types of adhesion factors present on the substrate, and therefore on the type of transmembrane proteins with which the cell binds. Many cell types can alter their own stiffness to match that of the substrate to which they adhere. The precise mechanisms of mechanosensing are not well defined, but simultaneous control of substrate stiffness and adhesive patterns suggests that stiffness sensing occurs on a length scale much larger than single molecular linkages and that the time needed for mechanosensing is on the order of a few seconds. The presence of hyaluronic acid within substrates that also contain specific adhesion proteins can reset the stiffness sensitivity of cells that adhere through integrins. The dynamic response of cell morphology to substrate mechanics results from an integration of chemical and physical stimuli, and control of material elasticity provides an additional control parameter by which to optimize cell and tissue growth and function.

Abstract Number: O26

Post-doctoral Research

TGF-ß1 IS ACTIVATED BY INTEGRIN-MEDIATED CONTRACTION OF HUMAN CARDIAC FIBROBLASTS

Vincent Sarrazy, Anne Koehler, and Boris Hinz

Laboratory of Tissue Repair and Regeneration, Matrix Dynamics Group, Faculty of Dentistry, University of Toronto

Contraction and stiffening of the heart tissue by myofibroblasts is a principle cause of cardiac fibrosis progression, ultimately leading to organ failure. Myofibroblasts differentiate from cardiac fibroblast under the action of transforming growth factor beta1 (TGF-β1). TGF-β1 is secreted into the extracellular matrix as inactive large latent complex. Fibroblast traction forces mediated via transmembrane integrins have been shown to induce a conformational change in the latent complex, resulting in TGF-β1 release and thus activation. The identity of the integrins implied is not known. In cardiac fibrosis, the latent TGFβ1 binding integrins αvβ3, αvβ5, α8β1, and LTBP-1 have all been shown to be upregulated. We hypothesize that cardiac myofibroblast traction transmitted by either of these candidates will activate TGF-β1. Human cardiac fibroblasts were grown on soft culture substrates that mimic healthy heart tissue and fibrotic scar tissue. Integrin expression and myofibroblast differentiation were assessed by immunofluorescence and Western blotting. Activation of TGF-β1 by cardiac myofibroblasts was quantified using a luciferase reporter assay. Expression of integrins αvβ3 and αvβ5, as well as the myofibroblast marker α-SMA and latent TGF-β1 were up-regulated on stiff substrates, whereas α8β1 remained unaltered. Increasing integrin traction by Mn2+ increased active TGF-β1, whereas blocking integrin αvβ5 or the integrin binding site in the latent complex resulted in significantly reduced TGF-β1 levels and decreased α-SMA expression. To this end, blocking of integrin αvβ5 expression or interaction with latent TGFβ1 emerges as an attractive therapeutic strategy to selectively blocking TGF-β1 activation by fibrotic fibroblasts.

Abstract Number: O27

Graduate Research

A PHYSIOLOGICALLY RELEVANT MODEL OF COLLAGEN MINERALIZATION

Alexander J. Lausch, Bryan Quan, Eli D. Sone

Collagen biomineralization is a complex process and the controlling factors, at the molecular level, are not well understood. The periodontium displays nanoscale control over mineralization. More specifically, the periodontal ligament (PDL) becomes mineralized along a sharp front of about 200nm at the cementum/PDL junction. As such, the periodontium presents a unique opportunity for investigation into extracellular control over mineralization. We present a model of collagen biomineralization which employs excised mouse periodontium. Fixation of the tissue ensures both insoluble protein content and collagen structure of both mineralized and unmineralized tissues closely resemble native periodontium. Ultra thin sections of demineralized mandible are sectioned under cryo-conditions to reveal bone, ligament and dentin. When exposed to mineralizing solutions, transmission electron microscopy (TEM) has shown that the sections demonstrate selective remineralization. The bone and dentin remineralize preferentially to ligament, which remains unmineralized. Mineral is characterized by selected area electron diffraction (SAED) and energy dispersive x-ray spectroscopy (EDX). Banding patterns are visible in the remineralized collagen, suggestive of intrafibrillar mineral. Calcium phosphate solutions with high phosphate and polyaspartic acid, however, produce oriented crystalline mineral in all three mineralized tissues, as shown by electron diffraction. Our model demonstrates that extracellular matrix control directs selective remineralization and that the soluble protein content (which is lost during demineralization) is required to direct oriented crystalline mineral formation. This model allows nanoscale characterization of the mineral formation within the collagen matrix.

Abstract Number: O28

Graduate Research

TRANSGLUTAMINASE ENZYMES REGULATE ADIPOCYTE DIFFERENTIATION VIA MODULATING FIBRONECTIN SYNTHESIS AND ACTIN CYTOSKELETON

Myneni VD [#], Keillor JW [$], and Kaartinen MT [#,*]

[#]Faculty of Dentistry, McGill University, Montreal, QC, Canada, [$]Department of Chemistry, Faculty of Science, University of Ottawa, Ottawa, ON, [*]Faculty of Medicine (Experimental Medicine), McGill University, Montreal, QC

Adipose tissue is a specialized form of connective tissue that contains adipocytes, fat cells. The function of adipocytes is to store and release lipids. Preadipocytes have a fibroblastic shape in culture and upon differentiation they acquire rounded morphology. This change of shape is due to changes in cell-matrix interactions and cytoskeletal re-orgnization. Transglutaminases (TGs) are a family of enzymes that catalyze covalent cross-linking of proteins and they are known to play a role in cellular differentiation, matrix synthesis, cell-matrix interactions and matrix stabilization. In this study we asked if adipocytes (3T3-L1 cells) express TGs and, if so, do they have a role in adipocytes differentiation. We identified two TGs enzymes, transglutaminase 2 (TG2) and cellular FXIIIA in both preadipocytes and adipocytes at the mRNA and protein levels. Total transglutaminase activity during adipocyte differentiation increased 40-fold with adipogenesis induction media and activity was higher during first 4 days of differentiation. NC9, a transglutaminase inhibitor, caused a dose dependent increase in fat deposition. The effect of inhibitor on fat deposition was only seen during early phase of differentiation, i.e., during first 4 days when cells have fibroblastic shape and deposit fibronectin matrix. A decrease in fibronectin matrix levels is required for adipocyte differentiation. Inhibition of TG activity at early differentiation stage reduced the levels of secreted fibronectin. Inhibition of TG activity reduced formation of focal adhesions and indiced reorganization of actin stress fibers to cortical actin network. Conclusion: Transglutaminase activity modulates fibronectin levels and cytoskeletal reorganization during early stages of adipocytes differentiation.

Abstract Number: O29

Invited Speaker

PGD_2 AND ITS ANALOGUES AS POTENTIAL ANABOLIC AGENTS FOR BONE

Artur Fernandes

Université de Sherbroke

With the exception of strontium ranelate, which has not been approved for clinical use in North America, all available agents capable of inducing bone formation, either by local or systemic administration are proteins. This implies high production and administration costs. Prostaglandins are interesting potential anabolic agents for bone, as systemic intravenous administration of some prostaglandins can induce periostal new bone formation. We studied the synthesis and actions of the different prostaglandins on human bone cells, focusing on PGD_2 during the last years. PGD_2 is produced by human osteoblasts under biologically relevant stimuli and induces osteoblast chemotaxis; its production is increased *in vivo* in conditions of increased bone remodeling. On osteoclasts PGD_2 decreases osteoclastogenesis, decreases bone resorption by mature osteoclasts and increases the apoptosis rate of these cells. These results suggest that PGD_2 or analogues acting on PGD2 receptors could be used as anabolic agents for bone.

Abstract Number: O30

Graduate Research

LOSS OF EQUILIBRATIVE NUCLEOSIDE TRANSPORTER 1 (ENT1) IN MICE LEADS TO PROGRESSIVE ECTOPIC MINERALIZATION OF SPINAL TISSUES RESEMBLING DIFFUSE IDIOPATHIC SKELETAL HYPEROSTOSIS (DISH) IN HUMANS

Warraich S[*], Bone DB[*], Quinonez D[*], Holdsworth DW[#], Drangova M[#], Dixon SJ[*], Séguin CA[*], Hammond JR[*]

[*] Western University, [#]Robarts Research Institute

DISH is a non-inflammatory spondyloarthropathy, characterized by ectopic calcification of spinal tissues that occurs in 6-12% of North Americans. Its etiology is unknown and there are no specific treatments. ENT1 mediates transport of hydrophilic nucleosides, such as adenosine, across plasma membranes. In mice lacking ENT1 (ENT1-/-), we observed development of calcified lesions with remarkable resemblance to DISH in humans. Our objective was to characterize these lesions. MicroCT analyses revealed that ENT1-/- mice developed ectopic mineralization, starting in the cervical-thoracic spine and extending to the lumbar region with advancing age. Histological examination of decalcified samples showed large, irregular accumulations of eosinophilic, amorphous material encapsulated by fibrocartilaginous cells, with no apparent inflammation. Advanced lesions involved intervertebral discs and, in severe cases, impinged on the spinal cord. In contrast, we found no evidence for ectopic mineralization of appendicular joints or blood vessels. Energy-dispersive X-ray spectroscopy of lesions revealed the presence of calcium and phosphorus with an atomic percent ratio of ~1.5. HPLC analyses of plasma showed 2.8-fold greater adenosine levels in ENT1-/- compared to wild-type mice. Additionally, quantitative RT-PCR analyses of spinal tissue from the cervical-thoracic region of 6-month-old mice revealed lower levels of adenosine A1 receptor in ENT1-/- compared to wild-type mice. Lesions in the ENT1-/- mouse resemble DISH in humans and point to a role for purine metabolism in the regulation of biomineralization. ENT1-/- mice may provide a model to investigate mechanisms and evaluate therapeutics for preventing pathological calcification in DISH and related disorders. Supported by CAN and CIHR.

Abstract Number: O31

Graduate Research

CHONDROADHERIN FRAGMENTATION AS A BIOCHEMICAL MARKER FOR EARLY STAGE DISC DEGENERATION

Alkhatib B[#], Gawri R[#], Roughley P[%], Ouellet J[$], Haglund L[#]

[#]Orthopaedics Research Laboratory, McGill University,Montreal, [%]Shriner's Hospital for Children, Montreal, [$]McGill Scoliosis and Spine Center, McGill University, Montreal.

Disc degeneration has been strongly associated with back pain. 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, and to characterize the cleavage site within CHAD at which fragmentation occurs.

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.

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 scoliosis. Normal tissue samples showed no anti-neoepitope antibody binding, confirming that CHAD fragmentation at this site was not present in the healthy disc.

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.

Abstract Number: O32

Invited Speaker

SPINAL METASTASES: STRUCTURAL INTEGRITY AND RESPONSE TO TREATMENT

Cari Whyne

Sunnybrook Research Institute

Metastatic cancer accounts for the majority of tumors that occur in bone and is diagnosed most frequently in the spinal column. Skeletal metastases present radiologically as bone destructive (osteolytic), bone forming (osteoblastic) or as a mixture of the two (mixed). Irrespective of presentation, normal bone architecture is significantly altered. Clinically, all patterns are associated with an increased risk of pathologic fracture. Computational image analysis, structural assessment and finite element modeling can be utilized to quantify the complex behaviour of the metastatic spine. In combination with advanced micro imaging, it is possible to both volumetrically quantify tumour burden and evaluate the influence of new minimally invasive treatment methods. Specifically, this work presents the impact of complex skeletal metastatic disease and photodynamic therapy (alone and in combination with bisphosphonates and radiation therapy) on vertebral architecture and fracture risk.

Abstract Number: O33

Invited Speaker

GROWTH FACTOR AMBUSH: HOW SHORT CAN TGFB PULSES GET AND HOW LONG DO THEY LAST (IN VITRO)?

Michael RAGHUNATH

Department of Bioengineering, Faculty of Engineering & Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore

Scarring and fibrosis has remained a clinical problem in a variety of settings and still awaits a therapeutic solution. TGFβ-1, a crucial mediator of this pathology drives differentiation of fibroblasts into alpha-SMA expressing and collagen I overproducing myofibroblasts. Most in vitro fibrogenic studies are done with continuous exposure of cells to TGFβ-1 over several days. However, this is a poor reflection of pulsatile cytokine exposure prevailing in vivo. We therefore wanted find out more about the effects of pulsed TGFβ-1 exposure in the creation and maintenance of the myofibroblast phenotype. Growth-arrested fibroblasts (0.5$^{\%}$ serum) were pulsed once with 5ng/ml of TGFβ-1 in serum free media for 30 minutes or 4h. Alternatively, we pulsed them again the following day with the same duration (2 x 30 min) and 4h (2 x 4h). Thereafter, the pulsed cultures were washed with HBSS and maintained in 0.5$^{\%}$ FBS for 1, 7 and 14 days. At the indicated days the 24h collagen secretion rate was monitored and α-SMA analysed by immunoblotting and immunocytochemistry. Selected fibrogenic genes were analyzed by RT-PCR. Epigenetic modification on acetylated Histone H3 expression was determined by a sandwich ELISA assay and DNA methylation levels on fibrogenic genes were analyzed extensively using MALDI-TOF. We were surprised to find that a single 30 min pulse and a 4h pulse was sufficient to elevate collagen I secretion rate and α-SMA expression for 7 days. Furthermore a second pulse extended this period for up to 14 days. In all treatment types, fibrogenic genes were up-regulated 24h post pulse. When we looked into epigenetic modifications and were surprised again because we could not identify acetylation or methylation changes in TGFβ-1 pulsed fibroblasts. Customary models of in vitro fibrogenesis use 3–5 days continuous exposure of cells with TGFβ-1, a condition far removed from physiology. We show here, that already a single 30 min pulse is sufficient to effect long term changes towards the myofibroblast phenotype, and is potentiated by a second pulse a day later. The phenotypic change seems to swing back to normal under these conditions, so a dampening is occuring over time but the question remains what mechanism leads to the sustained phenotypic response to single or a repeat pulse. We currently speculate that a single TGFβ-1 pulse leads to sustained microenvironmental changes in the pericellular matrix resulting in a positive feed-back that is dampened in the course of ongoing extracellular matrix remodelling. We believe that the ECM in this case acts as a memory of a growth factor insult.

Abstract Number: O34

Undergraduate Research

THE EFFECT OF MAST CELL DEFICIENCY ON BONE TISSUE REGENERATION

Michael H. Wang[#,%], **Michael B. Sullivan**[$,%], **Xuejiao Li**[#,%], **Janet E. Henderson**[#,$,*,%], **Paul A. Martineau**[#,$,*,%]

[#] McGill University Faculty of Medicine, [$] McGill University Department of Surgery, [*] McGill University Division of Orthopaedic Surgery, [%] McGill University Health Centre JTN Wong Labs for Bone Engineering

Skeletal reconstruction is currently limited by the availability of donor tissues used in autologous bone graft transplantation. Since mast cells have an innate ability to home in on injury sites, mast cells are a potential adjunct therapy and drug delivery mechanism to augment bone regeneration. However, previous research has neither established the role of mast cells in bone healing nor the timing of their involvement.

1) Demonstrate whether bone regeneration is disturbed in mast cell-deficient (c-kit mutant) mice; and 2) Establish the timing of bone healing in wildtype and mutant.

Wildtype and mutant C57Bl6/J mice underwent bilateral femoral cortical defect surgeries to investigate bone regeneration. Mice were euthanized at post-operative zero, two, four, and six weeks. Femurs were extracted for analysis using micro-computed tomography (Micro-CT) and histology to assess bone healing. Micro-CT examined the micro-architecture of the healing cortex, while histology identified cellular contributions, including osteoblast activity, mineralization, and the presence of mast cells.

At baseline post-operative zero weeks, wildtype and mutant have the same bone volume fraction (BV/TV), a measure of bone healing. Compared to baseline, both genotypes showed significantly higher BV/TV at post-operative two and four weeks. Compared to mutants, wildtype femurs had significantly higher BV/TV at post-operative two weeks and both achieved similar BV/TV by post-operative four weeks. This indicates that wildtype regeneration was completed by week two and mutant regeneration by week four. Histology is preliminary but mineralization stains support Micro-CT data.

In the absence of mast cells, bone regeneration still proceeds but is significantly delayed.

Abstract Number: O35

Invited Speaker

CCN2: A MASTER REGULATOR OF TISSUE REPAIR AND FIBROSIS

Andrew Leask

Western University

CCN2 is a member of the CCN family of matricellular proteins. CCN2 is normally minimally expressed in mesenchymal cells, but markedly upregulated in vivo during tissue remodeling, repair and fibrosis. We have generated mice in which CCN2 is deleted in the whole body as well as mice in which CCN2 is deleted specifically in fibroblasts. We subjected mice to the dermal punch model of cutaneous tissue repair, and inflammatory (bleomycin-induced) and genetic (loss of PTEN) models of skin fibrosis. CCN2 is not required for skin development. However, mice deleted for CCN2 show delayed kinetics of cutaneous tissue repair and are resistant to both models of fibrosis. Although TGFbeta induces CCN2 and synergizes with CCN2 to promote fibrosis in vivo, loss of CCN2 does not affect the ability of TGFbeta to induce a-SMA or collagen production. Loss of CCN2, however, appears to affect the recruitment of NG2-positive pericytes. Thus CCN2 may be a common downstream pro-fibrotic mediator and thus may be a good target for anti-fibrotic therapy.

Abstracts Poster Presentations

(by order of poster numbers)

Friday	06:30 -	Posters Setup	
Friday	06:30 - 08:00 pm	Poster Session I	(all numbers)
Saturday	12:35 - 02:00 pm	Poster Session II	(viewing all poster numbers)
Saturday	04:00 - 05:40 pm	Poster session III	(presenting even poster numbers)
Sunday	09:45 - 11:10 am	Poster session IV	(presenting odd poster numbers)
Sunday	12:25 - 02:00 pm	Poster session V	(all poster numbers) tear-down by

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Posters

Abstract Number: P01

Post-doctoral Research

CONTRIBUTION OF PROLINE-POOR SEQUENCE MOTIFS TO ELASTIN SELF-ASSEMBLY AND MECHANICAL PROPERTIES

Muiznieks L.D.[#], Sitarz E.[#], Miao M.[#], Reichheld S.[#] and Keeley F.W.[#$]

[#]Molecular Structure and Function Program, The Hospital For Sick Children, Toronto, $Department of Biochemistry, The University of Toronto

Elastin is a hydrophobic, self-assembling protein of the extracellular matrix that provides large blood vessels, lung and skin with elasticity. A fundamental requirement for forming elastomeric materials is retention of a high degree of conformational disorder even when aggregated, where this disorder is strongly related to a high (50%) combined proportion of proline and glycine residues within hydrophobic domains. Indeed, reducing the number of proline residues within short elastin-like polypeptides disrupts elastin-like assembly and results in the formation of aggregates rich in β-structure, while removal of prolines promotes hydrophobic collapse into β-sheet-rich amyloid fibrils. \The majority of elastin hydrophobic domains have an average proline spacing of 4-8 residues however, the native sequence of hydrophobic domain 30 is uncharacteristically proline-poor across many species. Here we investigated the contribution of proline-poor domain 30 to elastin self-assembly and material properties. Increasing the number of copies of domain 30 within model elastin-like polypeptides substantially stabilized the surface of protein-rich colloidal droplets, the first step in elastin self-assembly, and promoted droplet interactions. Furthermore, materials cast from these polypeptides displayed a higher elastic modulus (were stiffer), consistent with a greater number of contacts between monomers in the polymer matrix. Taken together, we hypothesize that structured sequence motifs arising from proline-poor domains impart key constraints on conformationally disordered elastin domains, providing an essential contribution to self-assembly, including incorporation of the monomer into the growing elastin fibre, and tensile mechanical properties.

Abstract Number: P02

Post-doctoral Research

GENOME-WIDE EXPRESSION PROFILING OF IDIOPATHIC SCOLIOSIS ENDOPHENOTYPES

Kristen Fay Gorman[*], Qing Yuan[*], Cédric Julien[*], Saadallah Bouhnik[*], Anita Franco[*], Karen Choquet[*], Da Shen Wang[*], Marie-Yvonne Akoume[*], Alain Moreau[*,$]

[*]Viscogliosi Laboratory in Molecular Genetics of Musculoskeletal Diseases, Sainte-Justine University Hospital Research Center; Department of Biochemistry, Faculty of Medicine, Université de Montréal, Québec, Canada, [$]Department of Stomatology, Faculty of Dentistry, Université de Montréal, Québec, Canada.

Although the aetiology of idiopathic scoliosis (IS) remains unknown, we have demonstrated a signalling dysfunction linked to osteopontin (OPN), acting through Gi proteins. This dysfunction can be stratified into three distinct Groups (G1, G2, G3), which comprise the first non-clinical endophenotypes for IS. In order to elucidate the biology that distinguishes these endophenotypes, we compared the genome-wide expression profiles for each Group. Gene expression differences between Groups provides candidate genes important for the pathophysiology of IS.

We examined genomic expression for 15 patients and 3 controls using Affymetrix array chips with RNA from osteoblasts. Selection criterion for data exploration was a 3-fold expression change between Groups. Gene function was analyzed using DAVID software. Statistical analysis was performed using FlexArray software. We conducted an ANOVA on all 54,000 probe sets and considered genes with a corrected p-value ≤ 0.05 as significantly differentially expressed. Quantitative PCR on an expanded cohort validated interesting genes.

We identified 347 genes with a 3-fold expression change. In general, the gene expression profile for G1 is dramatically different from that of G2 and G3. From the ANOVA we identified 25 genes and one miRNA that are significantly differentially expressed. Validation of candidate genes revealed a distinct expression difference related to osteopontin sensitivity.

We found that the gene expression differences reflect observed biochemical and signaling activities previously demonstrated. We expect that the genes and miRNA significantly differentially expressed between endophenotypes relate to the regulation of osteopontin and G-inhibitory signaling pathways related to the primary etiology in IS.

Posters

Posters

Abstract Number: P03

Post-doctoral Research

PROHIBITIN A NOVEL OSTEOARTHRITIS BIOMARKER FOR EARLY DETECTION AND STAGING OF OA PATIENTS

Lavoie JF[#$], Picard C[#$], Doucet R[#*], Taheri M[#], Pellicelli M[#$], Lavigne P[%], Moreau A[#$*§]

[#] Viscogliosis Laboratory in Molecular Genetics of Musculoskeletal Diseases, Sainte-Justine University Hospital Research Center, Montreal, QC, Canada, [$] Biochemistry Department, Faculty of Medicine, Universite de Montreal, [*] Molecular Biolology program, Universite de Montreal, [%] Maisonneuve-Rosemont Hospital and Department of Surgery, Université de Montréal, [§] Department of Stomatology, Faculty of Dentistry, Université de Montréal

Introduction: We have previously demonstrated a loss of PITX1 transcription factor in OA cartilage. Using functional in vitro analyses we showed that PHB1 over-expression in normal chondrocytes was sufficient to inhibit endogenous PITX1 expression. This prompted us to assess whether nuclear accumulation of PHB1 is an early event in OA and if this nuclear accumulation could be detected in other cell types where normally PITX1 is expressed.\

Methods: 88 consecutive OA and 33 healthy subjects were enrolled after IRB's approval. PHB was detected by immunohistochemistry (IHC) on paraffin sections, Western blotting (WB) on cytoplasmic and nuclear extracts or by immunofluorescence (IF) on cells and coupled with confocal microscopy. To examine whether PHB1 nuclear accumulation precedes PITX1 repression and OA symptoms, IHC and qPCR analyses were performed on STR-ort mice, a well known OA mouse model.\

Results: The IHC analysis in human cartilage showed a 40% increase in the number of articular chondrocytes exhibiting nuclear PHB1 in OA subjects. This result was confirmed by IF and WB. Similarly, PHB1 nuclear accumulation was also present in leucocytes of OA patients when compared to healthy or rheumatoid arthritis patients. Interestingly, nuclear accumulation of PHB1 in STR/ort articular chondrocytes correlated with a significant decrease in PITX1 expression as well as an increase in Mankin's score and loss of proteoglycan.\

Conclusion: Nuclear accumulation of PHB1 is present in chondrocytes and leukocytes of OA patients turning-off PITX1 expression. This finding paves the way to use PHB1 as a blood marker for the diagnosis of OA.

Abstract Number: P04

Other

ADAMTSL2$^{-/-}$;FBN1$^{+/-}$ MICE SHOW SEVERE GROWTH RETARDATION, JOINT CONTRACTURES, AND STIFF SKIN: A MODEL FOR HUMAN GELEOPHYSIC DYSPLASIA?

Dirk Hubmacher[*], Lauren W. Wang[*], Suneel S. Apte[*]

[*]Department of Biomedical Engineering, Lerner Research Institute, Cleveland Clinic

Geleophysic dysplasia (GD) is a rare human genetic disorder presenting with short stature, short distal limbs, joint contractures, and thick skin. High morbidity, and frequently, juvenile mortality results from cardiac valvular and tracheo-pulmonary anomalies. Mutations in the extracellular proteins ADAMTSL2 or fibrillin-1 lead to recessive or dominant GD, respectively. Fibrillin-1 assembles in microfibrils and modulates extracellular TGFβ and BMP signaling. Excess TGFβ signaling was described in cells derived from GD patients and we showed previously that ADAMTSL2 directly bound latent transforming growth factor-β binding protein (LTBP)-1 and fibrillin-1. The Adamtsl2 gene was targeted in mice by insertion of an IRES-lacZ-neomycin cassette, which also provided a gene expression reporter. β-gal staining showed Adamtsl2 expression in heart, lung, joints, tendons, and aponeuroses. Deletion of Adamtsl2 resulted in neonatal lethality due to abnormal lung and cardiac development. However, additional deletion of one allele of fibrillin-1 rescued the lethal phenotype. Adamtsl2-/-;Fbn1+/- mice appear normal at birth, but postnatal growth is severely delayed and affected animals die by 14 days of age. The mice walk on tiptoes and have joint contractures. The skin is stiff and exfoliative. Histology revealed a thickened stratum corneum combined with an attenuated hypodermis traversed by collagen extending from the dermis to hypodermal muscle. In summary, Adamtsl2-/-;Fbn1+/- mice present with many of the major manifestations seen in GD, and disclose a crucial functional relationship between ADAMTSL2 and fibrillin-1. In addition, they may identify pathways regulating fibrosis.

Posters

Posters

Abstract Number: P05

Other

TISSUE STRETCH INDUCES FIBROBLAST NUCLEAR TRANSLOCATION AND DOWN REGULATION OF ZIC-1

Helene M. Langevin[#,$], Nicole A. Bouffard[#], Cathryn Koptiuch[#]

University of Vermont Department of Neurology[#], Department of Orthopedics and Rehabilitation[$]

Zic-1 is a transcription factor primarily involved in embryonic neural and mesenchymal differentiation, but recently implicated in mechanotransduction in adult musculoskeletal tissues. We have previously shown that stretching of whole mouse areolar connective tissue causes dynamic cytoskeletal and nuclear remodeling with loss of nuclear invaginations. This study examined the effect of static tissue stretch on fibroblast gene expression (in vivo) and Zic-1 nuclear translocation (ex vivo).

C57B6 mice underwent static stretching of one side of the trunk (~50% strain) for 90 minutes while under anesthesia, euthanized and areolar connective tissues bilaterally processed for RNA analysis using Affymetrix gene arrays. Ex vivo, areolar connective tissue samples were randomized to tissue stretch vs. no stretch for 2 hours, then fixed, immunohistochemically stained for Zic-1 and imaged with confocal microscopy.

Zic-1 mRNA differential expression was significantly reduced (-1.95) on the stretched side of the mouse compared with the contralateral non-stretched side ($p < .01$, N=7 mice). In response to tissue stretch ex vivo, Zic-1 protein translocated into the nucleus while non-stretched tissue had nuclei void of Zic-1 leaving a ring of staining around the nucleus.

Our results indicate that dynamic cytoskeletal and nuclear remodeling of areolar connective tissue fibroblasts in response to static stretch is accompanied by nuclear translocation of Zic-1 protein and downregulation of Zic-1 mRNA. This suggests that Zic-1 may play a role in downstream mechanotransduction of the stretch signal in connective tissue fibroblasts. Further experiments will examine potential Zic-1 targets and cytoskeletal mechanisms that may link to its nuclear translocation.

Abstract Number: P06

Post-doctoral Research

KINDLIN-2 REGULATES CARDIAC FIBROBLAST ACTIVATION AND MECHANOTRANSDUCTION

Zimina E[*], Has C[#], Bruckner-Tuderman L[#], Clement S&, Hinz B[*]

[*]Laboratory of Tissue Repair and Regeneration, Matrix Dynamics Group, Faculty of Dentistry, University of Toronto, Toronto, Canada, [#]University Medical Center Freiburg, Freiburg, Germany, &Department of Pathology and Immunology Faculty of Medicine, CMU University of Geneva, Switzerland.

Heart fibrosis is one of the most common causes for heart failure. Fibrosis arises from a variety of pre-conditions that include mechanical stress, like pressure overload in chronic hypertension and post-infarct heart remodelling. In response to mechanical stress, cardiac fibroblasts become activated and differentiate into myofibroblasts, key fibrotic cells with excessive collagen secretion and contractile activities. Kindlins are a recently identified group of cytoplasmic proteins that connect integrin receptors to the contractile actin cytoskeleton. Kindlin-2 is expressed in cardiac fibroblasts and upregulated during myofibroblasts activation in conditions of experimentally induced rat heart fibrosis. Immunlocalization demonstrated accumulation of kindlin-2 in focal adhesions of primary human cardiac fibroblast. We hypothesize that kindlin-2 is central in cardiac fibroblast activation by playing an important mechano-transducing role within cell–matrix junctions. To test this we subjected primary human cardiac fibroblasts to different mechanical conditions in culture. Kindlin-2 levels were increased in human cardiac fibroblasts cultured on fibrosis-stiff substrates in compare with fibroblasts cultured on soft substrates. The expression levels of kindlin-2 and the myofibroblast marker α-SMA were co-regulated, being higher in stressed conditions. Down-regulation of kindlin-2 in cardiac fibroblasts reduced the levels of α-SMA and myofibroblast activation. Mechanical stimulation of cardiac fibroblasts using fibronectin-coated ferromagnetic microbeads or by stretching the cells on deformable silicone membranes resulted in a fraction of kindlin-2 translocating from focal adhesions to the nucleus. Together, our observations suggest that mechanical stress controls the expression and localization in activated myofibroblasts and may thereby contribute to development of fibrosis in the myocardium.

Posters

Posters

Abstract Number: P07

Graduate Research

BIOMECHANICAL PROPERTIES OF THE FLEXOR RETINACULUM

Brett A[#], Gordon K[#], Oliver M[#] and Agur A[$]

[#]School of Engineering, University of Guelph, [$]Division of Anatomy, Department of Surgery, University of Toronto

Carpal tunnel syndrome (CTS) is the most frequently occurring entrapment neuropathy affecting an estimated 3.8% of the adult population. Although recent studies have begun to illuminate the pathogenic tissue changes characteristic of CTS, its aetiology remains poorly understood. It is hypothesized that repetitive tendon movement results in insult to the subsynovial tissue which leads to pathogenic tissue remodeling and tissue thickening. However, examination of CTS affected flexor retinaculum (FR) samples has not revealed morphological or histological changes and it is unknown whether mechanical properties differ between healthy and diseased tissue.

Mechanical evaluation of the FR is not only important in understanding CTS but also for modeling of the wrist. There are few studies which have attempted to quantify the biomechanical properties of the FR, perhaps due to fixation difficulties.

Three cadaveric wrist joints (2-M;78±17.5 years) were dissected free of all other soft tissue. The medial and lateral halves of the wrist were mounted in a custom built jig in an Instron materials testing machine. Nine markers were fixed to the volar surface of the flexor retinaculum to allow for strain measurement via image analysis. Following preconditioning, a stress relaxation test and a tensile test to failure were conducted. Preliminary results were obtained for Stiffness (62.6 ±7 N/mm), Load to Failure (277.4±82.9 N) and Ultimate Strength (4.8±1.6 MPa).

Future work will include examination of regional strain patterns and a comparison of healthy to diseased tissue in an effort to explain the aetiology of carpal tunnel syndrome.

Abstract Number: P08

Graduate Research

DYNAMIC CYTOSKELETAL REMODELING OF CONNECTIVE TISSUE FIBROBLASTS IN RESPONSE TO STATIC STRETCH IS DEPENDENT ON MATRIX MATERIAL PROPERTIES

Cathryn Koptiuch[#], Rosalyn D Abbott[#*], Helene M Langevin[#$]

[#] Department of Neurology, University of Vermont, Burlington VT, USA, [$]2Department of Orthopedics & Rehabilitation, University of Vermont,Burlington VT, USA, [*]2Department of Engineering, University of Vermont, Burlington VT, USA.

In areolar “loose” connective tissue, fibroblasts rapidly (within minutes) remodel their cytoskeleton in response to static tissue stretch, resulting in increased cell body cross sectional area which relaxes the tissue to a lower state of resting tissue tension. Whether this loosely arranged collagen matrix is required for these active cytoskeletal responses remains unknown. This study evaluates cytoskeletal remodeling of fibroblasts in and derived from mouse areolar and dense connective tissue in response to static stretch in both native tissue and collagen gels of varying crosslinking.

Excised mouse tissue and fibroblast-seeded collagen gels were randomized to either 2 hours of static stretch (~20% strain) or no stretch then examined with histochemical staining with confocal microscopy. Tissue and collagen gel material properties were determined by rheometric testing.

In response to tissue stretch ex vivo, cells within areolar, but not dense connective tissue increased their cross sectional area in response to tissue stretch ($p<.001$). Fibroblasts dissociated from dense connective tissue regained their ability to respond to stretch when seeded into a compliant matrix ($p<.01$) while cells derived from areolar connective tissue lost their ability to respond within a stiffer matrix environment. Changes in cytoskeletal remodeling were dynamic and reversible.

Our results suggest that dynamic cytoskeletal remodeling of connective tissue fibroblasts in response to static stretch is dependent on extrinsic matrix material properties rather than the cells’ tissue of origin (areolar vs. dense connective tissue). These results have potential implications for connective tissue tension regulation in fibrotic conditions causing increase matrix density and/or stiffness.

Posters

Posters

Abstract Number: P09

Graduate Research

ADYNAMIC BONE DISEASE (ABD) WEAKENS BONE MORE THAN AGING ALONE IN A MOUSE MODEL

[1,2]Ng, AHM; [2,3]Willett, TL; [1,3,4]Alman, BA; [1,2]Grynpas, MD

1Department of Laboratory Medicine & Pathobiology, University of Toronto, Toronto, Ontario, 2Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario,, 3Department of Surgery, University of Toronto, Toronto, Ontario, , 4 Program in Developmental and Stem Cell Biology, Hospital for Sick Children, Toronto, Ontario, Canada

Adynamic Bone Disease (ABD) is characterized by a loss of bone turnover. A clinical concern of ABD is diminished bone quality and an increase in fracture risk. Our current study aims to investigate changes to bone quality in ABD and how bone quality changes with age in our mouse model of ABD.

Three groups representing young, middle-aged, and old mice were studied. To create the adynamic bone condition, Col2.3Δtk (DTK) mice were treated simultaneously with ganciclovir and pamidronate to mimic the loss of bone turnover. At the end of treatment, animals were sacrificed and bones were harvested for analyses.

Histomorphometry and TRAP-staining analyses confirmed a natural decline in both bone formation and resorption with age in Controls, whereas bone turnover was severely blunted at all ages in ABD. There were no differences in bone mineral density (BMD) between Control and ABD mice at all ages, but aging ABD mice had better preserved trabecular bone volume and microarchitecture compared to Control mice. However, a normal BMD and improved microarchitecture in aging ABD mice did not result in stronger bones. Three-point bending of the right femur showed aging ABD mice required less energy to failure than younger ABD mice and this decrease was only due to changes in post-yield strains.

Little is known about the effects of metabolic bone disease on bone toughness. Our data suggest that the bones of aging ABD mice are less tough than that of Control mice and this decrease may be due to changes in the collagen.

Abstract Number: P10

Graduate Research

C-TYPE NATRIURETIC PEPTIDE MODULATES TGF-β1-INDUCED SYNTHESIS OF PROTEOGLYCAN BY VALVE FIBROBLASTS

Blaser MC[#], Kwon JW[$], Sider KL[#], Kwong AV[#], Wei K[#], Simmons CA[#,§]

[#] Institute of Biomaterials and Biomedical Engineering, [$] Division of Engineering Science, [§] Department of Mechanical & Industrial Engineering, University of Toronto

The hallmark of aortic valve disease (AVD) is unregulated connective tissue deposition. Examining early AVD may identify novel treatments for use before end-stage disease develops. AVD occurs concomitantly with increased TGF-β1 expression and MAPK activation and loss of protective factors in the valve like C-type natriuretic peptide (CNP). We investigated links between TGF-β1, CNP, and matrix synthesis in a mouse model of early AVD and in vitro.

Male wild-type (WT) mice were fed control or BioServ F3282 (high-fat/high-carbohydrate, HF/HC) diets for four months, and aortic valve sections were assessed (immuno)histologically. Distal valve thickening occurred in HF/HC leaflets, due to solely to proteoglycan (PG) deposition ($p < 0.01$). TGF-β1 and Sox9 expression were increased in PG-rich lesions of HF/HC leaflets, which also had lower CNP expression than non-lesion regions. No leaflets were αSMA-positive.

Valve interstitial cells (VICs) from healthy porcine aortic valves were treated in vitro with 5 ng/ml TGF-β1 and/or 1 μM CNP or 10 μM of Erk1/2 inhibitor for 6 days, and PG synthesis was measured by Alcian Blue solubilisation. TGF-β1 increased PG expression by 29% ($p < 0.05$) and amplified Erk1/2 phosphorylation, both of which were significantly reduced by CNP. Blocking Erk1/2 signaling with U0126 significantly reduced PG expression ($p = 0.06$).

A high-fat diet induces early changes in mouse aortic valve ECM, without myofibroblast activation. TGF-β1 induces PG production in VICs, which is inhibited by CNP – possibly through Erk1/2. Insight into the mechanisms regulating formation of PG lesions helps to further our understanding of AVD progression.

Posters

Posters

Abstract Number: P11

Graduate Research

HYPOXIA REGULATES TGF-β SIGNALING PATHWAYS AND ECM PRODUCTION IN HUMAN CHONDROCYTES

Chi Y. and Philip A.

Department of Surgical Research, McGill University, Montreal General Hospital, Montreal, Quebec, Canada

Transforming Growth Factor-Beta (TGF-β) plays a critical role in maintenance and repair of articular cartilage, and deregulation of its activity is implicated in osteoarthritis (OA). Our previous work has shown that chondrocytes express two TGF-β type I receptors, ALK5 and ALK1, which signal through the Smad2/3 and the Smad1/5/8 pathway, respectively. It has been suggested that there is a shift from ALK5/Smad2/3 to ALK1/Smad1 pathway during the progression of OA. Articular cartilage is physiologically hypoxic and alteration in oxygen tension is associated with chondrocyte dysfunction and OA progression. We hypothesized that oxygen tension differentially regulates TGF-β signaling pathways in chondrocytes to modulate cartilage function. The purpose of the study was to determine whether low oxygen tension differentially regulates ALK5/Smad2/3 versus ALK1/Smad1 pathway and ECM production in human chondrocytes.

Human chondrocytes (C28/I2) were exposed to hypoxia (<2$^{\%}$ O2) or normoxia for 2 or 48 hours and cell lysates were analyzed for the expression of TGF-β signaling components and ECM production. Our results show that low oxygen tension increased the expression of HIF-1alpha, ALK5, Smad2/3, phospho-Smad2/3, type II collagen and decreased the expression of ALK1, Smad1 and phospho-Smad1. Our findings suggest that under low oxygen tension, the TGF-β signaling pathway is shifted to ALK5/Smad2/3 pathway associated with increased production of type II collagen in these cells. Identification of factors and understanding their role in regulating TGF-β signaling and responses in chondrocytes may provide a basis for the development of novel therapeutic approaches for the treatment OA.

Abstract Number: P12

Post-doctoral Research

FUNCTIONALIZATION OF DYNAMIC CULTURE SURFACES WITH A CARTILAGE EXTRACELLULAR MATRIX EXTRACT ENHANCES CHONDROCYTE PHENOTYPE

Derek H. Rosenzweig, Sofia Solar-Cafaggi and Thomas M. Quinn

Soft Tissue Biophysics Laboratory, Department of Chemical Engineering, McGill University, Montreal, QC, Canada

Culture and expansion of primary chondrocytes in monolayer is necessary for cell-based therapies and tissue engineering, yet it often leads to a rapid loss of chondrogenic phenotype termed dedifferentiation. Current culture techniques utilize collagen type I for functionalization of culture surfaces, although collagen type II is predominant within the cartilage extracellular matrix (ECM). We attempted to circumvent chondrocyte dedifferentiation on extendable culture surfaces functionalized with cartilage ECM extract. Articulate cartilage was decellularized, homogenized and partially digested to generate the ECM extract. First, primary bovine articular chondrocytes were isolated and cultured on silicone rubber cross-linked to either rat tail collagen type I (control) or ECM extract for either 6 days, or passaged 3 times. Viability, proliferation assays and gene expression analysis were performed to determine effects of the ECM extract. Second, chondrocytes were cultured on a high extension silicone rubber (HESR) dish functionalized with the ECM extract and compared to passaged controls. After six days of static culture on ECM extract there were no differences in viability, proliferation, or apoptosis compared to controls. Gene expression analysis of cells grown on ECM extract revealed elevated COL2A1 and downregulated COL1A2 expression compared to controls. Furthermore, cells cultured on static ECM extract secreted 3-fold more glycosaminoglycan (GAG) versus controls. Chondrocytes cultured on HESR dishes functionalized with ECM extract had enhanced cartilage-specific gene expression and redifferentiated in pellet culture more efficiently compared to passaged controls. Thus, ECM extract promoted growth and phenotypic maintenance of chondrocytes and may provide protective affects against dedifferentiation.

Posters

Posters

Abstract Number: P13

Graduate Research

A CONTINUOUSLY EXPANDING SILICONE CULTURE SURFACE GENERATES HIGH YIELDS OF NON-FIBROTIC DERMAL FIBROBLASTS FOR GRAFTING APPLICATIONS

CHAUDHRY S[@], MAJD H[#], PIETRAMAGGIORI G[#], ALMAN B[$], QUINN T[%], HINZ B[@]

[@] Laboratory of Tissue Repair and Regeneration Matrix Dynamics Group Faculty of Dentistry University of Toronto Canada, [#] Laboratory of Cell Biophysics École Polytechnique Fédérale de Lausanne Switzerland,, [$] Program in Developmental and Stem Cell Biology Hospital for Sick Children University of Toronto Canada, [%] Department of Chemical Engineering McGill University Montréal Canada

Background: The expansion of primary autologous dermal fibroblasts in culture is a pivotal step to obtain sufficiently high numbers for applications where large surface area tissue destructions exceed the body's repair capacity and demand grafting of regenerative cells. Stimulated by the stiff surface of conventional culture plastic, a substantial percentage of fibroblasts spontaneously differentiate into fibrotic myofibroblasts by producing excessive amounts of collagen, de novo expressing α-smooth muscle actin (α-SMA), and consequently developing high contractile force. Myofibroblasts cause pathological tissue contractures characteristic of hypertrophic scars and fibrosis. Hence, suppressing this phenotype during cell culture expansion is crucial for the success of subsequent grafting. We hypothesize that culture on highly elastic surfaces will deliver better quality fibroblasts by inhibiting myofibroblast development. Method: To rapidly produce high numbers of fibroblasts for cell therapy we implement a novel method combining dynamic enlargement of the culture surface with fibroblast growth on a highly compliant, and extendable, silicone rubber (HESR). Results: 1) Attachment and proliferation of human dermal fibroblasts on functionalized HESR is similar to conventional tissue culture plastic. 2) Repeated passaging on plastic augments the percentage of fibrogenic myofibroblasts. 3) HESR fibroblast cultures show differential gene expression of putative markers for fibrosis. 5) The pro-fibrotic cytokine TGF-β1 fails to activate myofibroblast differentiation on HESR. 6) HESR polymer rheology and not polymer chemistry is responsible for the mechanically-induced gene expression changes and 7) HESR dynamic expansion culture generates a multi-fold increase in daily cell yield while retaining the anti-fibrotic properties of static HESR.

Abstract Number: P14

Graduate Research

BEYOND MINERAL DENSITY: BONE QUALITY ASSESSMENT WITH THE MECHANICAL RESPONSE TISSUE ANALYZER

Gaspar AE[*#], Grynpas MD[*#], and Willett TL[*$]

[*] Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, Canada, [#] Institute for Biomaterials and Biomedical Engineering, University of Toronto, Toronto, ON, Canada, [$] Department of Surgery, University of Toronto, Toronto, ON, Canada.

Background: Current clinical methods for assessing bone fracture risk, (dual energy x-ray absorptiometry (DEXA), quantitative ultrasound (QUS)), assess only mineral density and demonstrate poor ability to predict fractures (16%). These techniques cannot detect bone collagen damage, which is known to increase fracture risk. The mechanical response tissue analyzer (MRTA) is an instrument capable of providing a direct in vivo assessment of the dynamic mechanical behaviour of long bones by measuring the mechanical impedance of the bone to a vibration stimulus. This technique can quantify the stiffness and viscoelastic damping of the bone, characteristics attributed to the mineral and collagenous phases, respectively.

Objective: Determine whether the MRTA can detect changes in the mechanical behaviour of bone due to specific alterations of the collagenous phase (without altering mineral content), which increase bone fragility but are undetectable by current tools.

Methods: Three treatments will be performed, each producing specific changes in bone: a) collagen fracture by irradiation, b) intermolecular crosslinking of collagen by advanced glycation endproducts, c) mechanical fatigue. Before and after each treatment, the bones will be measured using the MRTA, standard mechanical bending tests (in order to validate the MRTA's measurements), and current clinical tools, QUS and DEXA, which are not expected to detect such damage.

Results: Preliminary work suggests that the MRTA can detect irradiation damage whereas current clinical tools cannot.

Conclusions: Direct in vivo assessment of long bone dynamic mechanical behavior by MRTA may prove to be an advantageous tool for bone quality assessment beyond bone mineral density.

Posters

Posters

Abstract Number: P15

Graduate Research

BONE COLLAGEN MODIFICATION DUE TO HIGH DOSE GAMMA-IRRADIATION STERILIZATION

Burton BB[*#], Grynpas MD[*#], and Willett TL[*$]

[*] Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, Canada, [#] Institute for Biomaterials and Biomedical Engineering, University of Toronto, Toronto, ON, Canada, [$] Department of Surgery, University of Toronto, Toronto, ON, Canada.

Background: Bone allografts are used in orthopaedic reconstruction when bone stock is deficient. Gamma-irradiation sterilization is a widely-used safety measure; however, it embrittles bone. This is often attributed to collagen damage by free radicals/oxidation. Seeking a solution to this loss of toughness, it is important to more fully understand this bone collagen damage. Objective: Study the changes in molecular stability and integrity of bone collagen as a result of irradiation, and relate these to loss of bone toughness. Methods: Using bovine cortical bone, beams irradiated at 30 kGy were compared to native controls. Beams were tested in 3-point bending to fracture followed by characterization of the decalcified bone collagen by general fluorescence and absorbance readings, differential scanning calorimetry (DSC), hydrothermal isometric tension testing (HIT), high performance liquid chromatography (HPLC) and gel electrophoresis. Results: Irradiated samples lost 56% of their post-yield energy-to-fracture ($p<0.002$). General fluorescence increased. HPLC showed no significant change in pentosidine content (oxidation-dependent fluorescent advanced glycation endproduct crosslink). A 20% decrease in measures of thermal stability ($p<0.001$ for both) was measured with DSC, with a >300% increase ($p<0.001$) in heat of denaturation. HIT testing showed ~20% decreases in thermal stability and temperature of maximum isometric force ($p<0.001$ both). Decrease in these temperatures indicates destabilization and loss of integrity of the collagen network. Interestingly, the increase in heat of denaturation suggests irradiated collagen requires more energy to denature. Together these data suggest three levels of modification: fragmentation of collagen, loss of crosslinks, and formation of new non-covalent bonding.

Abstract Number: P16

other

MATRIX METALLOPROTEINASE-9 EXPRESSION FROM HUMAN KERATINOCYTE IS SUPPRESSED THROUGH KRE-M9 ELEMENT IN THE PROMOTER BY BINDING TO POLY(ADP-RIBOSE) POLYMERASE OF WHICH FRAGMENTATION CAUSED BY CASPASE ACTIVITY RESULTS IN UN-SUPPRESSION ON APOPTOSIS AND/OR O….

Takashi Kobayashi[#], Minoru Akiyama[#], Norihiro Fujimoto[#], Takahiro Satoh[#], Kazuhito Hayakawa[$,]

[#]Department of Dermatology, National Defense Medical College, Saitama, Japan, [$]Department of Dermatology, Teikyo University Chiba Medical Center, Chiba, Japan

Matrix metalloproteinase (MMP)-9 acts for a variety of pathological conditions in the skin, especially in apoptotic or inflammatory disorders. We previously reported the localization of MMP-9 in the peri-keratinized region in the epidermis, especially in the dyskeratotic and hyperkertotic lesions.

In this study, using differentiation model for human keratinocyte in culture by raising calcium concentration which could reflect a kind of apoptosis for karatinocyte as a programmed cell death, we report the importance of KRE-M9 element in the MMP-9 promoter as a repressing region for the transcription, in contrast to 12-o-tetradecanoyl-phorbol-13-acetate (TPA) responsive element (TRE) as an enhancing region adjacent to KRE-M9. The sequence of KRE-M9 is similar to that of KRE-4 in the promoter of involucrin, known as a differentiation marker. We purified the protein which specifically binds to KRE-M9 as well as KRE-4, and identified it as poly(ADP-ribose) polymerase-1 (PARP-1). The fragmented PARP-1 by caspase activity, however, is no longer capable of binding to KRE-M9, which results in the abrogation of the suppression for the transcription.

Such mechanism of MMP-9 expression through KRE-M9 element is also responsible for the stimulation by inflammatory cytokines of tumor necrosis factor (TNF)-α or of interleukin (IL)-1α. In addition, the induction of gelatinase activity, especially MMP-9, as well as caspase activitities was shown in the homogenized skin from the lesion of contact dermatitis in mice. To our knowledge, this is the first report showing the relation between MMP and caspase concretely in view of the gene regulatory mechanism.

Posters

Posters

Abstract Number: P17

Graduate Research

CELL ALIGNMENT WITHIN TISSUE-ENGINEERED VASCULAR SUBSTITUTES IMPROVES VESSEL PROPERTIES

Bourget J-M[#, $], Guillemette M[#,$], Gauvin R[#, %], Mounier M[$], Miville-Godin C[$], Auger FA[#],Veres T[$], Germain L[#]

[#] Centre LOEX de l'Université Laval, Génie tissulaire et régénération : LOEX - Centre de recherche FRSQ du Centre hospitalier affilié universitaire de Québec, and Département de Chirurgie, Faculté de Médecine, Université Laval, Québec, QC, Canada., [$] Industrial Materials Institute, National Research Council Canada, Boucherville, QC, Canada, [%] Quebec Center for Functional Materials (CQMF), Quebec, QC, Canada.

Cardiovascular diseases are the leading cause of death in the USA. There is a lack of an optimal transplant material for small caliber blood vessels. Tissue-engineered blood vessels, reconstructed by the self-assembly approach, demonstrated impressive results in human. Improvements in these constructs could lead to substitutes with improved physiological properties. Alignment of cells within native tissues is important for tissue functions such as resistance and contraction. This cell alignment can be recreated in vitro by contact guidance. Our hypothesis is that vessels reconstructed with circumferentially aligned cells will present improved contraction and mechanical properties.

We produced a pattern of hills and valleys by photolithography, followed by hot embossing of a bio-compatible thermoplastic elastomer, in order to produce a tissue culture substrate. Smooth muscle cells (SMCs) were grown with ascorbate and serum until they form cell sheets, on either patterned or flat polymer substrates. Cell sheets were rolled around a mandrel to form tissue-engineered media. After maturation, sections of the constructs were tested for contraction capabilities and mechanical resistance. Histological staining and immunofluorescence labelling of SMC marker's were also performed.

Circumferential and longitudinal alignments of SMCs within the reconstructed media modified the contractile properties of the construct. Mechanical properties of the vessel were also dependent upon cell alignment. Histological staining demonstrated the structural integrity of these constructs and immunofluorescent labelling showed alignment of cells within the tissue.

Alignment of cells is an important factor for tissue function, recreating this geometry will eventually allow tissue-engineered blood vessel to match their physiological counterparts.

Abstract Number: P18

Graduate Research

THE EFFECT OF MECHANICAL STIMULATION ON OSTEOCYTES CHEMO-SENSITIVITY

Zhang Jia Ning[#], Liu Chao[#] and You Lidan[#*]

[#] Institute of Biomaterials & Biomedical Engineering, University of Toronto, ON, Canada, [*] Department of Mechanical and Industrial Engineering, University of Toronto, ON, Canada

Osteocytes are believed to be the mechanosensory cells that detect and respond to mechanical loading. Mechanical loading results in oscillatory fluid flow (OFF) of interstitial fluid through the lacunar-canalicular networks and activates osteocytes to induce calcium mobilization and release prostaglandin E2 (PGE2), a key molecule in the regulation of bone remodeling. Osteocytes are also sensitive to chemical stimulation. Neurotransmitter serotonin, which plays roles in bone metabolism, can also increase PGE2 release in osteocytes. However, the combined effects of mechanical and chemical stimulation on osteocytes have never been studied. Since osteocytes in bone can experience both types of stimulation simultaneously, it is unclear whether mechanical stimulation can influence the osteocyte sensitivity towards chemical stimulation. In this study, we hypothesize that mechanical stimulation can affect the sensitivity of osteocytes to chemical stimulation. Specifically, we investigate the effect of OFF on the osteocyte response to serotonin in triggering calcium mobilization and releasing PGE2.

Methods: Osteocytes seeded on glass slides were subjected to serotonin with or without pre-treatment by OFF. Calcium response was measured by Fura-2AM loaded osteocytes and PGE2 levels were obtained from conditioned media.

Results: Without mechanical stimulation, serotonin induced calcium mobilization and increased PGE2 release by 3-fold. With the cells mechanically stimulated, serotonin treatment no longer affected PGE2 release, and induced lower calcium response.

Conclusion: Serotonin can stimulate osteocytes to increase calcium mobilization and release PGE2. Mechanical stimulation reduced osteocyte sensitivity to respond to serotonin in both calcium and PGE2. I am currently investigating the mechanisms behind these effects.

Posters

Posters

Abstract Number: P19

Graduate Research

COMPARISON OF THE BIOACTIVITY OF BMP-2 ISOFORMS

ZHOU AJ[#], LEE DWK[#], CLOKIE CML[#], PEEL SAF[#]

[#]Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, University of Toronto

Bone morphogenetic protein 2 (BMP-2) is a dimeric growth factor. Recombinant human BMP-2 (rhBMP-2) is produced as two N-terminal isoforms (18 or 22-kDa), potentially generating three different forms of dimers (18-18, 18-22 or 22-22). Clinical rhBMP-2 preparations can vary greatly in their isoform ratios. However, it is unknown whether these isoforms differ in their biological activity. To evaluate the bioactivity of rhBMP-2 isoforms in vitro and in vivo, two rhBMP-2 samples with different isoform ratios and a clinically available rhBMP-2 were obtained. Cell lines secreting different ratios of rhBMP-2 isoforms were also generated. The amount of rhBMP-2 was quantified by ELISA. Isoform ratios were determined by densitometry on SDS-PAGE gels or Western blots. BMP-2 bioactivity in vitro was determined using the C2C12 assay. BMP-2 osteoinductive activity in vivo was determined by implanting rhBMP-2 in the muscle pouch in mice (n=5). After 28 days, the induced bone ossicles were analyzed by microCT and histology. The BMP-2 samples tested showed a wide range of 18/22-kDa isoform ratios from 88/12 (mainly 18-kDa BMP-2) to 0/100 (only 22-kDa BMP-2). The samples with higher 18/22-kDa isoform ratios demonstrated a significantly higher biological activity in vitro ($P<0.05$). However, there were no significant differences in the induced ossicles produced by the samples of different isoform ratios in vivo. We conclude that small variations in rhBMP-2 isoform ratio do not alter BMP-2 bioactivity significantly. Larger variations (>20%) in isoform ratio produce small but significant differences in vitro; however, these were not seen in vivo.

Abstract Number: P20

Graduate Research

IN VITRO INVESTIGATION OF OSTEOCYTE RESPONSE TO MICRODAMAGE

Liu, C[#]; You, L

[#]Institute of Biomaterials and Biomedical Engineering, University of Toronto

Physiological loading creates microdamage in bone, which causes physical damage to osteocytes embedded within the bone. The damaged osteocytes may signal bone remodeling of the damage site. This process may be inflammatory in nature and unlikely to be the same as unloading-induced remodeling, since in vivo evidence suggested that mechanical loading is required for the repair of microdamage in bone and inflammatory cytokines such as TNFα and IL-6 were upregulated.

We hypothesize that osteocytes with physical damage similar to microdamage release increased levels of TNFα and IL-6, and this response is regulated by mechanical loading.

MLO-Y4 osteocyte-like cells were cultured in α-MEM supplemented with 2.5% FBS, 2.5% CS, and 1% P/S on collagen-I-coated glass slides at 37 ºC and 5% CO2. Physical damage alone and in combination with fluid shear (2Pa oscillatory at 1Hz) was applied to the cells for 1 hour. They were damaged by an array of 9 equally spaced 20G needles. TNFα and IL-6 mRNA levels were quantified with RT-PCR. Student's t-test was used to assess significance ($P < 0.05$).

Physical damage alone induced 140% increase in IL-6 and 200% increase in TNFα levels. Fluid shear stress alone induced 80% increase in IL-6 and 150% increase in TNFα levels. With both fluid shear stress and physical damage, IL-6 increased 60% from control, and TNFα increased 80%.

In summary, physically damaged osteocytes could signal remodeling through increased inflammatory cytokines IL-6 and TNFα. However the increases in both cytokines are modulated by fluid shear stress.

Posters

Posters

Abstract Number: P21

Graduate Research

PHOTOSENSITIZER CONJUGATED CHITOSAN FOR CROSSLINKING AND REINFORCEMENT OF DENTIN COLLAGEN

Annie Shrestha[#], Anil Kishen[#,$]

[#]Laboratory for Photo-therapeutics in Oral Diseases, Dental Research Institute, Faculty of Dentistry, University of Toronto, Toronto, Canada, [$]Discipline of Endodontics, Dental Research Institute, Faculty of Dentistry, University of Toronto, Toronto, Canada

Clinical studies have shown that loss of tooth structure, as well as dentin-collagen degradation with time are important factors associated with the structural failure in a root-filled tooth. Photodynamic crosslinking of collagen establishes bonds between collagen fibrils and link one polymer chain to another. Additionally, reinforcement of collagen matrix could be achieved by incorporating biopolymeric fillers such as chitosan. The aim of this study was to investigate the effect of photosensitizer conjugated chitosan nanoparticles to induce photodynamic crosslinking and reinforcement of dentin-collagen. Collagen obtained from demineralized dentin sections was photodynamically crosslinked with a non-coherent light source (540 nm) at 20 J/cm2 using rose bengal conjugated chitosan (CSRB) nanoparticles. The mechanical properties, enzymatic degradation and ultrastructural changes of dentin-collagen were evaluated. Photodynamically crosslinked collagen showed higher degree of resistance to enzymatic degradation ($p<0.01$) and increase in UTS ($p<0.05$). Incorporation of chitosan into crosslinked collagen matrix, improved the toughness and UTS of dentin-collagen ($p<0.01$). Under transmission electron microscopy, the dentin-collagen showed incorporation of these nanoparticles. This study highlighted the possibility of improving resistance to degradation and mechanical properties of dentin-collagen by simultaneous photodynamic crosslinking method and incorporation of photosensitizer conjugated chitosan nanoparticles into collagen microstructure.

Abstract Number: P22

Graduate Research

EFFECTS OF PROSTAGLANDIN EP4 RECEPTOR AGONIST - ALENDRONATE CONJUGATE IN TREATING OVARIECTIMIZED RAT MODEL OF OSTEOPOROSIS

Careesa Liu[#], Tom L. Willett&, Robert N. Young[*], and Marc Grynpas[#]&

[#]Institute of Biomaterials and Biomedical Engineering, University of Toronto, [*]Department of Chemistry, Simon Fraser University, &Samuel Lunenfeld Research Institute, Mount Sinai Hospital

Since both anti-resorptive and anabolic effects are desired in the treatment of osteoporosis, this study aims to determine the in-vivo effects of a novel conjugate drug containing alendronate (ALN) and a bone-forming agonist for the prostaglandin EP4 receptor (EP4-A).

Six weeks post-ovariectomy, 18-week-old female Sprague-Dawley rats were intravenously given the conjugate at low dose (CL, 5mg/kg/week) and high dose (CH, 25mg/kg week 1, 15mg/kg weeks 2, 4, 6), unconjugated EP4-A and ALN (2.5mg/kg/week each), and vehicle (OV, weekly). Subcutanous injections were given for prostaglandin E2 (PG, 4 mg/kg/day) and vehicle-treated sham-operated controls (SV). After 6 weeks, the right femur and 6th lumbar vertebrae were imaged using micro-computed tomography.

Conjugate treatment resulted in significant de novo bone formation along the endocortical wall in femoral mid-diaphysis. There was 144% increase in endocortical bone volume for CL compared to OV, and a 17% increase in cortical thickness. The CH group showed dramatic endocortical bone formation leading to closure of the marrow cavity, but a 30% decrease in peak cortical BMD compared to other groups. In vertebrae, conjugate treatment resulted in increased trabecular formation with doubling of trabecular number and volume in CH relative to OV, and exceeding SV levels.

These initial results indicate that conjugate treatment increased bone turnover with formation exceeding resorption, leading to de novo bone formation. However, too much resorption at the high dose leads to loss of peak cortical BMD and a significantly compromised cortical structure in both vertebrae and femurs.

Posters

Posters

Abstract Number: P23

Graduate Research

OSTEOPETROSIS MUTATION R444L CAUSES ER RETENTION AND MISPROCESSING OF VACUOLAR H+-ATPASE A3 SUBUNIT.

Ajay Bhargava[‡], Irina Voronov[‡], Yongqiang Wang[‡], Michael Glogauer[‡], Norbert Kartner[‡] and Morris F. Manolson[‡§1]

From the [‡]Dental Research Institute, Faculty of Dentistry, and the [§]Department of Biochemistry, Faculty of Medicine, University of Toronto, Toronto, Ontario M5G 1G6 Canada.

Osteopetrosis is a genetic bone disease characterized by increased bone density and fragility. The R444L missense mutation in the human V-ATPase a3 subunit is one of several known mutations in a3 and other proteins that can cause this disease. After introducing the R444L mutation into the yeast a3 homolog, Vph1p, for further study, a relatively small effect on function was observed, with no temperature sensitivity. Further mutations in the yeast system suggested that a basic amino acid at the homologous position (R462) was not necessary for Vph1p function. Modeling of the R444L mutation in mouse a3 (R445L) caused ER retention of a3 with attendant abrogation of maturation and trafficking of the glycoprotein and its ultimate degradation. In the RAW 264.7 mouse osteoclast differentiation model, the mutant a3 was found to degrade at an increased rate over the course of osteoclastogenesis. Limited trypsinolysis studies suggested that the R445L mutation alters a3 protein conformation. Together, these data suggest that R444 has some role in protein folding or stability that is of greater significance to mammalian a3 than to its equivalent in yeast, and that infantile osteopetrosis caused by the R444L mutation in the V-ATPase a3 subunit is another member of the growing class of protein folding diseases. This may have implications for treatment using protein rescue strategies.

Abstract Number: P24

Graduate Research

INSUFFICIENCY OF OP-1 FOR THE TREATMENT OF RADIATION INDUCED NON-UNION IN A RAT FEMORAL FRACTURE MODEL

Janic K [#$]; Nicholls FH [%]; Willett, T L [# %]; Grynpas, M D [#]; Ferguson P C [%]

[#] Samuel Lunenfeld Research Institute, Mt. Sinai Hospital, [$] Institute of Biomaterials and Biomedical Engineering, University of Toronto, [%] Department of Surgery, Division of Orthopaedic Surgery, University of Toronto.

Effective clinical management of soft tissue sarcoma includes surgery and external beam radiotherapy. Pathologic fracture is a serious, late complication and no reliable biologic solution exists for this problem.

Osteogenic protein 1 (OP-1) is used clinically to treat persistent non-union. It was the objective of this study to determine whether OP-1 would lead to an increased rate of union in rat femoral fractures subjected to periosteal excision and external beam radiotherapy.

Using a previously validated rat model of radiation induced non-union,144 female Sprague-Dawley, retired breeders were separated into four treatment groups (1.control, 2.combined therapy, 3.control + OP-1, 4.combined therapy + OP-1) and temporal end-points of 28, 35 and 42 days post-fracture.

For combined therapy,18 Gy of radiation was administered to the left femur followed by surgical excision of the periosteum three weeks later. Six weeks following radiation, left femurs were fractured and half had 80 µg of OP-1 implanted into the femoral canal. Samples were analyzed using MicroCT, Back Scattered Electron Microscopy (BSE) and Histomorphometric modalities.

As in our previous study, all animals subjected to combined modality therapy had persistent non-union of the left femur. Although OP-1 was effective in augmenting healing of fractures in control fractures, OP-1 did not improve healing in combination therapy fractures. Although, histological examination confirmed that OP-1 was active in combination therapy fractures, it was insufficient for union. This suggests that endogenous fracture repair mechanisms have been compromised and cannot be rescued with osteoinductive drugs.

Posters

Posters

Abstract Number: P25

Graduate Research

LUTEOLIN INHIBITION OF V-ATPASE A3–D2 INTERACTION DECREASES OSTEOCLAST RESORPTIVE ACTIVITY

Crasto G.J[‡], Kartner N[‡], Manolson M.F[‡§]

[‡]Dental Research Institute, Faculty of Dentistry and [§]Department of Biochemistry, Faculty of Medicine, University of Toronto, Toronto, Ontario M5G 1G6 Canada

V-ATPase-mediated acid secretion is required for osteoclast bone resorption. Osteoclasts are enriched in V-ATPase a3 and d2 subunit isoforms, and disruption of either of their genes impairs bone resorption.

Using purified fusion proteins of a3 N-terminal domain (NTa3) and full-length d subunits we determined in a solid-phase binding assay that half-maximal binding of d1 or d2 to immobilized NTa3 occurs at 3.1 ± 0.4 or 3.6 ± 0.6 nM, respectively, suggesting equally high-affinity interactions. A high-throughput modification of this assay was then used to screen chemical libraries for a3–d2 interaction inhibitors, and luteolin, a naturally occurring flavonoid, was identified, with half-maximal inhibition at 2.4 ± 0.9 μM. Luteolin did not significantly affect NIH/3T3 or RAW 264.7 cell viability, nor did it affect cytokine-induced osteoclastogenesis of RAW 264.7 cells or bone marrow mononuclear cells at concentrations ≤ 40 μM. Luteolin inhibited osteoclast bone resorption with an EC50 of approximately 2.5 μM, without affecting osteoclast actin ring formation. Luteolin-treated osteoclasts produced deeper resorption pits, but with decreased surface area, resulting in overall decreased pit volume. Luteolin did not affect transcription, or protein levels, of V-ATPase subunits a3, d2 and E, or V1V0 assembly. Previous work has shown that luteolin can be effective in reducing bone resorption, and our studies suggest that this effect of luteolin may be through disruption of osteoclast V-ATPase a3–d2 interaction.

We conclude that the V-ATPase a3–d2 interaction is a viable target for novel antiresorptive therapeutics that potentially preserve osteoclast–osteoblast signaling important for bone remodeling.

Abstract Number: P26

Graduate Research

POROUS TITANIUM SCAFFOLD PROMOTE BONE REGENERATION OF LONG BONE CRITICAL SIZED DEFECT IN RODENT MODEL

Saad AlQahtani[#, %], C Gao[$, #], Ed Harvey[#, %], Janet Henderson[$, #, %]

Departments of[$] Medicine and [%]Surgery, McGill University, [#]JTN Wong Laboratories for Mineralized Tissue Research, Montreal, QC

Reconstruction of skeletal defects arising from traumatic injuries or surgical resection imposes a major challenge to orthopaedic surgeons. Currently available strategies using autologous or allogeneic bone grafts to promote bone healing are inadequate. This study evaluated the potential of a novel porous titanium scaffold to promote bone regeneration of long bone critical sized defects in rodent models.

Five mm critical-sized segmental defects were generated in the femoral diaphysis of 20, five months old Fischer rats (mass 250±20g) and stabilized using a polyethylene plate and K wires. A novel porous titanium scaffold independently developed at the National Research Council of Canada was implanted in the skeletal defect, whereas control rats received no implants. Bone regeneration around and within the scaffold was analyzed by histological assessment using Von Kossa, alkaline phosphatase and tartrate resistance acid phosphatase, radiography and micro-computed tomography (Micro CT) at postoperative 12 weeks.

The porous titanium scaffold has a microscopic structure similar to native bone. Radiography revealed that the porous titanium promoted bone callus formation at postoperative 12 weeks, while the control group showed no callus formation. Micro CT demonstrated extensive bony ingrowth and wide-spread bone-titanium interface.

The novel porous titanium scaffold shows promise as a potential substitute to bone grafts for bone reconstruction in patients with critical sized defects.

Posters

Posters

Abstract Number: P27

Graduate Research

TRANSPLANTATION OF MESENCHYMAL STEM CELLS AND POSTOPERATIVE DELIVERY OF VASCULAR ENDOTHELIAL GROWTH FACTORS SYNERGISTICALLY PROMOTE FRACTURE HEALING IN RODENT MODEL

Gao C [*^]; Chua M[^%]; Jiang F[*^]; Wang H[^]; Li A[^]; Nazhat S[#]; Harvey EJ [$^]; Henderson JE[*$^]

[*]Departments of Medicine, [$]Surgery and [#]Mining & Materials Engineering, McGill University, ^JTN Wong Laboratories, McGill University Health Centre, Montreal, Quebec; [%] British Columbia Institute of Technology

Interventions using allograft bone to promote bone regeneration in skeletal defects, seen in fracture nonunion, are ineffective due to its poor osteoinductive capability.

This study was to determine if the combination of vascular endothelial growth factor (VEGF) and mesenchymal stem cells (MSCs) seeded in collagen scaffolds could enhance bone healing in rodent model.

MSCs from donor mice were seeded in collagen gels and the cellular viability and differentiation in osteogenic medium were monitored. MSCs-seeded scaffolds prepared were transplanted into 3mm x 1mm defects drilled in the RIGHT femurs of recipient mice. Similar defects in the LEFT femur without intervention were internal control. On postoperative D4, a subset of mice had the defects made on the RIGHT femurs further treated with 10ng VEGF while the LEFT defect receiving vehicle. Osteogenesis was evaluated after 4 weeks using micro computed tomography (MicroCT) and histology.

The collagen scaffold supported cell survival and osteoblastic differentiation in culture. MicroCT at postoperative 4 weeks showed the RIGHT defects, which received the cell-seeded scaffold only, have a trend toward more bone compared with the LEFT defects. VEGF led to significantly more bone formation in the RIGHT defects compared with the LEFT one ($P<0.001$). Histology proved more mineral and increased staining for osteoblast and osteoclast activity in the presence of cell-seeded scaffolds.

Transplantation of MSCs seeded in collagen vehicle and VEGF delivery enhanced bone regeneration synergistically in a rodent model of fracture repair showing promise as a potential strategy to substitute bone grafts for bone reconstruction.

Abstract Number: P28

Graduate Research

CHARACTERIZATION OF EARLY ONSET SCOLIOSIS IN MICE WITH TARGETED DISRUPTION OF FIBROBLAST GROWTH FACTOR RECEPTOR 3

Michael B. Sullivan[$%], Ali Esmaeel[$%], Marco Kneifel&, Jean Ouellet[$%], Neil Saran[$%], Janet E. Henderson[$#%]

[$] McGill University Department of Surgery, [#] McGill University Department of Medicine, [%] JTN Wong Lab for Bone Engineering, Research Institute of the McGill University Health Centre, & University of British Columbia

Scoliosis can be infantile or juvenile in onset; however, it is more frequently identified in older children, particularly girls as they enter puberty. Untreated scoliosis progresses with age and there are currently few non-operative therapies for severe scoliosis. Furthermore, current animal models inadequately represent human scoliosis.

Show that Fgfr3-/- mice, in addition to severe kyphosis and other skeletal abnormalities, develop progressive scoliosis and are an improved model of scoliosis.

Ninety-six male and female mice of wild-type and Fgfr3-/- backgrounds were radiographed with posterior-anterior and lateral views to measure cobb angles, body mass, and other anatomical dimensions. Mice were euthanized at 4 to 25 weeks old and processed for micro-CT and histological analysis to compare parameters such as vertebral and inter-vertebral disc (IVD) morphology, vertebral rotation, and cellular characterisation. All knockout mice were paired with an age and gender matched wild-type counterpart for micro-CT and histological analysis.\\

Fgfr3-/- mice developed scoliosis by 8 weeks ($p<0.05$) that progressed until the end of study, reaching a maximum of 40.9o±18.3 compared to wild-type counterpart of 5.1o±4.2. Micro-CT analysis of Fgfr3-/- vertebral body micro-architecture revealed decreased bone volume fraction of the convex side, decreased IVD thickness and relative body height on the concave side of the curve. Histology identifying mineralization supports these results.

Because the proposed model features spontaneously occurring scoliosis, we see it as more clinically relevant than surgically induced scoliosis. We propose the Fgfr3-/- mouse as an animal model that is inexpensive, easily available, non-invasive, and closely reproduces the disease.

Posters

Posters

Abstract Number: P29

Graduate Research

LINK-N PEPTIDE: CAN DEGENERATED HUMAN INTERVERTEBRAL DISCS BE REPAIRED?

[#]Gawri R, [#]Antoniou J, [%]Ouellet J, [#]Steffen T, [$]Roughley PJ, [#]Haglund L, [#]Mwale, F.

[#]Department of SurgeryMcGill University Montreal, [%] McGill Scoliosis and Spine Center Montreal, [$]Shriners Hospital for Children Montreal.

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.

Cells were isolated from lumbar discs and exposed to Link-N for 48 hours. Intact discs were prepared for organ culture and injected in the NP with 35SO4 and 1mg of Link-N/disc. Discs were also injected with Link-N conjugated to 5-TAMRA, and its distribution within the disc and in the surrounding medium was evaluated.

Proteoglycan synthesis increased in a dose dependent manner with the maximal response at 1000ng/ml 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 at one week post injection, suggesting a sustained effect of Link-N. The fluorescent peptide was found to localize in the NP and at the NP/iAF junction, but its lateral diffusion within the disc was restricted by the AF structure. Diffusion of Link-N into the surrounding medium appeared to occur by diffusion through the endplates.

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 a definitive regenerative effect and a significant cost advantage over growth factors such as BMP7, TGFβ and GDF5.

Abstract Number: P30

Undergraduate Research

ACUTE VIBRATION INDUCES TRANSIENT EXPRESSION OF ANABOLIC GENES IN THE INTERVERTEBRAL DISC IN A FREQUENCY DEPENDENT MANNER

Priya Patel[1+], Matthew R. McCann[1+], Kim L. Beaucage[1], Corey Bacher1, David W. Holdsworth[2], S. Jeffrey Dixon[1] and Cheryle A. Séguin[1]

1Department of Physiology and Pharmacology, 2Imaging Laboratories, Robarts Research Institute; Department of Medical Biophysics, and Department of Surgery, Schulich School of Medicine & Dentistry, The University of Western Ontario, London, Ontario, Canada; N6A 5C1. , + Authors contributed equally to work.

Objective: Degeneration of the intervertebral disc (IVD) is implicated in the etiology of back pain and current therapies are considered inadequate. Whole-body vibration has been adopted for the treatment of spine pathologies; however, there is limited knowledge of the direct effects of vibration on IVDs. We examined the effects of acute vibration on gene expression in IVDs and characterized the dependence of these changes on time and vibration frequency.

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, 15-90 Hz, constant acceleration 0.3 g) and IVDs were harvested at specific times post-vibration. Gene expression was quantified using real-time polymerase chain reaction.

Results: Acute vibration at 15 Hz induced the expression of anabolic genes (aggrecan, biglycan, decorin, type I collagen, Sox9) and suppressed expression of Mmp13, with the most pronounced changes detected 6 h post-vibration. These beneficial effects were frequency-dependent, with no significant changes in gene expression detected between 45-90 Hz. In vivo, the anabolic response was even more robust (up to 19-fold increases) and was accompanied by decreased expression of Adamts4/5 and Mmp3.

Conclusions: These findings demonstrate dramatic anabolic effects of acute vibration on IVD tissues, responses that are dependent on vibration frequency. Similarity of responses in vivo and ex vivo establishes that most, if not all, of these effects arise through the direct actions of vibration on spinal tissues.

Posters

Posters

Abstract Number: P31

Post-doctoral Research

INFLUENCE OF PLACEMENT TWO IMPLANTS ON THE RETENTION OF MAXILLARY OBTURATORS IN PATIENTS WITH MAXILLARY EDENTULOUS ARCH AND UNILATERAL MAXILLARY DEFECTS

Ass.prof. Salah AbdelFatah Hegazy [*],Dr. Ahmed Khalifa[**]

* Associate professor of Removable Prosthodontics ,Faculty of Dentistry , Mansoura University, ** Clinical demonstrator of Removable Prosthodontics ,Faculty of Dentistry , Mansoura University.

Aim of the study: To evaluate the influence of placement two implants on the retention of maxillary obturators.The two implants placed one on the resected and one in the non resected side of the patients

Material and methods: Twenty maxillary edentulous patients with unilateral maxillary defects of age ranged from 45-70 years, and of either sex were selected for study. The patients had completely dentulous mandibular arch. Two implants were placed for each patient, one in the first premolar region of the healthy side and one in the area of first, and second molar of the defect side. For all the patients, the retention was measured before superstructure placement for the two maxillary osseointegrated implants using soft liner alone, after placement of ball attachments without soft liner, and after placement of superstructure wit soft liner. All measures of retention were done after three months of obturator insertion using forcimeter gauge.

Results: The results of this study showed that there was significant difference when using two implants with soft liner in comparison with soft liner or implants alone where p less than .005 using one way ANOVA test.

Conclusion: The use of only two strategically placed implants in the remaining bone of the resected and non-resected side of unilateral maxillary defect can significantly affect the obturator retention.

Abstract Number: P32

Graduate Research

OPTIMIZATION OF BIOREACTOR DESIGN FOR NORMAL LOADING OF HUMAN INTERVERTEBRAL DISCS

Gawri R [#], Althukair R [#], Moir J [#], Roughley P [$], Ouellet J [%], Steffen T [#], Haglund L [#]

[#]Orthopaedics Research Laboratory, McGill University, Montreal, Canada. [$]Shriner's Hospital for Children, [%]McGill Scoliosis & Spine Center, McGill University, Montreal, Canada

Intervertebral disc (IVD) degeneration is a common cause of back pain. It's crucial to understand the interplay between mechanobiology, IVD composition and metabolism to understand underlying cause of IVD degeneration. A bioreactor is being developed that facilitates organ culture of intact human IVDs in controlled dynamically loaded environment. In this study, stress profilometry was used to evaluate optimal loading platen design for the bioreactor.

Intact human lumbar IVDs were prepared. Surface area was measured to calculate the loads to be applied to generate pressures of 0.3MPa and 0.6MPa. Two platen sets were tested, full coverage and partial coverage of only the nucleus pulposus region. Stress profiles were recorded at 0.3MPa and 0.6MPa static load. Vertical and horizontal stress profiles were generated for anterior-posterior and lateral diameters of the IVD.

In young healthy IVDs, stress profiles for full and partial covering platens were very similar to the stress profiles generated from the same disc with intact vertebral bone. Degenerate specimens showed an uneven load profile with regions of perturbation, when loaded with full coverage platens. Loading the degenerate discs with partially covering platens resulted in uneven load profiles and failure of the cartilaginous endplates at higher load.

A critical step in the development of bioreactor is validation of its components. Our findings indicate that choice of load platen is critical to provide in vivo-like load conditions when studying degenerate IVDs in organ culture. This bioreactor system provides a useful tool to study the mechanisms of degeneration and regeneration of human IVD.

Posters

Posters

Abstract Number: P33

Graduate Research

EFFECT OF INTERVERTECH-1 LEVELS ON PROTEOGLYCAN SYNTHESIS BY INTERVERTEBRAL DISC CELLS

Gawri R [#$], Haglund L [#], Madiraju P [$], Epure L [$], Ouellet J[%], Antoniou J [$], Mwale F[$].

[#]Orthopaedics Research Laboratory,McGill University, Montreal, Canada. [$]Lady Davis Institute,McGill University, Montreal, Canada. [%]McGill Scoliosis & Spine Center, McGill University, Montreal, Canada.

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.

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.

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.

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.

Abstract Number: P34

Undergraduate Research

INCREASED PARAVERTEBRAL CONNECTIVE TISSUE THICKNESS INDUCED BY MOVEMENT RESTRICTION

Woodworth SD, Langevin HM

Department of Neurology, University of Vermont, Burlington, VT

We have recently shown that human subjects with chronic low back pain had a 25% increase in ultrasound echogenicity of lumbar paravertebral connective tissue compared with control subjects, as well as a 25% reduction in shear strain within the thoracolumbar fascia during passive trunk flexion. This abnormal connective tissue structure and function may be due in part to movement restriction that could contribute to the cause or the effect of low back pain. We hypothesize that connective tissue remodeling can be induced experimentally in rats using a non-surgical method for restricting trunk and hindleg motion.

20 rats were randomized to movement restriction vs. no-restriction for 8 weeks. Movement restriction was achieved using a "hobble" device consisting of a metal collar surrounding one ankle connected to a chest harness resulting in a 50% reduction of the standing distance between the forefoot and ipsilateral hindfoot. At 8 weeks, rats were euthanized and the paravertebral connective tissue of the back examined with magnetic resonance imaging (MRI). The cross sectional area of connective tissue in the back was determined in transverse MRI images from L6 to L3.

Connective tissue cross sectional area was increased in the movement restriction group compared with non-restricted controls (p=.01). Further histological analyses will investigate pathological changes associated with this increase in connective tissue thickness.

Movement restriction can influence the thickness of connective tissue in the back in a rodent model. Possible causative pathological processes relevant to low back pain include fibrosis, inflammation and fatty infiltration.

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Abstract Number: P35

Graduate Research

OSTEOCYTES EXHIBIT MULTIPLE CALCIUM OSCILLATIONS IN RESPONSE TO MECHANICAL LOADING

Al-Dujaili SA[*], Liu C[*], Guenther A[*#], You L[*#]

[*] Institute of Biomaterials and Biomedical Engineering, University of Toronto, [#], Department of Mechanical and Industrial Engineering, University of Toronto

Calcium signaling is an early response of osteocytes to mechanical stimuli, where a transient increase in intracellular calcium concentration shortly follows the onset of stimulation. Magnitude, length and frequency of calcium oscillations have been implicated in regulating cell viability and functions. We hypothesize that calcium signaling in osteocytes is mechanical loading pattern dependent and regulated by intercellular-spacing.

In this study we investigated the effect of cyclic hydraulic pressure (CHP; 68kPa, 0.5Hz) and oscillatory fluid flow (OFF; 3Pa, 1Hz) on MLO-Y4 osteocyte-like cell calcium response. 21% of cells responded with minimum 2-fold increase in the calcium response to CHP over baseline (no stimulation) and 15% of cells had multiple calcium peaks. 69% of cells responded with minimum 4-fold increase in calcium response to OFF, and 20% of cells showed multiple calcium peaks. The differences in the magnitude and number of calcium peaks to loading suggests that calcium mobilization under OFF is regulated by a different mechanism than CHP.

We also varied cell density to model intercellular spacing during OFF-induced calcium signaling. The percentage of responding cells decreased 3.5-fold and cells with multiple calcium peaks completely abolished under low cell density, suggesting that intracellular calcium mobilization is largely dependent on intercellular spacing and cell-cell interactions.

This is the first study to examine the role of different mechanical and spatial conditions on osteocyte calcium response. Further elucidation of the involved mechanisms is needed to understand the role of osteocytic calcium signaling on bone remodeling and potential treatment of osteoporosis and other bone diseases.

Abstract Number: P36

Graduate Research

ROLE OF FIBRONECTIN IN MICROFIBRIL ASSEMBLY AND HOMEOSTASIS IN VIVO

Amani Hassan[#], Christine Fagotto-Kaufmann[#], Laetitia Sabatier[#], Takao Sakai[$], Dieter P. Reinhardt1[#*]

[#]affiliation 1: McGill University, Faculty of Medicine , Montreal,QC, Canada, [$]affiliation 2:Lerner Research Institute, Cleveland Clinic Cleveland, OH, USA, [*]affiliation 3:McGill University,Faculty of Dentistry, Montreal, QC, Canada

Substantial evidence using cell culture systems exist from our group and others demonstrating that fibronectin (FN) acts as a master organizer of matrix formation. In these cell culture assays, FN guides fibrillin-containing microfibril formation and function. Mutations in fibrillin-1 result in a number of fibrillinopathies, characterized by cardiovascular, skeletal and ocular systems. We aim to determine the role of FN on the formation, homeostasis and function of microfibrils in vivo. This information is essential to understand the pathogenesis in fibrillinopathies as it relates to potential modifiers of clinical severity. We have generated a conditional and tamoxifen-inducible FN knockout mouse model in the smooth muscle. FNflx/flx mice were crossed with the transgenic mouse line SMA-Cre-ERT2/+ in which the expression of the tamoxifen-dependent Cre-ERT2 recombinase is under the control of the mouse α-SMA gene. Deletion of FN gene in the FNflx/flx; SMA-Cre-ERT2/+ was induced by injecting tamoxifen intraperitoneally or subcutaneously (1 mg/day or 0.75mg/day) for 5 consecutive days into 5 or 3 week old mice. Initial analyses of experimental mice followed 3-5 days after tamoxifen injection. Homozygous mice are much smaller than their wild-type littermates and do not move in cage. Heterozygous show reduced mobility compared to non-treated control and 3 week old mice. Histological analysis of blood vessels (aorta) shows disrupted architecture of the vessel wall. Homozygous tamoxifen-injected mice were also characterized by enlarged and reduced alveolar spaces in the lung. These findings suggest that FN is a key matrix protein in the development but not homeostasis of blood vessels and lungs.

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Abstract Number: P37

Graduate Research

CRYO-TEM OF HYDRATED COLLAGEN FIBRILS IN DENTAL AND PERIODONTAL TISSUE SECTIONS

Quan B.D.[*], Sone E.D.[#$*]

[*]Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, ON, Canada, [#]Faculty of Dentistry, University of Toronto, Toronto, ON, Canada, [$]Department of Materials Science and Engineering, University of Toronto, Toronto, ON, Canada

Type I collagen is the major organic component of most mineralized vertebrate tissues including Bone, Dentin, and Cementum. In order to investigate the role of collagen structure in mineralization, we use cryo Transmission Electron Microscopy (cryo-TEM) to evaluate the structure of hydrated collagen fibrils from closely associated non-mineralized and mineralized mouse tissues: Periodontal Ligament (PDL), Cementum, and Dentin. The PDL, a non-mineralized connective tissue, inserts into the bone of the tooth socket and into the mineralized Cementum on the tooth root to anchor root to bone. Collagen fibrils are continuous between PDL and Cementum, yet there is a sharp transition between mineralized and unmineralized tissue. This juxtaposition of mineralized and non-mineralized tissues presents an opportunity to observe differences between collagen fibrils which span a mineralized interface. Using cryo-TEM and image averaging techniques, we show that there are surprisingly few differences in periodicity and banding structure in collagen fibrils from Periodontal Ligament, Cementum, and Dentin, pointing to the role of non-collagenous macromolecules in control of mineralization at this interface.

Abstract Number: P38

Graduate Research

THE ER STRESS INHIBITOR, 4-PHENYLBUTYRIC ACID (4-PBA), REDUCES KIDNEY INJURY IN A HYPERTENSIVE-PROTEINURIC MODEL OF CHRONIC KIDNEY DISEASE.

Victoria Yum[1], Danielle E. Jerome[1], Richard C. Austin[1] and Jeffrey G. Dickhout[1]

1Department of Medicine, Division of Nephrology, McMaster University, St. Joseph's Healthcare Hamilton, ON, Canada

The myofibroblast is a key cell type involved in fibrosis of many tissues. Myofibroblasts can be identified by de novo synthesis of α-smooth muscle actin (α-SMA), a contractile component that is also present in smooth muscle cells. The Dahl salt-sensitive (SS) rat develops hypertension upon salt loading and demonstrates renal pathology characterized by glomerular sclerosis, renal interstitial fibrosis and tubular atrophy. Using the Dahl SS background, salt-induced hypertension and severe proteinuria is reduced by the substitution of chromosome 13 from the normotensive Brown Norway rat (BN13). Dahl SS or BN13 rats were placed on a high ($8^{\%}$) or low ($0.4^{\%}$) salt diet for 4-weeks with or without 4-PBA (1g/kg/day) treatment. 4-PBA treatment did not affect mean arterial pressure of the Dahl SS on high salt diet ($8^{\%}$ NaCl, SS: 120.5±12.6 mmHg, SS + 4-PBA: 119.9 ±9.9 mmHg). However, 4-PBA treatment did significantly reduce total 24 hour protein excretion in the high salt Dahl SS rats and caused a 6-fold reduction in Periodic acid-Schiff (PAS) positive cast area density. Dahl SS rats showed greater amounts of fibrosis (as shown by α-SMA staining) compared to the BN13 control rats. The appearance of α-SMA around protein casts seems to indicate that the injury due to these casts results in myofibroblast differentiation. The effects of 4-PBA on myofibroblast differentiation and collagen deposition are being explored in this model.

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Abstract Number: P39

Graduate Research

SHORT FIBULIN PROTEOLYSIS AND CELL ATTACHMENT PROPERTIES

Djokic J[*], Fagotto-Kaufmann C[#], Reinhardt DP[*,#]

McGill University, [*]Faculty of Dentistry and [#]Faculty of Medicine, Montreal, Quebec, Canada

The fibulin family comprises seven extracellular matrix (ECM) 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. Knockout mouse studies have implicated the short fibulins, fibulin-3, -4, and -5, in elastic fiber assembly, stabilization, and function.

We recombinantly expressed full-length human short fibulins in human embryonic kidney cells, and purified them 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. 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).

We measured strong adhesion of human skin fibroblasts to the short fibulins by 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. We also observed strong, calcium-dependent binding of fibulin-4 to immobilized heparin in solid phase binding assays, suggesting that this fibulin may bind cell-surface heparan sulfate.

Abstract Number: P40

Graduate Research

CELLULAR MECHANOSENSING IN CONSTRAINED FLOATING COLLAGEN MATRICES

Mohammadi H[#], Janmey A P[$], McCulloch A C[#]

[#] Matrix Dynamics Group, University of Toronto, Toronto, Ontario, Canada,[$] Institute for Medicine and Engineering, University of Pennsylvania, Philadelphia, USA

Cells are mechanically sensitive, which enable critical roles in many cellular functions and human diseases. Currently little is known about the contribution of directional matrix deformations to cellular mechanosensing that extend beyond the cell-matrix interface. Here we present a new model in which adherent cells exert forces on the matrix and induce in- and out-of-plane deformations. In this model collagen gels are supported by circumferential nylon frames that enable the gel to float on growth medium. Cells respond to the matrix mechanics without interference from an underlying foundation, which could impose additional mechanical constraints on the matrix. The boundary conditions created by the nylon frame create a deformation gradient within the matrix so that the matrix is deformed more centrally than near the edges when acted upon by the same applied force. In this model cells respond to deformation gradients by extending cellular processes whose lengths and number depend on matrix width and bending rigidity which is, in turn, determined by matrix stiffness and thickness. Notably, when the width of a thin (50 um) and soft (80 Pa) matrix is increased (from 300 to 2000 um) the number of cell extensions decreases (from 9 to 3) while the maximum cell extension length increases (from 125 to 188 um). However, the generation of cellular processes is reduced on matrices of larger width (e.g. 5000 um) and/or bending rigidity. These data indicate that cells sense the bending rigidity of the extracellular matrix by inducing both horizontal and vertical deformations of the matrix.

Posters

Abstract Number: P41

Undergraduate Research

CHARACTERIZATION OF GASTROINTESTINAL DEFECTS IN THE FGFR2 W290R CROUZON MOUSE MODEL

Dab S[#], Sokhi R[$], Gong S-G[*]

[#]University of Toronto Faculty of Dentistry, [$]University of Toronto Faculty of Dentistry, [*]University of Toronto Faculty of Dentistry

Mutations in Fibroblastic Growth Factor Receptors (FGFR's) have been associated with human craniosynostotic birth defects such as Crouzon syndrome (CS). Several case reports have indicated the higher prevalence of gastrointestinal tract (GIT) disorders in these patients. However, the effects of FGFR mutations on GIT development in craniosynostotic patients have not been well documented in the scientific literature. Our laboratory has characterized a mouse model of human CS with a mutation in Fgfr2 (W290R). We hypothesize that this mutation has a direct and specific effect on the development of GIT. To characterize these defects, dissected stomachs and esophagus (6 wks.) of Fgfr2(W290R) mutant Heterozygotes (HET) and Wild type (WT) mice were analyzed morphologically, histologically and immunohistochemically (using the antibodies, Ki67 and FGFR2). The stomach demonstrated a disproportionately underdeveloped fundus, body and abnormal cytoarchitecture. Histologically, esophagus showed a wider lumen, comparatively fewer epithelial corrugations and differences in thickness and orientation of muscle layers. Immunohistochemically, decreased rate of cell proliferation in the epithelium and reduced expression of FGFR2. Efforts are currently underway to further characterize the other molecular defects in the FGFR2 W290R mutant mice. Taken together, our results provide scientific evidence for the importance of Fgf signaling in the growth and patterning of the GIT. Furthermore, our study offers a sound scientific rationale for any changes in the clinical management of GIT problems in patients with craniosynostotic defects.

Abstract Number: P42

Graduate Research

TRANSIENT LOCALIZED ELEVATION OF CYTOSOLIC FREE CALCIUM IS REQUIRED FOR UROPOD RETRACTION AND OSTEOCLAST MIGRATION

Benjamin D. Wheal[#], Natsuko Tanabe[$], S. Jeffrey Dixon[%], Stephen M. Sims[%]

[#]Graduate Program in Neuroscience, The University of Western Ontario, London, Canada; [$]Nihon University School of Dentistry, Tokyo, Japan; [%]Department of Physiology and Pharmacology, Schulich School of Medicine & Dentistry, The University of Western Ontario, London, Canada

Osteoclasts are large multinucleated cells responsible for the resorption of bone and other mineralized tissues. Mature osteoclasts are highly motile and alternate between cycles of bone resorption and migration. However, little is known regarding the subcellular mechanisms that regulate osteoclast motility. We hypothesized that changes in the concentration of cytosolic free calcium ([Ca2+]i) contribute to the control of osteoclast migration. Our purpose was to characterize subcellular changes in osteoclast [Ca2+]i and their possible role in regulating osteoclast motility. [Ca2+]i was monitored by digital fluorescence imaging of fura-2-loaded osteoclasts using alternating excitation wavelengths of 345/380 nm with emission at 510 nm. Migrating osteoclasts exhibited a polarized morphology with lamellipodia extending forward at the leading edge of the cell and the uropod undergoing retraction at the rear, generating net forward movement. Migrating osteoclasts displayed a distinct spatiotemporal pattern of [Ca2+]i that consisted of localized elevation of [Ca2+]i in the trailing edge of the cell that coincided with uropod retraction. This elevation of [Ca2+]i was blocked in osteoclasts loaded with the cytosolic calcium chelator BAPTA. Time-lapse recordings revealed that BAPTA-loaded osteoclasts continued to extend lamellipodia but failed to detach from the substrate, giving rise to dramatically elongated, highly branched morphologies. These findings reveal a novel spatiotemporal pattern of [Ca2+]i in osteoclasts, with transient localized elevation of [Ca2+]i being required for uropod retraction. This study reports a heretofore unrecognized role for subcellular Ca2+ signaling in the regulation of osteoclast migration. Supported by the Canadian Institutes of Health Research (CIHR).

Posters

Posters

Abstract Number: P43

Graduate Research

SURFACE TOPOGRAPHY OF BIOMIMETIC CALCIUM PHOSPHATE COATINGS REGULATES OSTEOCLASTIC RESORPTION

Daniel O. Costa[†], S. Jeffrey Dixon[§], Paul Prowse[§], Douglas Hamilton[§], and Amin S. Rizkalla[†,§]

Faculty of Engineering†, Schulich School of Medicine & Dentistry[§], The University of Western Ontario, London, Ontario, Canada

The behavior of bone cells is influenced by the surface chemistry, topography and roughness of implants and scaffolds. Bone-like biomimetic apatite coatings improve the osteoconductivity and bone bonding ability of biomaterials for applications in orthopedics and dentistry; however, their interactions with osteoclasts are poorly understood. Our purpose was to investigate how the topography of apatite coatings might regulate their resorption by osteoclasts. Synthetic polymer (polycaprolactone) was coated with calcium phosphate (CaP) by incubation in simulated body fluid. Surface roughness was controlled by modifying incubation time and ionic concentration. X-ray diffraction and Fourier-transform infrared spectroscopy showed the successful formation of CaP coatings, including carbonate-substituted hydroxyapatite (HA). SEM and AFM of coatings and mechanical profilometry revealed that increasing ionic concentration and incubation time caused significant increase in topographical complexity and surface roughness (Ra increased from ~1 to 2 μm). Osteoclasts were isolated from neonatal rabbits and incubated for 48 h on HA coatings having distinct surface topographies. Quantification of staining for the osteoclast marker, tartrate-resistant acid phosphatase, and organization of filamentous actin were consistent with greater osteoclast activity on coatings with less surface complexity as compared to rougher surfaces. Furthermore, SEM revealed the presence of resorption lacunae exclusively on the smoother HA coating. In conclusion, CaP coatings can be prepared with different topographies, which appear to regulate osteoclastic activity and hence susceptibility to resorption. Thus, it may be possible to design HA-coatings with optimal rates of degradation and, when used as a delivery system, release of bioactive compounds. Supported by NSERC.

Abstract Number: P44

Graduate Research

EXTRACELLULAR MATRIX REORGANISATION DURING ADIPOGENESIS: MICROARRAY ANALYSES OF RECONSTRUCTED ADIPOSE AND CONNECTIVE TISSUES ENGINEERED FROM HUMAN STEM CELLS

MARIE-ÈVE OUELLETTE[*$], MAUD VALLÉE[*$], JEAN-CHRISTOPHE BÉRUBÉ[%], YOHAN BOSSÉ[%], JULIE FRADETTE[*$]

[*]Génie tissulaire et régénération: LOEX-Centre de recherche FRSQ du CHA universitaire de Québec, Université Laval, Québec, QC, [$]Département de Chirurgie, Faculté de Médecine, Université Laval, Québec, QC, [%]Institut universitaire de cardiologie et de pneumologie de Québec

The self-assembly method of tissue engineering takes advantage of the endogenous capacity of mesenchymal cells to produce and organize ECM upon ascorbic acid stimulation. We used adipose-derived stem cells to reconstruct both connective and adipose tissues. We investigated variations in ECM mRNA expression associated with adipocyte formation in our 3D human model. Microarray experiments were performed on reconstructed tissues as well as on subcutaneous fat using the Illumina HumanWhole Genome-6 v3 array. Fold changes in expression were calculated with normalized data extracted by ArrayStar software. We used ANOVA and SAM for statistical analysis of genes differentially expressed (fold change > 2.0). Pathway analysis was performed using Ingenuity system 8.5. By assessing the mRNAs that were modulated in reconstructed tissues containing adipocytes compared to their respective non-induced connective tissues, important variations in ECM elements were observed. As expected, basal lamina collagens such as COL4 (2.3x) and COL7 (2.7x) were upregulated with adipocyte presence. In contrary, COL10 (-9.0x) and COL8 (-5.3x) were downregulated. The metalloproteases MMP1 (-6.7 fold), MMP3 (-2.8 fold), MMP7 (20x), MMP9 (-4.7x) as well as the MMP inhibitor TIMP1 (18x) were modulated with adipogenesis. A disintegrin and metalloproteinase ADAMTS-1 (2.0x), ADAMTSL2 (-11x) also varied with adipocyte differentiation. Other MEC components were modulated: LAMA2 (4.8x), syndecan (-5.8x), versican (-2.6x), lumican (-2.4x). Further investigation is needed to describe matrix expression and remodeling during adipogenesis. Comparative analyses of tissues reconstructed by the self-assembly method will help define the major actors involved during adipogenic differentiation in a tissue-like context. Supported by NSERC.

Posters

Posters

Abstract Number: P45

Other

DYNAMIC LOADING OF INTACT HUMAN INTERVERTEBRAL DISCS AND DISC VIABILITY

Moir J[#], Beckman L[#], Gawri R[#], Mulligan K[#], Wismer N[#], Roughley PJ[$], Ouellet J[*], Steffen T[#], Haglund LA[#]

[#]Orthopaedic Research Laboratory, [$]Shriners Hospital for Children Canada, [*]McGill Scoliosis & Spine Center, McGill University, Montreal, Canada

Back pain due to intervertebral disc (IVD) degeneration is a socioeconomic burden that requires remedy for individuals and society. A system for intact disc culture could discern the interplay between disc metabolism, biochemical composition and mechanobiology.

The dynamic loaded bioreactor system developed for bovine discs (Haglund et al. Tissue Eng Pt C 17(10): 1011-1019 2011) was used with human discs and full coverage platens allowing investigation of disc and cell response to load.

IVDs donated through Transplant Quebec were isolated within 8 hours of death, x-rays annotated degree of degeneration. Discs were prepared following methodology of Jim et al. Eur Spine J 20:1244-1254 2011. Image J (NIH) determined disc surface area was used to calculate the force applied. The discs were left for a minimum of 48 hours without load followed by 48 hrs static load at 0.1 MPa. Cyclic load was then applied at 0.1Hz at 0.1-0.3 MPa for 2 hrs twice per day for a total culturing period of two weeks. Cell viability was determined by Live/Dead Assay (Invitrogen).

Cell viability assay demonstrated at least 80% of cells alive in both nucleus pulposus and annulus fibrosis at the termination of the experiment. This indicates sufficient nutrient supply to the cells in a system designed to reflect physiological loading.

This bioreactor system maintained cell viability in intact human intervertebral discs. This important step allows the long term culture necessary to investigate responses to biologically based therapies.

Abstract Number: P46

Undergraduate Research

FAK/SRC INHIBITION ALLEVIATES THE PERSISTENT FIBROTIC PHENOTYPE OF LESIONAL SCLERODERMA FIBROBLASTS

Katherine Thompson[$,*], Xu Shi-wen[#], Korsa Khan[#], Shangxi Liu[$], Murray Baron[%], Christopher P Denton[#], David J. Abraham[#],& and Andrew Leask[$,&]

[#]Centre for Rheumatology, University College London (Royal Free Campus), Rowland Hill St., London UK, NW3 2PF, [$%]Department of Rheumatology, SMBD-Jewish General Hospital, Room A-216, 3755 Cote Ste Catherine Road, Montreal, Quebec, CANADA, H3T 1E2, &Co-senior authors

Fibrotic diseases such as systemic sclerosis (SSc, scleroderma) are characterized by the abnormal presence of the myofibroblast, a specialized type of fibroblast that overexpresses the highly contractile protein α-smooth muscle actin, which displays excessive adhesive properties. The precise contribution of adhesive signaling, which requires integrin-mediated activation of focal adhesion kinase (FAK)/src, to fibrogenic gene expression in normal and fibrotic SSc fibroblasts is unclear. Herein, we use FAK wild type and knockout fibroblasts and normal and SSc fibroblasts with the FAK/src inhibitor PP2 to show that FAK/src operates downstream of both integrin beta1 and reactive oxygen species to promote the expression of genes involved with matrix production and remodeling including CCN2, alpha-smooth muscle actin (SMA) and type I collagen. Moreover, we show that blocking FAK/src with PP2 alleviates the elevated contractile and migratory capability of lesional SSc dermal fibroblasts. We conclude that the excessive adhesive signaling is intimately involved with the fibrotic phenotype of SSc fibroblasts; blocking adhesive signaling may be beneficial in controlling fibrosis.

Posters

Posters

Abstract Number: P47

Graduate Research

EXPRESSION OF PPARα, β, AND γ IN THE HARTLEY GUINEA PIG MODEL OF PRIMARY OSTEOARTHRITIS

Fatima Ezzahra El Mansouri[#], Salwa Sarah Nebbaki[#], Nadia Zayed[#], Mohamed Benderdour[$], Johanne Martel-Pelletier[#], Jean-Pierre Pelletier[#], and Hassan Fahmi[#]

[#]Osteoarthritis Research Unit, CRCHUM, Notre-Dame Hospital, 1560 Sher- brooke Street East, Montreal QC H2L 4M1, Canada, [$] Centre de recherche, Hôpital Sacré-Coeur de Montréal, 5400, boul. Gouin Ouest, Montréal QC H4J-1C5, Canada.

Background: Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor superfamily. Three isoforms have been identified: PPARα, PPARβ/δ and PPARγ. Several in vitro and in vivo studies suggest that PPARγ may have protective roles in osteoarthritis (OA). So far, little is known about the pattern of PPAR expression during the progression of OA and cartilage degradation.

Objective: To investigate the expression of PPARα, β, and γ in cartilage over the course of OA in the spontaneous Hartley guinea pig model.

Methods: Hartley guinea pigs were sacrificed at 2 (control group), 4, 8, and 12 (n = 6 per group) month-old of age. Cartilage was obtained from the central portion of the medial tibial plateau. Cartilage degradation was evaluated histologically using the Osteoarthritis Research Society International (OARSI) guidelines. The expression of PPARα, β and γ was analyzed by immunohistochemistry. The non-parametric Spearman test was used for the correlation analysis between the protein expression levels and histological scores.

Results. PPARα, β and γ, were detected in medial tibial plateaus from control animals. There was no significant changes in the levels of PPARα and PPARβ over the course of OA. In contrast, PPARγ expression decreased during the progression of OA. Correlation analysis revealed a negative correlation between PPARγ levels and histological score of OA.

Conclusion. Expression of PPARγ in cartilage decreased during the course of OA. These data suggest that loss of PPARγ expression in cartilage may contribute to the pathogenesis of OA.

Abstract Number: P48

Graduate Research

EGR-1 MEDIATES THE SUPPRESSIVE EFFECT OF IL-1 ON PPARγ EXPRESSION IN HUMAN OA CHONDROCYTES

Salwa Sarah Nebbaki[#], Fatima Ezzahra El Mansouri[#], Nadia Zayed[#], Mohamed Benderdour[$], Johanne Martel-Pelletier[#], Jean-Pierre Pelletier[#], and Hassan Fahmi[#].

[#] Osteoarthritis Research Unit, CRCHUM, Notre-Dame Hospital, 1560 Sher- brooke Street East, Montreal QC H2L 4M1, Canada. [$] Centre de recherche, Hôpital Sacré-Coeur de Montréal, 5400, boul. Gouin Ouest, Montréal QC H4J-1C5, Canada.

Background: Peroxisome proliferator-activated receptor gamma (PPARγ) is a ligand activated transcription factor and member the nuclear hormone receptor superfamily. Several lines of evidence indicate that PPARγ have protective effects in osteoarthritis (OA). Indeed, PPARγ has been shown to down-regulate several inflammatory and catabolic responses in articular cartilage and chondrocytes and to be protective in animal models of OA. We have previously shown that IL-1 down-regulated PPARγ expression in OA chondrocytes. In the present study we will investigate the mechanisms underlying this effect of IL-1.

Methods: Chondrocytes were stimulated with IL-1, and the level of PPARγ and Egr-1 protein and mRNA were evaluated using Western blotting and real-time reverse-transcription polymerase chain reaction, respectively. The PPARγ promoter activity was analyzed in transient transfection experiments. Egr-1 recruitment to the PPARγ promoter was evaluated using chromatin immunoprecipitation (ChIP) assays. Small interfering RNA (siRNA) approaches were used to silence Egr-1 expression.

Results: We demonstrated that the suppressive effect of IL-1 on PPARγ expression requires de novo protein synthesis and was concomitant with the induction of the transcription factor Egr-1. ChIP analyses revealed that IL-1 induced Egr-1 recruitment at the PPARγ promoter. IL-1 inhibited the activity of PPARγ promoter and overexpression of Egr-1 potentiated the inhibitory effect of IL-1, suggesting that Egr-1 may mediate the suppressive effect of IL-1. Finally, Egr-1 silencing with small interfering RNA blocked IL-1-mediated down-regulation of PPARγ expression.

Conclusion: These results indicate that Egr-1 contributes to IL-1-mediated down-regulation of PPARγ expression in OA chondrocytes and suggest that this pathway could be a potential target for pharmacologic intervention in the treatment of OA and possibly other arthritic diseases.

Posters

Posters

Abstract Number: P49

Other

PARADOXICAL MOTION OF THE THORACOLUMBAR FASCIA INDUCED BY LATERAL TRANSLATION OF THE SKIN

Fox JR[#], Langevin HM[#], Koptiuch C[#]

[#] University of Vermont, Department of Neurology, Burlington, Vermont, USA

The thoracolumbar fascia (TLF) s a complex structure composed of several layers of dense connective tissue separated by "loose" areolar connective tissue allowing the dense layers to glide past one-another. This independent motion, quantified as shear strain, is reduced in chronic low back pain (LPB). Recent evidence suggests that the TLF is abundantly innervated and that pathology within the tissue may be implicated in LBP.

Two human subjects without LBP were tested, with 10 more subjects with/without LBP planned before June 2012. Ultrasound elastography was used to quantify motion within TLF induced by manually translating the skin surface (3-4mm) and by pulling a string attached to adhesive tape on the skin.

A 3 mm cephalad translation of the skin 2cm lateral to the L3-4 interspace resulted in ~0.075mm cephalad translation of the superficial layers of the fascia, while the deep layers moved by approximately the same amount, but directionally caudad.

That motion of the fascia can be induced by moving the skin is hardly surprising. What is surprising, and for which the mechanism is unresolved, is that while the motion of the superficial layers move in-phase with the input motion, the deep layers move in the contrary direction. This phenomenon suggests the existence of a semi-rigid connection between the skin and deeper tissues that could be important for understanding the sensory input that arises from the TLF. We are currently investigating this further to better understand the biomechanical mechanism underlying this phenomenon and the potential implications for better understanding LBP.

Abstract Number: P50

Post-doctoral Research

THE ROLE OF TAK1 IN NORMAL TISSUE REPAIR AND FIBROSIS

Fen Guo, Shangxi Liu, and Andrew Leask [#]

[#]Departments of Dentistry , Dental Sciences Building, University of Western Ontario, London, ON, Canada, N6A 5C1

Introduction: Fibrotic diseases are a significant problem in Canada and in the western world and there is no therapy for fibrosis, in part because fundamental mechanism underlying fibrosis is unknown. The cytokine transforming growth factor (TGF)b plays a major role in fibrogenesis. We have shown that TGFb1 exerts fibrogenic effects in mouse embryonic fibroblasts at least in part via TGFb1 activated kinase (TAK)1. Moreover, we have also shown that TAK1 activation is increased in fibrotic fibroblasts isolated from scleroderma patients. However, the precise contribution of TAK1 to fibrogenic responses in vivo is unknown.

Objective: To assess whether TAK1 is required for cutaneous tissue repair and fibrosis in adults in vivo using mice deleted for TAK1 in adult fibroblasts and explore whether inhibiting TAK1 alleviates the persistent fibrotic phenotype of cultured SSc dermal fibroblasts using a selective TAK1 inhibitor.

Methods: Mice harboring a fibroblast-specific deletion of TAK1 were generated and used to evaluate their wound-healing and fibrogenic responses. In addition, the effect of TAK1 deletion on TGFb1 signaling in the fibroblasts was investigated.

Results: Our preliminary data showed that TAK1-conditional-KO mice exhibited delayed cutaneous wound closure, less granulation formation, decreased collagen production, decreased myofibroblast formation, and reduced phosphorylation of JNK and p38 during wound healing. TAK1-deficient fibroblast showed decreased adhesion, migration, and proliferation.

Conclusion: TAK1 is essential for normal wound healing, and that targeting of TAK1 may be a viable antifibrotic strategy in the treatment of certain disorders, including scleroderma.

Posters

Posters

Abstract Number: P51

Post-doctoral Research

DELETION OF CCN2 IN FIBROBLASTS RESCUES THE FIBROTIC PHENOTYPE OBSERVED IN PTEN DEFICIENT MICE

Liu S[#], Parapuram S I K[#], Sha W[#], Shi-Wen X[$], Abraham D [$] and Leask A[#]

[#]University of Western Ontario, Division of Oral Biology and Department of Physiology and Pharmacology, London, Ontario, N6A 5C1, Canada, [$]University College London, Centre for Rheumatology, Rowland Hill St, London, NW3 2PF, United Kingdom

Background: Fibrosis is characterized by excessive production of collagens and their contraction by fibroblasts. It is the major cause of internal organ failure. There is no effective therapy till now. The matricellular protein connective tissue growth factor (CTGF/CCN2) is a marker of fibrotic cells and is considered playing an important role in fibrogenesis. Our previous studies showed that (a) mice with specific deletion of CCN2 in fibroblasts were resistant to bleomycin induced skin fibrosis and (b) deletion of pten in fibroblasts resulted in skin fibrosis. We would like to know weather deletion of CCN2 could rescue the fibrotic phenotype caused by pten deficiency.

Materials and Methods: Fibroblast specific pten knockout and pten/CCN2-double knockout mice were created by crossing mice carrying floxed pten and floxed CCN2 alleles with mice carrying a tamoxifen-inducible Cre-recombinase under the control of a fibroblast-specific regulatory sequence from the pro a2(I) collagen gene. Two months after gene deletion, animals were sacrificed and dermal thickness, collagen production, and a-smooth muscle actin (a-SMA) were determined.

Results: Loss of pten resulted in significant increase of dermal thickness, collagen production and the number of a-SMA positive myofibroblasts. However pten/CCN2 double knockout mice showed essentially normal dermal thickness, collagen production and number of myofibroblasts.

Conclusion: Our results indicate that CCN2 is required for two models of skin fibrosis, namely bleomycin-induced and PTEN-gene deficiency. These data suggest that therapeutic strategies blocking CCN2 in vivo may be of benefit in combating fibrotic skin disease such as in scleroderma.

Abstract Number: P52

Graduate Research

PROSTAGLANDIN D2 INDUCES APOPTOSIS OF HUMAN OSTEOCLASTS BY ACTIVATING THE CRTH2 RECEPTOR AND THE INTRINSIC APOPTOSIS PATHWAY

Li Yue[#] and Artur J de Brum-Fernandes[*]

[#]Université de Sherbrooke, [$]Université de Sherbrooke.

Prostaglandin D2 (PGD2) is a lipid mediator synthesized from arachidonic acid that activates two specific receptors, the D-type prostanoid (DP) receptor and chemoattractant receptor homologous molecule expressed on T-helper type 2 cells (CRTH2). PGD2 can affect bone metabolism by regulating osteoblast and osteoclast (OC) functions, both of which are involved in bone remodeling and fracture repair. The objective of this study was to determine if PGD2 regulates human OC apoptosis through its two receptors. Human OCs were generated through the differentiation of human peripheral blood mononuclear cells in the presence of receptor activator for nuclear factor κB ligand (RANKL) and macrophage-colony stimulating factor (M-CSF), and treated with PGD2, its agonists, and antagonists. After treatment, cellular apoptosis was determined using the TACS Blue Label Kit. Caspase activity was also examined using the caspase fluorogenic substrate assay. Western blot was performed to assess cleaved caspase-3, caspase-8, and caspase-9 protein levels. Treatment with PGD2 for 24 hours in the presence of naproxen (10 μM) to inhibit endogenous prostaglandin production increased the percentage of apoptotic OCs in a dose-dependent manner, as did the CRTH2 agonist DK-PGD2 but not the DP agonist BW 245C. In the absence of naproxen, the CRTH2 antagonist CAY10471 reduced OC apoptosis, whereas the DP antagonist BW A868C had no effect. The induction of PGD2-CRTH2 dependent apoptosis was associated with the activation of caspase-9, but not caspase-8, leading to caspase-3 cleavage. These data indicate that PGD2 induces human OC apoptosis through activation of the CRTH2 receptor and the intrinsic apoptotic pathway.

Posters

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Abstract Number: P53

Graduate Research

ROLE OF RAC IN DETERMINING OSTEOCLAST RESORPTION LACUNAE VOLUME AND STRUCTURE REVEALED BY CONFOCAL IMAGING OF PICRO SIRIUS RED REPORTER FOR COLLAGEN

Stephanie R. Goldberg[1,2,3,†], John Georgiou[1, †], Michael Glogauer[3,*], and Marc D. Grynpas

1Samuel Lunenfeld Research Institute, Mount Sinai Hospital, 60 Murray St., Box 42, Toronto, Ontario, M5T 3L9, Canada, 2Department of Laboratory Medicine, Pathobiology, and Institute for Biomaterials and Biomedical Engineering, University of Toronto, 3Faculty of Dentistry, Faculty of Medicine, University of Toronto, Room 221 Fitzgerald Building, 150 College Street, Toronto, Ontario, M5S 3E2, Canada, †These authors contributed equally to this work.

Modulation of Rho GTPases Rac1 and Rac2 impacts bone development, remodeling, and disease. In addition, GTPases are considered treatment targets for dysplastic and erosive bone diseases including Neurofibromatosis type 1. While it is important to understand the effects of Rac modulation on osteoclast function, two-dimensional resorption pit area measurements fall short in elucidating the volume aspect of bone resorption activity. Bone marrow from wild-type, Rac1 and Rac2 null mice was isolated from femora. Osteoclastogenesis was induced by adding M-CSF and RANKL in culture plates containing dentin slices and later stained with Picro Sirius Red to image resorption lacunae. Osteoclasts were also plated on glass cover slips and stained with phalloidin and DAPI to measure their surface area and nuclei. Volumetric images were collected on a laser-scanning confocal system. Sirius Red confocal imaging provided an unambiguous, continuous definition of the pit boundary compared to reflected and transmitted light imaging. Rac1- and Rac2-deficient osteoclasts had fewer nuclei in comparison to wild-type counterparts. Rac1-deficient osteoclasts showed reduced resorption pit volume and surface area. Lacunae made by Rac2 null osteoclasts had reduced volume but surprisingly surface area was unaffected. Surface area measures are deceiving since volume changed independently in resorption pits made by Rac2 null osteoclasts. Our innovative confocal imaging technique allows us to derive novel conclusions about Rac1 and Rac2 in osteoclast function. The data and method can be applied to study effects of genes and drugs including Rho GTPase modulators on osteoclast function and to develop pharmacotherapeutics to treat bone lytic disorders

Abstract Number: P54

Other

THE EFFECT OF LINK N ON THE CHONDROGENIC DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELLS

Hong Tian Wang PhD[1], Lisbet Haglund PhD[2], Peter J Roughley PhD[3], John Antoniou MD, PhD[1], Fackson Mwale PhD[1]

1 Lady Davis Institute for Medical Research, SMBD-Jewish General Hospital, 3755 Chemin de la Cote Ste-Catherine, Montreal, QC, Canada, H3T 1E2 , 2Orthopaedic Research Laboratory, Royal Victoria Hospital, McGill University, 687 Pine Avenue West Montreal, QC, Canada, H3A 1A1, 3 Genetics Unit, Shriners Hospitals for Children, 1529 Cedar Avenue, Montreal, QC, Canada, H3G 1A6

[Introduction] Link N is the amino terminal peptide of link protein in the extracellular matrix of cartilage. It can stimulate the biosynthesis of collagens II, IX and aggrecan in IVD cells. We hypothesized that Link N can enhance the chondrogenic differentiation of mesenchymal stem cells (MSCs).

[Objective] To determine the effect of Link N on gene expression in human bone marrow-derived MSCs.

[Methods] MSCs were cultured in chondrogenic differentiation medium with 0 (control), 0.1 or 1 µg/ml Link N (experimental). The medium was changed every 3 days, and the quantity of GAG secreted into the medium was analyzed. After the cells were cultured for 7, 14 and 21 days, gene expression was analyzed by real-time PCR. Experiments were conducted three times, and One-Way ANOVA was performed to determine the difference. $p<0.05$ was considered significant.

[Results] With concentrations of 0.1 and 1.0 µg/ml, no toxic effect of Link N was observed. Comparing the experimental cells with control cells, the expression of aggrecan, COL2A1 and SOX9 increased significantly at day 7 and 14, but not at day 21. The quantity of GAG increased significantly at day 9, 12 and 15, but not at day 3, 6, 18 and 21. The expression of alkaline phosphatase (ALP) was not affected significantly, while the expression of osteocalcin (OC) was decreased significantly at day 21. No significant difference was observed between 0.1 and 1.0 µg/ml Link N.

[Conclusion] Link N can improve the chondrogenic differentiation of MSCs and inhibit their osteogenic differentiation at late stage.

Posters

Abstract Number: P55

Other

INTERVERTECH 1 CAN AFFECT GENE EXPRESSION IN HUMAN INTERVERTEBRAL DISC CELLS

Hong Tian Wang[1]; Padma Madiraju[1]; Rahul Gawri[2]; Abdulaziz Almaawi[1]; Lisbet Haglund[2]; John Antoniou[1]; Fackson Mwale[1*]

1 Lady Davis Institute for Medical Research, Jewish General Hospital, McGill University, Montreal, QC, H3T 1E2, Canada, 2 Orthopaedic Research Laboratory, Royal Victoria Hospital, McGill University, Montreal, QC, H3A 1A1, Canada., *Corresponding author: fmwale@ldi.jgh.mcgill.ca

[Introduction] Intervertech 1 (IVT1) is a short peptide produced in mammals. It is involved in a wide variety of physiological functions, and can heal injured tissues and increase cell resistance to hypoxic episodes. We hypothesized that IVT1 can be applied in intervertebral disc (IVD) regeneration.

[Objective] To determine the effect of IVT1 on human disc cell survival and the expression of genes during extracellular matrix synthesis.

[Methods] Human annulus fibrosus (AF) cells and nucleus pulposus (NP) cells were cultured in DMEM/high glucose with 10% FBS in the absence (control) or presence of 50, 100, 150 or 200 nmol/L IVT1 for 48 hours. Then the cells were lysed in Trizol and total RNA was isolated. The existence of IVT1 receptor (IVT1R) was analyzed by western blot. Gene expression was analyzed by real-time PCR. Data were the mean of three experiments and the difference was determined by One-Way ANOVA statistical method. $p<0.05$ was considered significant.

[Results] Human AF and NP cells express the IVT1R. After the cells were cultured in DMDM with 50, 100 nmol/L IVT1 for 48 hours, the expression levels of IVT1R, aggrecan and COL2A1 increased significantly compared with control cells. No significant difference was observed in cells cultured with 150, 200 nmol/L IVT1.

[Conclusion] IVT1 receptors are present in human disc cells and the expression can be affected by IVT1 in the culture medium. IVT1 can increase disc regeneration by enhancing the expression of aggrecan and COL2A1 in human IVD cells.

Abstract Number: P56

Graduate Research

WOUND HEALING POTENTIAL OF HUMAN NEONATAL MESENCHYMAL CELLS IN AN ANIMAL MODEL OF HYPERGLYCEMIA

Fouadi M[$] and Davies JE[$,%]

[$] Faculty of Dentistry, University of Toronto, [%] Institute of Biomaterials and Biomedical Engineering, University of Toronto.

The use of bone marrow mesenchymal stem cells for impaired wounds has yielded promising results in preclinical and clinical studies. However, the potential of these cells is affected by host factors (e.g. age) and in vitro factors (e.g. expansion). Human umbilical cord perivascular cells (HUCPVCs) may represent a potent alternative.

To study the in vitro impact of various glucose conditions on proliferation and senescence status of HUCPVCs and hBM-MSCs. Also, to investigate the in vivo healing potential of HUCPVCs and hBM-MSCs in hyperglycemic mice.

Both cell types were cultured for 7 days under 2, 5.5, and 25mM glucose conditions. Ki-67 and SA-β-gal were used to detect proliferation and senescence respectively. Hyperglycemia was induced in NSG mice using Streptozotocin and 4 full-thickness wounds were created and treated with: cells, sham (methylcellulose), or left untreated. Image analysis and histomorphometry were employed for analysis.

HUCPVCs had higher proliferation indices under the 5 and 25mM conditions compared to hBM-MSCs. Under all conditions, SA-β-gal [%] was higher in hBM-MSCs compared to HUCPVCs. Hyperglycemia was successfully induced in mice. Wound closure [%] was higher in cell and sham treated wounds compared to the control, but with no significant differences. Disease tolerance varied among mice which affected wound healing and eventually all parameters measured.

HUCPVCs proliferate better than hBM-MSCs under the in vitro hyperglycemic conditions with lower expression of the senescence marker. The animal model was not suitable to detect and compare the healing potential of both cell types in an in vivo hyperglycemic milieu.

Posters

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Abstract Number: P57

Other

SPATIAL REDISTRIBUTION OF FIBROBLAST NUCLEAR CHROMOCENTERS IN RESPONSE TO TISSUE STRETCH: COMPONENT TREE ANALYSIS

Robert R. Snapp[#], Lindsay S. Peet[$], Gary J. Badger[*] and Helene M. Langevin[$]

[#] Department of Computer Science, University of Vermont, Burlington, VT 05405, USA, [$] Department of Neurology, University of Vermont, Burlington, VT 05405, USA, [*] Department of Medical Biostatistics, University of Vermont, Burlington VT, 05405, USA

Chromocenters are zones of heterochromatin that remain condensed throughout the cell cycle. Genes within chromocenters are generally repressed and characterized by specific epigenetic signatures that establish a transcriptionally repressed chromatin state, while euchromatin surrounding chromocenters tend to be heavily acetylated and transcriptionally active. Changes in the spatial organization of chromatin may influence gene expression. We had previously reported that, in response to static stretching of connective tissue ex vivo, fibroblasts actively remodel their nucleus within 30 minutes with an increase in nuclear cross-sectional area and loss of nuclear invaginations. In this study, we hypothesized that static tissue stretch would also affect the spatial distribution of fibroblast chromocenters.

In histological images of tissue excised from 26 mice and randomized to ex vivo stretch or no stretch, then fixed and stained with DAPI, we measured the spatial distribution of chromocenters within fibroblast nuclei using component trees: an image segmentation algorithm that organizes high-intensity voxels according to their topological proximity.

The average distance between chromocenters was significantly greater in tissue stretched for 2 hours ex vivo ($p<0.01$) compared with tissue incubated for 2 hours without stretch. There was no significant difference in chromocenter number or average size between stretch and no stretch. Average chromocenter distance was positively correlated with nuclear cross-sectional area ($r=0.69$, $p<0.0001$).

These results demonstrate that static stretching of whole mouse connective tissue can induce a measurable increase in chromocenter separation. Future studies will examine the relationship between fibroblast chromocenter separation and histone acetylation in response to connective tissue stretch.

Abstract Number: P58

Graduate Research

CONVERSION OF TRANSIENT FIBRONECTIN MATRIX INTO PERMANENT MATRIX IN OSTEOBLAST CULTURES IS PROMOTED BY FXIIIA TRANSGLUTAMINASE

Cui C[#] and Kaartinen MT [# $]

[#]Faculty of Dentistry, McGill University, Montreal, QC, Canada, [$]Faculty of Medicine (Division of Experimental Medicine), McGill University, Montreal, QC, Canada

Transglutaminases (TGs) are a group of enzymes that catalyze covalent crosslinking between peptide-bound glutamine and lysine residues in substrate proteins. Fibronectin (FN) is the main TG-substrate in bone matrix and plays a major role in cell adhesion and differentiation. We have shown that in MC3T3-E1 osteoblast, FXIIIA transglutaminase contributes to the extracellular matrix stabilization. Its activity is important at osteoblast differentiation checkpoint (days 4-6). In order to check how FXIIIA mediated crosslinking contributes to osteoblast differentiation, we aimed to test if it affects FN conversion from transient (soluble) to permanent (insoluble) matrix this having consequences for cellular behavior. For this, cells were grown and labeled with monodansylcadaverine (MDC) for 24h before end point at day 5. MDC is a probe that crosslinks to glutamine-residues. MDC-labeling was analyzed by immunofluorescence microscopy (IF) and Western blotting (WB). Both IF and WB results showed that MDC incorporated into FN matrix and that labeling was less in differentiating osteoblasts although FN levels in general increased. Immunoprecipitation with fibronectin and dansyl antibodies confirmed the labeling to FN. Analysis of DOC-soluble and SDS-soluble FN matrix by WB showed that differentiating osteoblasts had less SDS-extractable FN, indicating FN might get incorporated into highly insoluble matrix. MDC - also a competive inhibitor of protein-protein crosslinking - increased FN solubility indicating that FN crosslinking by FXIIIA is part of this insolubilization. Therefore, the observation demonstrates that FN is no longer accessible for labeling during differentiation and crosslinked to itself or other proteins and become part of the permanent matrix.

Posters

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Abstract Number: P59

Graduate Research

CELLULAR FACTOR XIIIA TRANSGLUTAMINASE REGULATES COLLAGEN MATRIX SECRETION AND LOCALIZES INTO CAVEOLAE ON OSTEOBLAST SURFACE

Wang S [#] and Kaartinen MT [# $]

[#] Faculty of Dentistry, McGill University, Montreal, QC, Canada, [$] Faculty of Medicine (Division of Experimental Medicine), McGill University, Montreal, QC, Canada

Transglutaminases(TGs) are a family of widely distributed enzymes that catalyze protein crosslinking by forming a covalent isopeptide bond between the substrate proteins. Osteoblasts express Factor XIIIA (FXIIIA) which is found both in cells and secreted to the matrix. Cellular FXIIIA is located on the plasma membrane of osteoblasts in rounded patches and its crosslinking activity is involved in regulating matrix secretion and deposition. The mechanism how this occurs is not fully known. We hypothesized that cellular FXIIIA is involved in caveolae-mediated endo- and exocytosis. In this study we investigated if FXIIIA co-localizes into caveolae and if its activity is involved in maintaining their stability on the osteoblast surface. Our immunofluorescence data shows that FXIIIA colocalizes with caveolin-1 in rounded patches specifically in differentiating MC3T3-E1/C14 osteoblasts. 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. Since inhibition of transglutaminase activity (with inhibitor NC9) blocks matrix secretion in osteoblasts cultures and trafficking of secretory vesicles onto cell surface, it is likely that this trafficking is mediated through caveolae-FXIIIA interaction. NC9 inhibitor did not affect the caveolin-1 levels in caveolae-enriched fractions or presence of caveolae on the cell surface and therefore it is likely not a structural component, but a factor for the function of caveolae.

Abstract Number: P60

Post-doctoral Research

MECHANICAL STIMULATION AND SAMPLE STIFFNESS MEASUREMENT IN A MICROFLUIDIC TISSUE CULTURE PLATFORM

MacQueen L[#], Chebotarev O[#$], Sun Y[#$] and Simmons C[#$*].

[#] University of Toronto Department of Mechanical and Industrial Engineering, [$] University of Toronto Institute of Biomaterials and Biomedical Engineering, [*] University of Toronto Faculty of Dentistry.

Mechanical forces are key regulators of cell and tissue development but are underrepresented in ex vivo culture platforms. Application of mechanical loading ex vivo is a promising approach for stem cell-based tissue engineering of load-bearing tissues like bone and cartilage, but optimization is challenging as regeneration is influenced by a large number of variable and interacting components, including cell type, matrix or scaffold properties, culture medium, and mechanical loading conditions.

To address the needs for increased experimental throughput and biomimicry in tissue culture platforms, we developed a microfluidic device that contains chamber arrays for growing tissue constructs; the platform provides dynamic mechanical stimulation to growing tissues and simultaneously measures sample stiffness. Precisely-controlled mechanical forces are applied to growing tissues using membranes that are deformed by pressure supplied through an underlying channel network. Integrated strain sensors measure membrane deflection and are used for in situ estimation of sample stiffness.

To demonstrate reliable stiffness measurements, we performed compression tests using silicone and gelatin hydrogel samples and thereby estimated their elastic moduli; the results were in excellent agreement those obtained using commercially available mechanical testing equipment. We then cultured tissue constructs under continuous mechanical loading in our device for one month demonstrating that the platform can be used in aqueous environments. These initial tests proved that our microfluidic bioreactor can be used for culture of arrayed tissue constructs under mechanical loading while providing real-time monitoring of tissue mechanical properties.

Posters

Posters

Abstract Number: P61

Other

A PORCINE MODEL COMBINING BACK INJURY AND MOVEMENT RESTRICTION: EFFECTS ON PARAVERTEBRAL CONNECTIVE TISSUE

Rhonda Maple[#], Samuel Woodworth[#], James Fox[#] and Helene Langevin[#]

[#]Department of Neurology, University of Vermont, Burlington

We have previously reported that paravertebral connective tissue thickness and ultrasound echogenicity are increased in human subjects with chronic low back pain. We hypothesize that, following a surgical injury, restricted movement combined with inflammation leads to connective tissue fibrosis and thus contributes to low back pain chronicity. We have developed a porcine model to examine the response of paravertebral connective tissue to a surgical injury with or without movement restriction.

Weanling pigs were paired by age and weight and randomized to injury alone, movement restriction, injury plus movement restriction or control. Paravertebral connective tissue injury was created by surgically separating the connective tissue layers via a 4 cm skin incision. Movement restriction was achieved using a “hobble” device connecting a leg strap on one hindleg to a chest harness which causes a decreased stride length. Pigs were euthanized after a five week treatment period and paravertebral connective tissues were examined with ultrasound, MRI and histology.

Preliminary observations using ultrasound and MRI on 13 pigs suggest that injury plus movement restriction leads to bilateral thickening of paravertebral connective tissue compared with injury alone or movement restriction alone. We anticipate having quantitative measurements on 21 pigs by June 2012.

This model is effective in studying combined movement restriction and connective tissue injury in pigs. In future studies we will examine specific pathological changes underlying the increase in connective tissue thickness, as well as its potential reversibility with movement-based treatment.

Abstract Number: P62

Other

SEVERE OSTEOPENIA ATTRIBUTABLE TO INCREASED BONE RESORPTION IN MICE LACKING BOTH TG2 AND FXIIIA TRANSGLUTAMINASES

Mousa A[#], Myneni VD[#], Murshed M[#], McKee MD[#], Melino G[*], Dickneite G[§], and Kaartinen MT[#,$]

[#] Faculty of Dentistry, McGill University, Montreal, QC, Canada, [$] Faculty of Medicine (Experimental Medicine), McGill University, Montreal, QC, [*] MRC ToxicologyUnit, University of Leicester, UK, [§] CSL Behring Gmbh, Marburg, Germany.

Bone is a specialized connective tissue formed by osteoblasts. Osteoblasts produce transglutaminases, TG2 and FXIIIA, which are protein crosslinking enzymes. They regulate matrix stabilization and osteoblast differentiation in vitro. Bone phenotype of TG2 and FXIIIA knockouts is very mild. To examine if TG activity is important for bone formation and if the two enzymes work synergistically to maintain bone mass, we have generated TG2-FXIIIA double-knockout mice for skeletal phenotyping. Here we report that the mice are severely osteopenic. Bone mineral density is significantly decreased (-13.1%) at 3 months. Analysis of trabecular bone by micro-computed tomography shows dramatic and highly significant osteopenic values: 57% bone loss and 49% increase in trabecular spacing. Trabecular number was decreased to 51% of controls. Mineralization was unaffected, with no evidence of osteomalacia. Histomorphometric analyses showed that osteoblast number was significantly increased (+35% per bone perimeter) indicating agumented proliferation. Quantification of mineral apposition rate showed no differences from normal mice demonstrating no alteration of bone matrix synthesis by osteoblasts. This implies that increase amount of osteoblasts do not result in more bone and cells are likely proliferating but not differentiating. This can be a result of fibronectin matrix alterations observed in these mice. Osteoclast number was dramatically increased by 93% (trabecular area) and by 104% (bone perimeter). Analysis of bone resorption markers also showed a significant, 2-fold increase. The resorption phenotype can arise from abnormal osteoblast-osteoclast coupling or it can also be osteoclast-autonomous as TG2 and FXIIIA are both also expressed by osteoclasts.

Posters

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Abstract Number: P63

Graduate Research

SKELETAL PHENOTYPING OF TRANSGENIC MICE OVER-EXPRESSING Gα11 PROTEIN IN OSTEOBLASTS

Ariana dela Cruz[*], Kim S. Sugamori[*], Michael Mattocks[*], Marc Grynpas[#], Jane Mitchell[*]

[*]Department of Pharmacology and Toxicology, University of Toronto, ON., [#]Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Canada

Gα11 (G11) is a heterotrimeric G protein that mediates phospholipase C-dependent signaling downstream of G protein-coupled receptors, including the parathyroid hormone 1 receptor (PTH1R). Our group has previously demonstrated that dexamethasone, a potent glucocorticoid, increases Gα11 expression in osteoblasts and can therefore enhance parathyroid hormone signaling through the PTH1R to phospholipase C . To determine the effects of increased G11 protein in bone, we developed transgenic mice that over-express G11 (+G11) in osteoblasts under control of the 3.6kb Col1A1 promoter.

The aim of our study is to characterize the skeletal phenotype of young and adult +G11 mice. DEXA and microCT scans of 3- to 20-week old +G11 mice mid-diaphysis femur and lumbar vertebrae revealed significant reductions in bone mineral density (BMD), trabecular thickness, and cross-sectional geometry. To investigate the effects of Gα11 over-expression on static and dynamic bone histomorphometric parameters, mice were injected intraperitoneally with calcein green (30 mg/kg) at 9 and 2 days before sacrifice. Lastly, biomechanical testing was performed using three-point bending of the femur and vertebral compression of the L6 vertebrae to analyze bone structural and material properties.

Together, our results suggest that overexpression of Gα11 in osteoblasts results in an osteopenic phenotype. Studies to evaluate the cellular and molecular basis of these changes in +G11 bone are ongoing.

Abstract Number: P64

Graduate Research

MECHANISM OF CYCLOSPORIN A – INDUCED GINGIVAL OVERGROWTH

LA Staudinger, SJ Spano, W Lee, T Moriarty, CA McCulloch

Matrix Dynamics Group, Faculty of Dentistry, University of Toronto

BACKGROUND: In cyclosporin A (CsA)-induced gingival overgrowth, collagen degradation by fibroblasts is inhibited, resulting in fibrosis. Since fibrillar collagen is the primary ligand for the discoidin domain receptor 1 (DDR1), we hypothesized that CsA perturbs DDR1 function.

OBJECTIVES: 1) Determine whether CsA affects DDR1 expression; 2) Examine whether CsA inhibits collagen binding to DDR1; 3) Assess whether the effects of CsA on DDR1 are independent of β1 integrins.

METHODS: NIH3T3 cells expressing DDR1 or null for DDR1, or GD25 cells expressing DDR1 and null for β1 integrins, were treated with vehicle (dimethyl sulfoxide, DMSO) or with CsA (10μM in DMSO). Collagen binding was measured by flow cytometry of cells incubated with type-I collagen or fibronectin-coated fluorescent beads. Immunostaining with 9EG7 and KMI6 antibodies and flow cytometry were used to measure β1 integrin activation and β1 integrin surface expression, respectively. Total DDR1 and β1 integrin protein levels were assessed by immunoblotting 18h and 3d after treatments.

RESULTS: CsA significantly reduced collagen binding in DDR1 expressing cells only. Fibronectin binding was significantly reduced in DDR1 null cells only. CsA significantly reduced β1 integrin activation in DDR1 expressing cells and DDR1 null cells. CsA significantly increased β1 integrin surface expression but not total protein expression in DDR1 expressing and null cells, 18h or 3d post-treatment. CsA treatment did not significantly increase DDR1 protein expression 18h post-treatment; however, 3d post-treatment, DDR1 protein expression was significantly increased.

CONCLUSIONS: CsA promotes upregulation of DDR1 protein expression and inhibits collagen I binding independent of β1 integrins.

Posters

Posters

Abstract Number: P65

Other

THE W290R MUTATION OF FGFR2 RESULTS IN THE PRODUCTION OF ADDITION CLEAVAGE PRODUCT(S) AND/OR SPLICED VARIANTS.

Arudchelvan Y and S-G Gong

Faculty of Dentistry, 124 Edward Street, Toronto, Ontario, Canada, M5G 1G6

Mutations in Fibroblast Growth Factor Receptor 2 (FGFR2) have been identified in human craniosynostotic syndromes such as Crouzon (CS). FGFR2 has two major isoforms, IIIb and IIIc, with specific ligand and tissue-specificities. Our laboratory characterized a mouse model of CS containing a mutation in codon 290 of murine FGFR2 (W290R) and showed that the phenotypic defects were due to disruption of signaling in both IIIb and IIIc isoforms. We found that Fgfr2W290R mutant tissues contained additional protein bands in Western blot (WB) analysis. Hypothesis: The W290R mutation of FGFR2 results in aberrant proteolytic cleavage and/or alternatively splicing. Aims: To (i) characterize the protein domains present in the additional band products with region-specific antibodies against FGFR2, and (ii) determine their protein sequence by mass spectrometry. Whole body protein lysates of E16.5 Fgfr2W290R wild type and mutant mice, before and after deglycosylation with N-Glycosidase F, were subject to WB analysis using antibodies against the IIIb, IIIc, tyrosine kinase and extreme C-terminal domains of FGFR2. Our results so far showed that FGFRr2W290R mutant receptors were hypoglycosylated. Additional protein bands found in mutant tissues were around 65-75 kDa and contained only the extreme C-terminal and tyrosine kinase domains of FGFR2 and missing most of the extracellular domain. Efforts are currently underway to determine the protein sequence of the additional products by mass spectrometry. We propose that the truncated FGFR2 product, likely missing the extracellular part, may act as a dominant-negative receptor to result in aberrant signaling in W290R mouse mutants.

Abstract Number: P66

Undergraduate Research

LUBRICANT COMPOSITION IN CANINE SYNOVIAL FLUID 12 WEEKS POST ACL-TRANSECTION: ALTERATIONS IN PROTEOGLYCAN 4 AND HYALURONAN

Chau SB[#], Ludwig TE[#], Ponjevic D[#], Matyas JR[#], Schmidt TA[#$]

[#$] University of Calgary, Calgary, AB, Canada, tschmidt@ucalgary.ca

Introduction: Synovial fluid (SF) contains two critical lubricants, proteoglycan 4 (PRG4) and hyaluronan (HA), whose concentration decrease post injury in a rat model of osteoarthritis (OA). The objective of this pilot study quantifies PRG4 and HA in an established large animal model of OA.

Methods: SF samples were aspirated from skeletally mature canine (N=4) joints both prior to ACL-transection surgery (operated knee) and 12 weeks post-surgery (both operated and contralateral knees). Concentration of PRG4 and HA in SF was determined using a custom and commercially available sandwich ELISA, respectively.

Results: PRG4 and HA concentrations in SF from the operated knee decreased post-surgery compared to pre-surgery, although differences were non-significant ($p>0.05$). Upon examination of alterations within each canine, both PRG4 and HA concentrations decreased in three of the four canines, while one increased. The canine sample that demonstrated increased in PRG4 concentration was not the same canine that increased in HA.

Conclusion: While no statistically significant difference in HA and PRG4 concentration post-surgery was observed in this small group of animals, three out of the four canines demonstrated decreased concentrations of PRG4 and HA. No correlation was observed between increases in PRG4 and HA post-surgery, suggesting an interrelation is unlikely. Additional studies with increased sample size are needed to fully elucidate changes in PRG4 and HA in this canine model. Understanding such changes, and how they relate to joint health and potential biomarkers of cartilage degradation, will further contribute to both veterinary and human osteoarthritis research.

Posters

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Abstract Number: P67

Graduate Research

REGULATION OF GENE EXPRESSION BY MODULATION OF CELL SHAPE AND ACTIN IN PASSAGED CHONDROCYTES

Justin Parreno[#$%], Sneha Raju[$%], and Rita Kandel[#$%]

[#]Department of Laboratory Medicine and Pathobiology, University of Toronto, [$]Samuel Lunenfeld Research Institute, Toronto, Ontario, [%]CIHR-Bioengineering of Skeletal Tissues Team, Canada

Articular cartilage has limited ability for self-repair and when damaged degeneration progresses until joint replacement is necessary. Unfortunately, these synthetic materials will ultimately fail. As well, the generation of large clinically relevant bioengineered cartilage is limited by the lack of a sufficient numbers of cells to generate hyaline cartilage. Expansion of chondrocyte numbers in monolayer culture results in cell shape change and the formation of actin stress fibers. Furthermore, these cells generate fibrocartilage, which is biomechanically inferior to articular cartilage and will breakdown. The hypothesis of this study is that chondrocyte shape regulates fibrocartilage biomarker mRNA levels via the actin cytoskeleton.

Confocal microscopy revealed that passaged chondrocytes were large, spread and had developed actin stress fibers unlike primary cells which were round with cortical distribution of actin filaments. Passaged cells also showed increased type I collagen and tenascin C (fibrocartilage biomarkers), but downregulated type II collagen and aggrecan mRNA levels. Passaged chondrocytes seeded in either suspension culture or exposed to latrunculin b (actin depolymerizing agent) in monolayer culture promoted the primary chondrocyte morphology. These cells were smaller, rounder, and lacked stress fibers. Also, type I collagen and tenascin C mRNA levels were downregulated ($p<0.05$) with treatments. These findings suggest a role for actin in the regulation of collagen type I and tenascin C. Elucidating the mechanism(s) leading to expression of these molecules, may result in novel ways to facilitate maintenance of chondrocyte phenotype during monolayer culture and generate sufficient cells suitable to use for biological repair.

Abstract Number: P68

Graduate Research

INTERACTION BETWEEN LUNG MACROPHAGES AND FIBROBLASTS AT DIFFERENT LEVELS OF MYOFIBROBLAST ACTIVATION

Cambridge Elizabeth, Michael Glogauer, and Boris Hinz

Matrix Dynamics Group, Faculty of Dentistry, University of Toronto, Toronto, Canada

Pulmonary fibrosis is associated with chronic inflammation and micro-scarring in the alveolar sacs, ultimately leading to loss of lung function and death. Previous studies indicate that the immune system exacerbates the fibrotic process by promoting fibroblast activation to pro-fibrotic myofibroblast through soluble factors. In co-cultures from explants of rodent lung, cell-cell adhesion between (myo)fibroblasts and macrophages via yet uncharacterized mechanisms were regularly observed, indicating direct cell-cell communication. This interaction varies with the stiffness of cell culture substrates, a major determinant of myofibroblast activation. We hypothesize that the activation state of fibroblasts to myofibroblasts will influence the efficiency of macrophage attachment and thus the progression of fibrosis. To mimic chronic inflammation in vitro, we directly co-cultured primary mouse lung fibroblasts with macrophages, differentiated from bone marrow monocytes. Immunofluorescence staining for adhesion proteins was used to identify potential mechanisms of attachment. The level of fibroblast to myofibroblast activation was evaluated by assessing alpha-SMA, the key marker for myofibroblasts. Our first results, obtained from jet-wash treatments, show that macrophages express cell adhesion molecules that promote strong attachment to both fibroblasts and myofibroblasts. Negative immunofluorescence staining of beta-catenin indicates that cadherin-mediated adherens junctions are not implicated in attachment. These preliminary results reinforce previous observations of cell-cell interaction between macrophages and fibroblasts/myofibroblasts. We are currently investigating the role of fibroblast-to-myofibroblast activation regarding the ability of macrophage adhesion by using silicone substrates of various stiffnesses, representing different stages of pulmonary fibrosis.

Posters

Posters

Abstract Number: P69

Post-doctoral Research

THE ROLE OF FAK IN RESTRICTION OF IL-1β-INDUCED SIGNALING AND MMP3 EXPRESSION

Dominik K. FRITZ, Christopher A. McCULLOCH

Matrix Dynamics Group, Faculty of Dentistry, University of Toronto

Background: Interleukin-1 (IL-1)β is a pro-inflammatory cytokine that mediates destruction of the extracellular matrix (ECM) in rheumatoid arthritis and periodontitis by enhancing the expression of matrix metalloproteinases (MMP). IL-1β enhances MMP expression through extracellular-signal regulated kinase (ERK)- and Ca2+-dependent signaling pathways, which in adherent cells require the formation of focal adhesion complexes. Objective: To define the role of the focal adhesion kinase (FAK) in restricting IL-1β-induced ERK activation, Ca2+ release and MMP3 expression. Methods: Wild type and FAK knockout mouse embryonic fibroblasts (MEF) were treated with vehicle or with IL-1β. Fibroblasts were assessed for FAK and mitogen activated protein kinase (MAPK) phosphorylation by immunoblot analysis, Ca2+ release by ratiometric photometry and MMP3 expression by ELISA and qPCR. Results: Treatment of wild type MEF with IL-1β stimulated phosphorylation of FAK at Y397 but not Y925, residues implicated in ERK signaling. IL-1β induced ERK phosphorylation in wild type but not in knockout MEF. In contrast, phosphorylation of p38MAPK and JNK was enhanced in knockout fibroblasts. IL-1β increased Ca2+ release and MMP3 expression in wild type but not in FAK knockout MEF. Conclusion: FAK is required for IL-1β-induced ERK activation, Ca2+ release and MMP3 expression in mouse fibroblasts. Selective targeting of phosphotyrosine residues on FAK that are required for IL-1β-mediated signaling could provide therapeutic targets in diseases characterized by ECM degradation. Supported By CIHR Operating Grant to CAM.

Abstract Number: P70

Graduate Research

ACTIN AND TUBULIN POLYMERIZATION STATUS REGULATES DE-DIFFERENTIATED CHONDROCYTE PHENOTYPE

Mortah Nabavi Niaki[#], Justin Parreno[$], and Rita Kandel[*]

[#] IBBME, Clinical Engineering, University of Toronto, Samuel lunenfeld esearch institute, CIHR-Bioengineering of Skeletal Tissue Team, Canada, [$] Laboratory Medicine and Pathobiology, University of Toronto, Samuel lunenfeld esearch institute, CIHR-Bioengineering of Skeletal Tissue Team, [*]Laboratory Medicine and Pathobiology, University of Toronto, Samuel lunenfeld esearch institute, CIHR-Bioengineering of Skeletal Tissue Team.

Articular cartilage has limited capacity for repair; using tissue engineered cartilage to repair damaged cartilage would be a potential therapeutic approach. However, this will require access to a sufficient number of chondrocytes capable of producing hyaline cartilage. Unfortunately, passaging chondrocytes in monolayer culture to expand cell number results in dedifferentiation as characterized by altered cell morphology, cytoskeleton, aberrant gene expression, and poor cartilage extracellular matrix accumulation. We hypothesize, modulating cytoskeletal components such as F-actin and tubulin might aid in reestablishing chondrogenic phenotype. This study examines the organization of F-actin, tubulin and lamin in primary and passaged chondrocytes as well as the effect of depolymerization agents on reestablishing primary chondrocyte morphology, cytoskeleton organization and gene expression. Primary chondrocytes are round with cortical actin whereas cells passaged twice in monolayer spread, develop stress fibers and show increased gene expression of type I collagen (dedifferentiation marker). Passaged chondrocytes have an average cell area of 885±36 μm2 compared to 300±14 μm2 for primary cells. Deploymerization of actin fibers via cytochalasin D treatment in P2 cells results in cell rounding, decreased cell size (138±7μm2), cortical arrangement of actin, aberrant lamin organization and a decrease in Col I expression. Nocodazole treatment resulted in tubulin disassembly but no apparent changes in cell size, actin or lamin organization and a decrease in Col I. This study indicates that both actin and tubulin organization contribute to maintaining chondrogenic shape, size and gene expression. However, none of the treatments alone were able to fully restore the articular chondrocyte phenotype.

Posters

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Abstract Number: P71

Graduate Research

INTERACTION OF TGF-ß1 WITH FIBRILLIN-1 FRAGMENTS

Kaur Jasvir[#], Hubmacher D[#], and Reinhardt DP[#$]

McGill University, Faculty of Medicine[#] and Faculty of Dentistry[$]

Fibrillins are extracellular matrix glycoproteins that assemble to form microfibrils in elastic and non-elastic tissues. Among several roles, microfibrils can regulate the bioavailability of various members of the TGF-β/BMP superfamily. Mutations in fibrillin-1 lead to a number of fibrillinopathies, most notably, Marfan syndrome, an autosomal dominant connective tissue disorder affecting 1 in 5000 individuals.

In order to study the role of fibrillin-1 in both microfibril assembly and modulation of the TGF β superfamily, recombinant proteins covering the N-terminus (rF23), central region (rF20), and C terminus (rFBN1-C) of fibrillin-1 are expressed using mammalian HEK293 cells and purified by metal chelating chromatography. Analysis by ELISA and cell culture assays demonstrated that these protein preparations contained significant amounts of active and latent TGF ß1. The levels of TGF-β1 found in rF23 and rFBN1-C were comparable to those found in non-transfected HEK293 cells, which were mock-purified in exactly the same manner, whereas rF20 was associated with significantly higher levels of latent TGF-β1. To eliminate copurified TGF β1, the protein preparations were subjected to gel filtration chromatography under high salt conditions. This procedure removed active and latent TGF-β1 associated with rF23 and rFBN1 C, however, excess latent TGF-β1 remained associated with rF20, indicating that this region has high affinity for latent TGF-β1. These results were corroborated by cell culture assays, and we hypothesize there is a direct interaction between the TGF-β1 and the central region of fibrillin-1. We have also elucidated that TGF ß1 is copurified with protein preparations by interacting with the nickel ions during metal chelating affinity chromatography. These findings illustrate that recombinant protein preparations require rigorous quality control before using them in cell culture assays and other experimental procedures.

Abstract Number: P72

Graduate Research

EFFECTS OF LOW INTENSITY PULSED ULTRASOUND ON FRACTURE HEALING; A SYSTEMATIC REVIEW

Bashardoust Tajali S.[1], Houghton P.[1], MacDermid J.[1,2], Grewal R.[3]

[1]The University of Western Ontario, Department of Physical Therapy, London - Ontario, Canada, [2] St Joseph's Health Centre, Hand and Upper Limb Centre, Clinical Research Laboratory, London - Ontario, Canada, [3] St Joseph's Health Centre, Department of Surgery, Hand and Upper Limb Centre, Clinical Research Laboratory, London - Ontario, Canada.

Objectives: To identify clinical trials relevant to the effects of low intensity pulsed ultrasound on bone regeneration.

Methods: The Five international electronic databases including Medline (1966-June 2010), and PubMed, EMBase, CINAHL, Cochrane Database of Randomized Clinical Trials (1980-June 2010) were searched to identify the relevant studies to effects of low intensity pulsed ultrasound on bone healing. The inclusion criteria were human clinical trial, all types of bones, fractures and outcome measurements, low intensity pulsed ultrasound application, and English language. Overall, 260 potentially eligible abstracts were identified and 65 articles were retrieved in full text. Of the 65 studies, 23 met the inclusion criteria and were critically appraised by two raters independently using PEDro quality measurement method. The results of all eligible studies were categorized in three groups: fresh, delayed or non-union, and distraction fractures. Seven trials were identified eligible for meta analysis due to varieties of outcome measurements and clinical situations. Time of third cortical bridging in radiographic healing among fresh fracture trials was our common criteria for the meta analysis.

Results: Time of third cortical bridging was statistically earlier following low intensity pulsed ultrasound therapy (mean random effect size 2.263, 95% CI 0.183 to 4.343, p 0.033). Although there is a moderate evidence of LIPUS effect on radiographic healing in delayed and nonunions, it was not possible to pool the data because of varieties among the trials and lack of information.

Conclusions: Low intensity pulsed ultrasound can stimulate radiographic bone healing in fresh fractures.

Posters

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Abstract Number: P73

Other

STRUCTURE/FUNCTION MAPPING OF BBK32 FUNCTION IN BORRELIA VASCULAR ADHESION

R EBADY, J FARNCOMB , A BANSAL, T MORIARTY

Matrix Dynamics Group, University of Toronto

Background: Dissemination of the Lyme disease pathogen Borrelia burgdorferi is initiated by endothelial-bacterial interactions permitting bacteria to decelerate and extravasate. B. burgdorferi adhesion is dependent on host plasma fibronectin (pFn), glycosaminoglycans (GAGs) and the Fn-, GAG-, and fibrinogen (Fg)-binding adhesin BBK32. BBK32 is the only bacterial vascular adhesin identified to date, and is conserved in other disseminating pathogens. The mechanisms that allow BBK32 to mediate B. burgdorferi adhesion to vascular endothelial cells under fluid shear force are largely uncharacterized. Objective: Construct a library of BBK32 variants expressed in E. coli and B. burgdorferi to investigate BBK32 sequences mediating shear force-regulated adhesion to GAGs, Fn, Fg, endothelial cells and the murine vasculature. Methods: 1) Introduce a series of BBK32 mutants into an E. coli expression vector encoding an N-terminal H6-MBP-TEV affinity-tag and investigate their ability to interact with GAGs, Fn and Fg under fluid shear force conditions in vitro. 2) Introduce mutants into a GFP-expressing non-infectious B. burgdorferi strain to identify BBK32 sequences critical for interaction with endothelial cells in a flow chamber model system. 3) Use intravital microscopy and qPCR measurement of bacterial burden to investigate BBK32 sequences mediating vascular adhesion in vivo, evasion of the intravascular immune system and dissemination into extravascular tissues. Results: Built a library of 24 BBK32 variants expressed in E. coli and B. burgdorferi, and developed an in vitro fluid shear force binding assay using purified interaction components, as well as a flow chamber system for investigating endothelial interactions under controlled shear force conditions. Conclusions: These experiments will provide a functional map of BBK32 interactions with host ligands under shear force conditions in vitro and in vivo, and invaluable insight into the BBK32 vascular adhesion mechanism.

Abstract Number: P74

Graduate Research

ELUCIDATING THE ROLE OF CCN PROTEINS DURING INTERVERTEBRAL DISC DEVELOPMENT

J.M. Bedore[*], W. Sha[*], M.R. McCann[*], A. Leask[*], C.A. Séguin[*]

[*]Department of Physiology and Pharmacology, Western University

Chronic back pain is the most common musculoskeletal disorder reported in Canada. Our ability to effectively treat intervertebral disc (IVD) disease is hampered by an incomplete understanding of disc development and function. Precise regulation of extracellular matrix metabolism within IVDs is essential to the ability of disc tissues to confer flexibility and mechanical stability. In tissues such as cartilage and bone, members of the CCN family of matricellular proteins are crucial for development and tissue repair.

We examined the expression and localization of CCN proteins in the embryonic and postnatal IVD of mice to delineate their role in tissue development. Furthermore, the expression of CCN proteins and extracellular matrix molecules were examined in mice with notochord-specific CCN2 deletion (NotoCRE;CCN2fl/fl). We demonstrate that CCN2 expression in the IVD is specific to notochord-derived cells from embryonic day 8.5 through to postnatal day 1. In newborn animals, CCN1 was detected in all components of the IVD, whereas CCN3 was detected only in the annulus fibrosus and cartilage endplates. Mice lacking CCN2 in notochord-derived cells showed deregulated extracellular matrix synthesis within the IVD, with decreased accumulation of type II collagen and aggrecan and concomitant increase in type I collagen. In the absence of CCN2, CCN3 expression was upregulated whereas CCN1 expression was reduced within the nucleus pulposus of newborn animals.

Given their ability to regulate the anabolic activity of IVD cells, we propose CCN proteins as essential to IVD development and/or function, representing intriguing candidate molecules for disease-modifying treatment of disc degeneration.

Posters

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Abstract Number: P75

Graduate Research

SIMULATION OF MUSCULOSKELETAL TISSUE DIFFERENTIATION WITHIN A STEM CELL SEEDED COLLAGENOUS SCAFFOLD UNDER CONFINED COMPRESSION

Saghar Nasr[#%], Neil A. Duncan[*%]

[#] Department of Mechanical and Manufacturing Engineering, University of Calgary, Calgary, [*] Department of Civil Engineering, University of Calgary, Calgary, Canada, [%] McCaig Institute for Bone and Joint Health, Health Sciences Centre, Calgary, Canada

Several mechanoregulatory algorithms have been proposed to predict differentiation pathways into musculoskeletal tissues. The goal of this study was to examine the effect of confined compression driving stem cells to differentiate within a collagenous scaffold. The FX-4000™ Flexcell® compression plus™ system was modified to apply confined compression to soft gels. A finite element model (FEM) of the setup was created (ABAQUS v6.11) and a biphasic algorithm regulated by shear strain and fluid flow was implemented into the model to predict temporal and spatial strain and tissue distributions within the gel. A cyclic pressure of 5 kPa (1 Hz) was applied at the bottom of the gel. As the gel was compressed, the interstitial fluid permeated out, from the top, through a porous plug. The FEM was initially validated in comparison to load-deflection results of the experimental setup. Due to the relatively fast rate of loading, the strain was distributed non-uniformly within the gel. As the fluid was forced out, the superficial layers experienced higher levels of strain and fluid velocity. These mechanical conditions, as predicted by differentiation algorithms, are conducive to cartilaginous tissue formation which was promoted in the top regions. However, in the deeper regions, the smaller values of mechanical stimuli delayed the differentiation process into cartilaginous tissues. These predictions of tissue formation and stem cell differentiation will be correlated with tissue culture investigations examining the biosynthetic activity to develop a greater understanding of the role of mechanical factors in the differentiation of stem cells into musculoskeletal tissues.

Abstract Number: P76

Graduate Research

CHARACTERIZING OSTEOCLAST PRECURSOR FUSION: CONTRIBUTION OF E-CADHERIN AND MIGRATION

C. E. Fiorino[*] and R. E. Harrison[*]

[*]Dept. of Cell and Systems Biology, University of Toronto, Toronto, ON, Canada.

Fusion between multiple precursor cells is an important aspect of osteoclast differentiation. Although considered a key commitment step, little is known about the mechanisms and molecules involved in osteoclast precursor fusion. Our strategy was to observe individual fusion events using long-term imaging with an epifluorescent Zeiss AxioObserver equipped with a humidified, temperature-regulated CO2 incubation chamber throughout the 6-day differentiation process. 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 previously documented. Further observation revealed fusion preferentially occurred at the leading edge of migrating precursor cells or at sites of active pseudopod extension. 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. Application of functional blocking antibodies against E-cadherin also caused a significant reduction in overall cell fusion. Diminished fusogenic capacity was also confirmed with classic calcium switch experiments. 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.

Posters

Abstract Number: P77

Graduate Research

CHARACTERIZATION OF EARLY EXTRACELLULAR MATRIX CHANGES IN A PORCINE MODEL OF AORTIC VALVE DISEASE

Kwong AV[#], Sider KL[#] and Simmons CA[#$]

[#]Institute of Biomaterials and Biomedical Engineering, University of Toronto, [$]Department of Mechanical and Industrial Engineering, University of Toronto

The hallmark of calcific aortic valve disease (CAVD) is maladaptive remodeling of valve tissue extracellular matrix (ECM), which leads to stiffened leaflets and valve dysfunction. While late-stage CAVD is well-characterized as fibrotic and calcific, little is known about initial cellular and ECM changes. Characterization of early pathological changes may lead to novel targets for the treatment of CAVD before excessive fibrosis and calcification.

To characterize early CAVD, a normal or high-fat/high-cholesterol (HF/HC) diet was fed to 24 male Yorkshire barrows for up to 5 months to induce early valve disease. Valve leaflets were then assessed (immuno)histologically.

As in humans, proteoglycan (PG)-rich lesions formed in the fibrosa layer of valve leaflets, with more prominent lesions in swine on the HF/HC diet than those on normal chow ($p<0.05$). These early lesions formed before the appearance of myofibroblasts, inflammatory cells and significant lipid accumulation, suggesting ECM changes are an initial step in disease progression. Within PG-rich lesions, versican and hyaluronan were frequently present. In contrast, biglycan and decorin tended to localize directly at the base of lesions. PG lesions also co-localized with cells that expressed Sox9, a chondrogenic transcription factor associated with calcification in human valves.

In conclusion, ECM remodeling occurs in a porcine model of early CAVD in the absence of lipid deposition, inflammatory cells, or myofibroblasts, but with significant versican- and hyaluronan-rich lesions, and chondrogenic cell presence. Further investigation of the roles of the specific PGs/glycosaminoglycan in CAVD lesions will provide new insights into early disease progression.

Abstract Number: P78

Graduate Research

OVEREXPRESSION OF ONCOSTATIN M (OSM) INDUCES MATRIX REMODELING AND COLL1+CD45+ FIBROCYTE ACCUMULATION IN BALB/C MOUSE LUNGS

Botelho F[*], Rodrigues R[#], Wong S[#], Ask K[$] and Richards CD[#]

[#]McMaster Immunology Research Centre, McMaster University, Hamilton, Ontario. [$] Department of Medicine, McMaster University. [*]College of Veterinary Medicine, Cornell University

Background: Increases in extracellular matrix (ECM) in lungs occurs with various chronic inflammatory lung conditions. Although TGFbeta and CTGF are recognized as important in controlling ECM, other cytokine pathways may also generate pathology. The gp130 cytokine (or IL-6/LIF cyokine) family includes OSM which is a potent regulator of connective tissue in C57Bl/6 mouse lungs in a manner independent of TGF-beta, IL-4/13 and STAT6 (published data). Other models have implicated the recruitment of CD45+collagen1+ fibrocytes in fibrogenesis. Our objective here was to assess OSM induction ECM in Balb/C mice, of fibrocyte accumulation, and of chemokines associated with fibrocytes.

Methods: We used Adenovirus vector encoding OSM (AdOSM) to over-express OSM in Balb/C mouse lungs, stored bronchoalveolar lavage (BAL) samples for subsequent ELISA, measured RNA levels of various genes by qRT-PCR, and completed FACS analysis of whole lung mononuclear cell populations.

Results: OSM markedly induced mRNA levels of collagen1A1,1A2, elastin, fibronectin, MMP13, TIMP-1 at Days 5 and 7 while TIMP-3 was reduced. This was associated with increases in histological staining for ECM proteins. FACS analysis showed 3-fold elevation of CD45+coll1+ fibrocyte accumulation in lung at day 7 that was still elevated at Day 14. Paradoxically, the fibrocyte chemokine SDF-1 (CXCL12) expression was reduced at the RNA and protein level in lungs.

Conclusions: OSM induces rapid expression of ECM-associated genes in lungs of Balb/C mice and increased fibrocyte accumulation. However, chemokine SDF-1 expression was reduced at the protein and mRNA level, suggesting that fibrocyte accumulation is SDF-1–independent in this system of ECM accumulation.

Posters

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Abstract Number: P79

Undergraduate Research

IN-VITRO REMINERALIZATION OF DENTAL TISSUE EXTRACELLULAR MATRIX: THE ROLE OF SOLUBLE PROTEIN-MIMICS

Callie J. Bazak[#], Erin B. McNeil[#], Bryan D. Quan[$], Alex J. Lausch[$], Eli D. Sone[#$%*]

[#] Materials Science Engineering- University of Toronto, [$] IBBME- University of Toronto, [%] Dentistry- University of Toronto

The anchorage of the tooth root to the underlying jawbone via periodontal ligament (PDL) fibres involves an exquisitely controlled mineralization process: unmineralized collagen fibrils (Sharpey's fibres), perpendicular to the root surface, become a fully mineralized part of the cementum layer of the tooth at an unusually sharp mineralization front (~200 nm). The molecular factors that control this mineralization process are still not well understood. We have been researching mineralization control in this system using thin sections of demineralized murine periodontal tissues that are remineralized in vitro. Using solutions containing calcium and phosphate stabilized by poly-aspartic acid (polyAsp), the previously mineralized tissues (dentin and cementum) are remineralized, in close proximity to the periodontal ligament (PDL), which remains unmineralized. Furthermore, the use of polyAsp results in the deposition of oriented and crystalline mineral, as in the native mineralized tissues. In order to probe the role of polyAsp in the model system, we compared sections mineralized with polyAsp to sections mineralized with other biomimetic additives with similar or differing characteristics. Stability and mineralization experiments were conducted with citrate, poly-acrylic acid, poly-glutamic acid, poly-lysine, and β-casein to determine effective mineralization concentrations and model suitability. We find that although the mineralization parameters (quantity, specificity, morphology, phase & orientation) of the different additives varied greatly, other poly-anionic acids that stabilize the calcium and phosphate solution to a similar extent as polyAsp behave most similarly in terms of tissue remineralization. This highlights the importance of highly acidic proteins in collagen mineralization.

Abstract Number: P80

Graduate Research

LUNG DENSITY CT IMAGING ANALYSIS CORRELATES WITH MATRIX PROTEIN DEPOSITION IN MOUSE LUNGS UPON OVEREXPRESSION OF ONCOSTATIN M (OSM)

Lauber SE[#], Richards GL[#], Guerette J[#] and Richards CD[#]

[#]McMaster Immunology Research Centre, McMaster University, Hamilton, Ontario

Background: Induction of extracellular matrix (ECM) in mouse lungs can be initiated by various methods as models of pulmonary fibrosis. We have been studying lung overexpression of cytokines such as TGF-beta and OSM using endotracheal administration of Adenovirus (Ad) encoding such cytokines. To follow development of lung fibrosis in single animals non-invasively over time, we developed a method to quantitatively assess changes in lung density using small animal CT imaging analysis. Our objective here was to measure cytokine-induced changes in mouse lung density and correlate CT generated data to histological assessment of ECM.

Methods: Animals were treated with AdOSM endotracheally (n=3). CT scans (McMaster small animal imaging centre) were generated at Day 0-Day14, data converted to hounsfeld units (HU) and analyzed using Amira software. The spectrum of densities were examined and voxels within the HU ranges >-100 were determined at each time point and differences from Day 0 were averaged. The densities within designated cylinder volumes (defined by anatomical reference) of left and right lungs were determined. Results showed 4 fold increases at Day 7 in densities >-100HU and 3 fold at those >0HU as a result of overexpression of OSM. These increases were maintained at Day14 but were more variable. Histological assessment of Day 14 lungs showed increases in collagen content by approximately 3 fold (digital analysis of picrosirius red stain). We conclude that CT image analysis correlates with increases in collagen content in this model, and thus can be used to monitor changes in ECM in single animals over time.

Posters

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Abstract Number: P81

Graduate Research

INTERFACES THE OSSEOUS ENDPLATE FORMS WITH THE VERTEBRAL BODY AND THE ANNULUS FIBROSUS

Elisabeth Rok[#], J. Paul Santerre[#], Rita A Kandel[$]

[#]Institute of Biomaterials and Biomedical Engineering, University of Toronto, [$] CIHR-BioEngineering of Skeletal Tissues Team, Mount Sinai Hospital

Bioengineering tissues of the IVD requires an understanding of the physiological loads experienced in vivo by the tissues and interfaces of the IVD. Little is known about the interfaces the osseous endplate (OEP) forms with the vertebral body (VB) and the annulus fibrosus (AF). We sought to determine whether the OEP interface with the AF or VB is stronger in uniaxial tension.

VB-AF-VB samples from the anterior and posterior regions of the most cephalad motion segments of the immature bovine (6-9 month) and adolescent (18-24 month) caudal spine were obtained. To determine tensile strength, VB ends were embedded in polymethacrylate and tested to failure in uniaxial tension at a rate of 0.02 mm/sec. Native bovine interfaces were assessed histologically and biochemically.

On average failure occurred at 1.45 ± 0.31 MPa at the interface between the OEP and VB in immature samples (n=10) with the fracture occurring through the proliferative and hypertrophic zones. Adolescent samples failed the OEP-VB interface at 1.72 + 0.50 MPa (n=5) and in the mid substance AF at 2.54 + 0.77 MPa (n=3). With increasing age, both native OEP-VB and OEP-AF interfaces decreased in proteoglycan content and increased in collagen content.

Both immature and adolescent samples failed at the OEP-VB interface while the AF failed only in adolescent samples. Understanding why the OEP-AF interface is stronger than the OEP-VB interface is essential to our continued development of an engineered IVD.

Abstract Number: P82

Graduate Research

EXTRACELLULAR MATRIX FORMATION BY INNER AND OUTER ANNULUS FIBROSUS CELLS IN VITRO

Iu J[#], Santerre JP[#], Kandel R[#$].

[#]Institute of Biomaterials and Biomedical Engineering, University of Toronto, Canada, [$]CIHR-BioEngineering of Skeletal Tissues Team, Mount Sinai Hospital

Degeneration of the intervertebral disc (IVD) is associated with chronic low back pain, for which there is no optimal treatment. Replacement with tissue engineered IVD is a novel alternative treatment. The IVD is composed of annulus fibrosus (AF) which consists of lamellae of collagen. The inner lamellae are composed predominately of type II collagen, separated by abundant proteoglycans. Progressing outward type II collagen and proteoglycan decrease while type I collagen increases, resulting in inner (IAF) and outer (OAF) zones which allow the AF to withstand both compressive and tensile stresses. Using aligned polycarbonate urethane (PU) nanofibres, the lamellar structure for the AF can be recapitulated but not the zonal architecture of the AF. Engineered tissues with appropriate properties are imperative to restore normal functions.

The aim of this study was to determine if AF cells can maintain their phenotypes in vitro.

OAF and IAF cells were isolated from OAF and IAF tissues harvested from bovine caudal discs. Seeded onto aligned PU nanofibres and cultured for 2 weeks, OAF and IAF issues formed in vitro were analyzed for gene expression, and accumulation of extracellular matrix molecules.

Both OAF and IAF cells show increase in DNA content over time, synthesize and retain similar amounts of collagen and proteoglycans. Both cell types synthesize type I collagen while only IAF cells synthesize type II collagen.

Selected phenotypic characteristics of the OAF and IAF cells are maintained in vitro. Further study is required to determine if this affects tissue formation.

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Abstract Number: P83

Graduate Research

THE ROLE OF FHL2 IN STIFFNESS DEPENDENT MESENCHYMAL STEM CELL OSTEOGENIC DIFFERENTIATION

Alan Y. L. Lam [#], Jan-Hung Chen [#$], Xiaofan Jin &, and Craig A. Simmons [#$*]

[#] Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, Ontario, Canada, [$] Department of Mechanical and Industrial Engineering, University of Toronto, Toronto, Ontario, Canada, & Division of Engineering Science, University of Toronto, Toronto, Ontario, Canada, [*] Faculty of Dentistry, University of Toronto, Toronto, Ontario, Canada

Osteogenic differentiation of mesenchymal stem cells (MSCs) is modulated by the stiffness of the substrate on which the cells are grown. This is mediated via the small GTPase RhoA, but the downstream signals that direct stiffness-dependent MSC fate are poorly defined. New insights into the mechanotransduction mechanisms that regulate MSC osteogenesis may lead to novel strategies for both bone regeneration and the treatment of ectopic calcification. Recent studies have shown that FHL2, a LIM-only member of the LIM protein superfamily, can act as an endogenous activator of MSC osteogenesis; we therefore hypothesized that FHL2 mediates stiffness-dependent osteogenesis downstream of RhoA. Murine, porcine, and human MSCs were grown on type I collagen-coated polyacrylamide gels of varying elastic moduli, E. For all MSC types, alkaline phosphatase activity, as a measure of osteogenesis, was greater on stiffer ($E \geq 22$ kPa) substrates than on softer (< 22 kPa) substrates. MSCs had increased FHL2 nuclear localization and RhoA activation with increasing substrate stiffness. Since FHL2 acts downstream of RhoA in other cell types to transmit cell membrane signals to the nucleus, we investigated this relationship in MSCs by knocking down RhoA levels pharmacologically and with adenoviruses and found that stiffness-mediated FHL2 nuclear translocation was abolished. On-going experiments are testing the role of FHL2 in stiffness-dependent MSC osteogenesis by siRNA knockdown of FHL2. Together, these results support the hypothesis that MSC osteogenesis occurs in a stiffness dependent manner via a RhoA/FHL2 pathway.

Abstract Number: P84

Graduate Research

EFFECT OF CERULENIN ON OSTEOCLASTS ACTIVITY

Komarova S[$], Sadvakassova G[#], Vu L[%]

[#]McGill

Cerulenin is an effective drug in the therapeutic treatment of a breast cancer. However, Cerulenin has been recently recognized as a trigger of Osteoclast fusion. We investigated Cerulenin [10-6 mM] and Piruvate [10-6 mM] into an Osteoclast development from RAW 264.7. We determined the optimum concentration of RAW 264.7 cells as 28K. On day fifth of Osteoclasts development, studied cells were fixed and stained. After that Osteoclasts were analyzed under a digital microscope in order to identify the quantity of Osteoclasts. Moreover, Osteoclasts were analyzed with the software PixeLink in order to determine the size of Osteoclasts and number of nucleoids. Our Data showed that under an influence of Cerulenin Osteoclasts are way bigger then control cells and an amount of nucleoids accordingly. Under an influence of Cerulenin and Piruvate the size of Osteoclasts and number of nucleoids were greater than in absence of Piruvate. We have gathered evidences which proved that Cerulenin has a significant impact on Osteoclasts genesis which manifests itself as an exhibitor of Osteoclasts fusion.

Posters

Abstract Number: P85

Graduate Research

ORGANIC SOL-GEL COATING IMPROVES INTEGRATION OF TISSUE-ENGINEERED CARTILAGE ON CALCIUM POLYPHOSPHATE SUBSTRATE

Lee WD[#], St-Pierre JP[$], Pilliar RM[#], Stanford WL[#*], Kandel RA[#$]

[#] Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, Ontario, [$] Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, [*] Sprott Centre for Stem Cell Research, Ottawa Hospital Research Institute, Ottawa, Ontario

Damage to articular cartilage does not heal spontaneously. We previously developed a biological substitute for damaged cartilage by engineering cartilage tissue on porous calcium polyphosphate (CPP) substrate. However, to withstand the shear stress exerted upon the implant during joint movement, the tissue-engineered cartilage must be properly integrated onto its substrate. In healthy joints, a zone of calcified cartilage between articular cartilage and subchondral bone facilitates this integration; however, in our construct, CPP releases polyphosphate ions that interfere with the formation of this calcified zone at the interface. Coating the CPP with thin calcium phosphate (CaP) film deposited via inorganic sol-gel method could limit its release, but this method was labour-intensive and occluded the pores of the substrate, limiting tissue ingrowth. To overcome this problem, this study sought to investigate the effectiveness of an alternative, organic sol-gel method in enhancing the construct's ability to withstand shear stress.

Electron microscopy confirmed that the organic sol-gel deposited CaP coating evenly and throughout the thickness of the CPP substrate. Histological examination of tissues formed by deep zone-enriched bovine chondrocytes confirmed that tissue mineralized closer to the interface than those cultured on uncoated substrates. Tissues were characterized biochemically for cartilaginous matrix components and mineral content. Finally, they were subjected to mechanical testing for compressive and resistance to shear stress at the cartilage-CPP interface. Taken together, we demonstrated that the organic sol gel method is better suited for applying the thin CaP coating on the CPP substrate.

Abstract Number: P86

Graduate Research

RAPAMYCIN IN THE MANAGEMENT OF BONE PAIN ASSOCIATED WITH METASTATIC BREAST CANCER MOUSE MODEL

Abd Elaziz, D[#], Komarova SV[#], Stone LS[#]

[#] Faculty of Dentistry, McGill University

Metastatic breast cancer patients suffer from irreversible osteolytic metastases. The most devastating symptom of osteolysis is growing pain. Rapamycin, which inhibits mammalian target of rapamycin (m-TOR), has been shown to reduce pain in inflammatory and neuropathic models. Also, it decreased osteolysis associated with metastatic breast cancer mouse models. Therefore, our aim was to evaluate the effectiveness of Rapamycin in reducing pain associated with osteolytic metastases. In order to address this, we had to first characterize a mouse model of bone cancer pain. Three groups of 6-week old balb/c mice received intra-tibial injections of either saline (control group) or 10^3 or 10^4 murine mammary carcinoma cells (4T1). Hypersensitivity to mechanical and thermal stimuli using the von Frey and radiant heat tests, respectively, were assessed bi-weekly for 4 weeks. By 4th week limbs injected with 10^4 4T1 cells were significantly more sensitive to mechanical and heat stimulation compared to the ipsilateral limbs of the control group. Radiographs taken weekly revealed tibial radiolucency which appeared 2 weeks after injection with 10^4 4T1 cells, while was observed after 3 weeks in (10^3) group. No radiological changes were found in the control group. After relating pain to osteolysis, the effects of Rapamycin on both osteolysis and pain can be assessed. Preliminary results from animals treated with Rapamycin suggest that it may have efficacy in the management of pain as well as osteolysis associated with metastatic breast cancer.

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Abstract Number: P87

Graduate Research

CHARACTERIZATION OF THE ELASTICITY OF VALVULAR FIBROBLASTS ON SOFT SUBSTRATES USING ATOMIC FORCE MICROSCOPY

Haijiao Liu[*], Craig A. Simmons[*], Yu Sun[*]

[*]Department of Mechanical and Industrial Engineering, University of Toronto, Toronto, ON M5S3G8, Canada

Cell mechanical properties change dynamically in response to microenvironmental cues, and therefore we hypothesized that fibroblastic valve interstitial cells (VICs) would change their elasticity in response to that of their extracellular matrix. Atomic force microscopy (AFM) was used to measure the elasticity of VICs (9-12 cells per group) grown on type I collagen-coated polyacrylamide hydrogels of different stiffness (3 to 144 kPa). Global cell elasticity was measured with spherical tips to yield a single value for cell elasticity. We also made local measurements with pyramidal tips to map cell elasticity by indenting up to 64 selected spots per cell; these measurements were averaged to produce an "average local cell modulus" for comparison with the global modulus.

Cell modulus measured globally or locally increased with substrate stiffness ($p<0.05$), with the average local cell modulus being consistently 2 to 3 times greater than the global modulus ($p<0.05$). Measuring 64 spots was time consuming, so we determined the average local modulus as a function of number of test spots. The average local modulus calculated from a single spot was greater than that from four spots ($p<0.02$), but there was no difference in the average local cell modulus when more than four spots were tested ($p>0.78$). Thus, four measurements with a pyramidal tip were adequate to characterize the average cell local elastic modulus compared with that obtained with many more measurements. These studies confirm that VICs tune their elasticity to that of their matrix and provide practical guidelines for AFM-based measurement of cell elasticity.

Abstract Number: P88

Graduate Research

THE EFFECTS OF DELAYED ADMINISTRATION OF RHO-KINASE INHIBITOR FASUDIL ON SURGICALLY INDUCED OSTEOARTHRITIS IN RATS

Michael Pest[#], Vasek Pitelka[#], Anusha Ratneswaran[#], Frank Beier[#]

[#] Western University Canada

Introduction: Osteoarthritis is a degenerative joint disorder with no disease modifying treatments. Our studies have demonstrated that transforming growth factor alpha (TGFα) is upregulated in a subset of human cases. Downstream targets for TGFα include rho-associated protein kinase (ROCK) which when inhibited in cartilage explant cultures has been shown to decrease catabolic degradation of collagen II and aggrecan.

Purpose: To evaluate the protective effects of delayed administration of ROCK inhibitor fasudil following surgical induction of rat OA in vivo.

Methods: OA was induced surgically in the right knee joint of adult male rats by anterior cruciate ligament transection with partial medial meniscectomy. Treatment began at 4 weeks post-surgery using osmotic pumps administering vehicle or fasudil with an additional sham-operated control group. Development of OA was evaluated histologically in safranin-O/fast green stained knee sections using a modified OARSI scoring system and by immunohistochemistry.

Results: Rats treated with fasudil at a low dose for 3 weeks exhibited significantly lower cartilage damage compared to vehicle and high dose treatment groups. This effect was lost at 6 weeks of treatment. Treatment with high dose fasudil showed no significant difference from vehicle at any time point.

Conclusions: Treatment with a low dose of fasudil slows the progression of cartilage damage at 3 but not 6 weeks treatment time in rats with established OA. A higher dose does not seem to protect against cartilage degeneration at either 3 or 6 weeks treatment, which may be due to toxicity or other dose related effects.

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Abstract Number: P89

Graduate Research

FIBRILLIN-1 SEQUESTERS RANKL AND INHIBITS OSTEOCLAST DIFFERENTIATION

Boraschi-Diaz I[1], Tiedemann K[1], Reinhardt DP[1,2], Komarova, SV[1]

McGill University, [1]Faculty of Dentistry and [2]Faculty of Medicine, Montreal, Quebec, Canada

Introduction: Fibrillin-1 constitutes the major backbone component of extracellular microfibrils. Mutations in the fibrillin-1 gene lead to Marfan syndrome, which is characterized by long bone overgrowth and osteopenia. Osteoclasts are multinucleated bone resorbing cells that release proteolytic enzymes and protons to cleave and dissolve the bone matrix. Key regulator of osteoclast differentiation is receptor activator NF-kappa-B ligand (RANKL).

Objective: To assess if fibrillin-1 can affect osteoclast differentiation.

Methods: Osteoclasts were induced to differentiate from murine monocytic Raw 264.7 cells with treatment of RANKL (50 ng/ml). Recombinant N-terminal and C-terminal halves of fibrillin-1 (50 µg/ml) were added during the course of osteoclastogenesis.

Results: N-terminal (but not C-terminal) half of fibrillin-1 inhibited osteoclast formation by 80 ± 10%. Since transforming growth factor β (TGFβ) is known to stimulate osteoclast differentiation and to bind to fibrillin-1, we assessed if potential sequestering of TGFβ by fibrillin-1 mediated inhibition of osteoclastogenesis. However, osteoclast formation was not affected by the addition of recombinant TGFβ, or by blocking the effect of TGFβ. We hypothesized that the N-terminal half of fibrillin-1 may bind RANKL thus interfering with osteoclastogenesis. Using a solid phase binding assay, we demonstrate that RANKL binds to N-terminal (but not C-terminal) half of fibrillin-1 in a calcium dependent manner, this was corroborated by immunoprecipitation assay. Finally, this inhibition induced by the N-terminal half of fibrillin-1 was rescued by increasing the concentration of RANKL 300-fold.

Conclusions: This study demonstrates that the N-terminal half of fibrillin-1 binds RANKL thus sequestering it from the microenvironment and inhibiting osteoclastogenesis.

Abstract Number: P90

Graduate Research

MYOFIBROBLASTS REMODEL THE TGF-β1 STORAGE COMPLEX IN THE EXTRACELLULAR MATRIX

Klingberg F[#], Koehler A[#], Buscemi L[$], Hinz B[#]

[#]Laboratory of Tissue Repair and Regeneration, Matrix Dynamics Group, Faculty of Dentistry, University of Toronto, [$]Laboratory of Cell Biophysics, Ecole Polytechnique Fédérale de Lausanne, Switzerland

Background: Myofibroblasts produce the detrimental tissue contractures characteristic of fibrosis. Myofibroblast differentiation from various precursors depends on two major factors, active TGF-β1 and a stiff extracellular matrix (ECM). Latent TGF-β1 is stored in the ECM by associating with the latent TGF-β1 binding protein-1 (LTBP-1). We previously demonstrated the myofibroblasts use integrins to mechanically activate TGF-β1 by pulling on the latent complex. We hypothesize that the organization level of LTBP-1 in the ECM will impact on the availability of TGF-β1 for activation.

Objective: To test whether organization of LTBP-1 into pre-stressed ECM fibrils improves mechanical activation of latent TGF-β1 by myofibroblasts.

Methods: First, we stably transfected HEK293 cells to express fluorescently labeled LTBP-1 (LTBP-1-EGFP). Dermal fibroblasts and myofibroblasts were then seeded onto HEK293-produced ECM after HEK293 removal or in direct co-culture with the HEK293. After another two days of culture, fibril formation by myofibroblasts was assessed by immunofluorescence staining for LTBP-1 and EGFP. Second, fibroblastic cultures were either supplemented or grown on surfaces coated with LTBP-1-EGFP for 2-5 days. Live video microscopy of LTBP-1 organization was performed to visualize the organization process. Third, integrin blocking experiments were carried out to identify key integrins involved in LTBP-1 ECM remodeling.

Results: Within two days, myofibroblasts generated fibrillar LTBP-1 structures exclusively from unorganized HEK293-produced ECM, demonstrated by co-localization of EGFP and LTBP-1. In control mono-cultures, myofibroblasts did not secrete or form fibrillar LTBP-1 before day five of culture. LTBP-1 can be incorporated into pre-existing ECM and an active reorganization process could be visualized.

Conclusion: Myofibroblasts remodel pre-existing or supplemented LTBP-1 into fibrillar structures. Studies of early events suggest a fibrillar adhesion associated ECM remodeling. Our next step will be to test whether LTBP-1 pre-organization improves latent TGF-β1 activation from the ECM by fibroblasts.

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Abstract Number: P91

Graduate Research

ISOLATION, CHARACTERIZATION AND CHONDROGENIC DIFFERENTIATION OF HUMAN BONE MARROW DERIVED HUMAN MESENCHYMAL STROMAL CELLS

C.Mahapatra1 and K.Pramanik2

1.Department of Biotechnology and Medical engineering, National Institute of Technology, Rourkela, India, 2.Department of Biotechnology and Medical engineering, National Institute of Technology, Rourkela, India

Cartilage tissue has limited self repair capability .Its current treatment involves development of tissue engineered cartilage construct by seeding of autologous mesenchymal stem cells into scaffold The tissue engineeried cartilage construts thus mimics the native cartilage tissue thereby is a good substitute to other prosthetic devices used which have fixed or lower load bearing capability and less integration to host environment multipotent mesenchymal stem cell (MSC) have been isolated from bone marrow(BM) ,adipose tissue ,umbilical cord blood ,placenta etc. with BM found to be better source of MSC. The present study involves the isolation of MSCs from Human bone marrow . Mononuclear cells (MNCs) were isolated from BM firstly by RBC lysis method where lyses of whole BM was done with Ammonium chloride. Secondly by Ficoll-hypaque method MNCs were isolated and cultured in 5% CO2 at 37 cultured MNCs were subculture and passaged to expand and select the pure population of precursor MSCs .. The results showed variation with regard to 3rd and 4th passage where expression of CD markers and morphological characters varied significantly. MSCs in the 3rd passage showed three types of morphology like epitheloid, spherical and spindle shaped type morphology CD markers in 3rd passage were negative for CD 45.and CD 34 and found positive for CD 73,CD 105,CD 90 Morphological characteristics in 4th passage was largely found to be spindle shaped fibroblast type .Increased expression of Positive CD marker was observed for CD 73,CD 105,CD 90 which showed MSCs maturation Sorted cells when cultured in chondrogenic inducing media morphologically showed hypertrophy and development of precursor chondrocytes.

Abstract Number: P92

Graduate Research

MECHANICAL STRESS MODULATES INTEGRIN-MEDIATED ADHESION OF MYOFIBROBLASTS TO THE LATENT TGFβ1 ASSOCIATED PEPTIDE

Y KWON[*1], F KLINGBERG[1], L BUSCEMI[2], B HINZ[1]

1Laboratory of Tissue Repair and Regeneration, Matrix Dynamics Group, Faculty of Dentistry, University of Toronto, 2Laboratory of Cell Biophysics, Ecole Polytechnique Fédérale de Lausanne, Switzerland

Heart fibrosis is characterized by excessive extracellular matrix (ECM) production and contraction by myofibroblasts, which differentiate from cardiac fibroblasts under the action of TGFβ1. Myofibroblasts activate TGFβ1 by integrin-mediated pulling on the latency-associated pro-peptide (LAP) of TGFβ1 which is ECM-bound. Mechanical TGFβ1 activation requires a stiff ECM and high cell contraction. We hypothesize that the mechanical stress determined by ECM stiffness and cell contraction will modulate the binding affinity of cardiac fibroblast integrins to LAP. First, we microcontact printed arrays of LAP protein islands with the features of giant myofibroblast adhesions (stressed) and small fibroblast adhesions (relaxed). We assessed localization of different LAP-binding integrins to LAP islands in cultured cardiac fibroblasts using immunofluorescence. Second, we applied stress to LAP-coated magnetic microbeads seeded onto cardiac fibroblasts and compared the recruitment of integrins to non-stressed controls. Restriction of cell adhesion to small LAP islands reduced myofibroblast stress and modulated the density of LAP-binding integrins. Application of stress to LAP-coated microbeads bound to the myofibroblast surface recruited LAP-binding integrins. Collectively, our data indicate that stress augments the binding of specific integrins to latent TGFβ1 which is a precondition for efficient TGFβ1 activation. Reducing myofibroblasts stress is a potential strategy to interrupt the autocrine production of active and pro-fibrotic TGFβ1 without impeding its beneficial effects.

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Abstract Number: P93

Graduate Research

LOSS OF TDAG51 CONFERS PROTECTION AGAINST VASCULAR SMOOTH MUSCLE CELL CALCIFICATION AND APOPTOSIS

Imtisal Al-Bondokji[1], Jeffery G. Dickhout[1], Alistair Ingram[1], Joan Krepinsky[1] and Richard C. Austin[1]

1Nephrology, St Joseph's Healthcare Hamilton and McMaster University, Hamilton, Ontario, Canada

Vascular calcification (VC) contributes to cardiovascular disease in renal failure. VC occurs by the accumulation of calcium and phosphate. SMC apoptosis induced by calcium and phosphate accumulation contributes to VC. This increases arterial stiffness and pulse pressure, contributing to elevated risk of heart failure and stroke. T cell death-associated gene 51 (TDAG51) is a pro-apoptotic factor expressed in atherosclerotic lesions. Furthermore, a significant reduction in intimal VC was observed in advanced atherosclerotic lesions from TDAG51-deficient (TDAG51-/-) mice. Based on these findings, we hypothesize that SMC apoptosis mediated by TDAG51 contributes to medial VC. TDAG51's effect on medial VC was examined with inorganic phosphate loading. Apoptosis was measured by LDH release and TUNEL staining, calcification by von Kossa staining and colorimetric calcium assays, osteoblast differentiation, and extracellular matrix protein expression through immunohistochemistry, western blotting and RT-PCR. Cultured aortic SMC derived from TDAG51-/- mice aortas treated with inorganic phosphate had a significant decrease in the expression of CHOP, BAX and BAK, but not BCL-2. Consistent with these findings, TDAG51-/- aortic SMC were significantly protected from apoptotic cell death following exposure to inorganic phosphate. Further, TDAG51-/- aortic SMC had reduced expression of osteoblast differentiation markers RUNX2 and Osterix and extracellular matrix proteins Collagen 1A1 and Elastin. In addition, aortic valves as well as thoracic and abdominal arteries from TDAG51-/- mice treated with high dose vitamin D3 showed no features of medial VC. Thus, our results suggest that TDAG51 deficiency protects aortic SMC from apoptosis-mediated mineralization and osteoblast-like cell differentiation associated with VC.

Abstract Number: P94

Graduate Research

THE SUPERFICIAL ZONE CHONDROCYTES SUPPRESS MINERALIZATION IN THE DEEP ZONE OF ARTICULAR CARTILAGE THROUGH SOLUBLE FACTORS PRODUCED BY SUPERFICIAL ZONE CHONDROCYTES

Bromand S,[1,2]Kandel, RA[1,2]

1CIHR-BioEngineering of Skeletal Tissues Team, Mount Sinai Hospital, University of Toronto, Toronto, Canada. 2Institute of Biomaterials and Biomedical Engineering, University of Toronto, Canada.

Background: Loss of the superficial zone of articular cartilage is one of the earliest changes in osteoarthritis and with disease progression the deep zone of cartilage shows progressive mineralization. Currently, the mechanisms controlling post-natal mineralization of articular cartilage are poorly understood.

Objective: To test the hypothesis that superficial zone (SZ) chondrocytes suppress mineralization in the deep zone of articular cartilage through soluble factors produced by superficial zone chondrocytes.

Methods: SZ and DZ chondrocytes were isolated from bovine articular cartilage; each subpopulation was seeded on separate membrane inserts, placed in side-by-side co-culture and grown for up to 10 days. The effect of the SZ on DZ cartilage tissue formation was determined histologically for tissue appearance and presence of apatite mineral deposits by Von Kossa staining. The effects of co-culture on both mineral and matrix contents were quantified biochemically. Gene expression of mineralization-related and matrix molecules were determined using real time PCR.

Results: Co-culture of DZ with SZ inhibited mineral formation in the DZ cartilage. Moreover, DZ co-cultured with SZ accumulated matrix with significantly higher proteoglycan and collagen content relative to DZ co-cultured with DZ.

Conclusion: The results confirm that superficial zone chondrocytes inhibit mineral formation in deep zone cartilage. This may explain why with progressive OA and loss of superficial zone cells that there is progressive mineralization of cartilage.

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Abstract Number: P95

Graduate Research

EFFECTS OF WHOLE-BODY VIBRATION ON BONE AND BODY COMPOSITION IN MICE

K.L. Beaucage[#], H.N. Nikolov[$], S.I, Pollmann[$], M.W. Grol[*], S.M. Sims[#], D.W. Holdsworth[$], S.J. Dixon[#]

Department of Physiology & Pharmacology, University of Western Ontario, London, Canada, $ Imaging Research Laboratories, Robarts Research Institute, London, Canada, * Department of Anatomy and Cell Biology, University of Western Ontario, London, Canada

Whole-body vibration (WBV) has been shown to significantly promote bone formation and suppress adipogenesis, leading to favorable changes in body composition. Our long-term objective is to investigate the mechanisms underlying the response of tissues to vibration. Our first goal was to build a platform for WBV of small animals, and to assess effects on body composition of mice. We developed a platform that delivers acceleration up to 1 g in the 10-100 Hz range. The acceleration accuracy was measured to be within 4% and precision at 45 Hz was better than 0.70%. Male 8-week-old C57BL/6 mice were placed on the vibration platform or a control platform for 15 min/day, 5 days/week for 18 weeks. The experimental group was subjected to WBV at 45 Hz and 0.3 g, parameters suggested by others to induce changes in body composition. Mice were scanned by micro-CT to quantify bone, lean and adipose tissues prior to and during the experimental period. Over a period of 15 weeks, there were no significant differences in the mass of adipose, lean or skeletal tissues between mice subjected to WBV compared to sham. Moreover, there was no significant change in whole-body volumetric bone mineral density (BMD). However, higher-resolution micro-CT scans of hind limbs after 15 weeks revealed a small but significant increase in BMD in the vibrated animals. Ongoing analyses include assessment of microarchitectural parameters (e.g. BV/TV, trabecular spacing and thickness) and histology. Contrary to previous reports, high-frequency low-magnitude vibration induces only slight changes in body composition of mice.

Abstract Number: P96

Graduate Research

DOES THE ENAMEL PROTEIN AMELOTIN DIRECTLY AFFECT HYDROXYAPATITE MINERALIZATION?

Abbarin N[*], Ganss B[*]

[*] Matrix Dynamics Group, Faculty of Dentistry, University of Toronto

Background: We have recently identified amelotin (AMTN) as a novel protein that is specifically expressed during the maturation stage of dental enamel. Transgenic mouse models developed in our lab, which overexpress or lack AMTN, have shown specific defects to the structure and organization of the enamel hydroxyapatite microstructure. Objective: The specific enamel defects in AMTN-overexpressing and –deficient mice suggest a possible involvement of AMTN in regulating hydroxyapatite mineralization directly. The objective of this project is to develop an in vitro calcium phosphate crystallization assay and to study the effects of recombinant AMTN protein on the shape, structure, habit, and phase of crystalline calcium phosphate deposits. Material and Methods: Recombinant human AMTN protein was expressed in E. coli cells and affinity purified to near-homogeneity. The protein was adsorbed to polystyrene surfaces and/or added to different formulations of simulated body fluid (SBF) containing various concentrations of calcium and phosphate ions and different pH. The forming calcium phosphate deposits were analyzed by light microscopy and SEM. Results: Recombinant AMTN protein production yielded sufficient amounts of recombinant protein at high purity. Initial analyses indicate that AMTN significantly inhibited calcium phosphate crystal deposition, but did not have an obvious effect on the shape and habit of formed crystals. Conclusion: Calcium phosphate deposition is inhibited in the presence of AMTN at given pH and Calcium phosphate concentrations. Further detailed studies will focus on characterizing the microstructure and phase of calcium phosphate deposits using techniques such as Raman spectroscopy and TEM.

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Abstract Number: P97

Graduate Research

THE PRIMARY CILIUM IS REGULATED BY AND PLAYS A CRITICAL ROLE IN EPITHELIAL-MYOFIBROBLAST TRANSITION (EMYT)

Rozycki M[#], Lam J[#], Speight P[#], Kapus A[#]

[#]:Surgery, University of Toronto, Li Ka Shing Knowledge, Institute, St. Michael's Hospital, Toronto, ON, Canada

Epithelial-myofibroblast transition (EMyT), a key process in fibrogenesis, involves the activation of a myogenic program (-smooth muscle actin (SMA) expression) in the injured epithelium. EMyT is an important pathologic feature of polycystic kidney disease, the prototypic ciliopathy. However, the impact of EMyT on the cilium or conversely the role of the cilium in EMyT remains largely unknown. Previously we developed a two-hit model of EMyT, wherein the injury (uncoupling) of adherens junctions (AJs) and the presence of the fibrogenic cytokine TGFß are both necessary to induce SMA expression in kidney tubular (LLC-PK1) cells. These inputs activate myocardin-related transcription factor (MRTF) and Smad3 respectively, the interplay of which elicits SMA expression. In this study we followed ciliary changes in this two-hit EMyT model and tested effects of deciliaition on SMA expression. We show that EMyT is associated with a biphasic change in the primary cilium, characterized by initial growth followed by dramatic ciliary loss. The loss of the cilium is Smad3-dependent since downregulation of Smad3 suppressed the LCM+TGFß-provoked deciliation. While the emergence of myofibroblasts is eventually associated with ciliary loss, the primary cilium itself is not dispensable for EMyT. Deciliation prior to induction of EmyT by Chloral Hydrate or the Hedgehog Pathway Inhibitor-4 (HPI-4) abolished the TGFß+LCM-triggered SMA-promoter activation and SMA protein expression. These deciliating agents caused substantial reduction in MRTF and Smad3 protein levels concomitant with a robust increase in cytosolic acetylated tubulin. Specifically, cellular acetylation appears to be influenced by ciliation status which in-turn regulates expression of myogenic proteins.

Abstract Number: P98

Graduate Research

ROLE OF PROTEOGLYCANS IN MICROFIBRIL ASSEMBLY

Hong Pham[*], Laetitia Sabatier[*], Dieter P. Reinhardt[*,#]

Mcgill University, [*] Faculty of Medicine and [#]Faculty of Dentistry, Montreal, Quebec, Canada

Fibrillins are the major constituent of the extracellular microfibrils. They contribute to structure and function of microfibrils in elastic and non-elastic extracellular fibers. Therefore, proper expression, assembly and homeostasis of fibrillins are important to guarantee the functional role of microfibrils in tissues. Mutations of fibrillin can disrupt microfibril assembly and lead to several connective tissue disorders, such as Marfan syndrome. In this study, we are investigating the role of heparan sulfate proteoglycans in microfibril assembly.

Our laboratory has previously shown that heparin/heparan sulfate inhibits the formation of fibrillin-1 network in human skin fibroblasts. We also demonstrated that fibrillin-1 interacts with heparin/heparan sulfate in vitro.

In the present study, we further identify and characterize the interaction of heparin/heparan sulfate with fibrillins. We demonstrate that the N- and the C-terminal halves of fibrillin-2 and -3 interact with heparin/heparin sulfate in solid phase binding assay. Through affinity chromatography, we will investigate the calcium-dependency of fibrillin interaction with heparin. We also show that fibrillin C-terminal multimerization increases the avidity for heparin/heparan sulfate. Through heparin inhibition assay, we demonstrate that heparin/heparan sulfate acts as a regulator of fibrillin homo- and heterotypic interactions, which are critical for microfibril assembly.

We will use siRNA transfection to identify potential cell surface heparan sulfate proteoglycans involved in microfibril assembly. Some of the candidates which will be tested are: syndecan 2 and syndecan 4. We will also test enzymes important for heparan sulfate production such as EXT1, EXT2, NDST1 and NDST2. We will observe effects of the knock-downs on the fibrillin-1 network by using immunofluorescence.