

What we have done TOGETHER, as the SRMT

CIHR-SRMT Progress Report
September 2008 – March 2010



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CIHR-SRMT Mission

As a team, we will establish a strong research program focused on skeletal repair incorporating the biology, mechanics, and transplantation of stem cell-derived bone and cartilage progeny, and we will measure the efficiency of repair approaches through sophisticated and quantitative longitudinal studies in animal and human models. This will involve:

- The development of a unique, trans-disciplinary research program involving cell and animal biologists, cell and tissue engineers, imaging physicists/mathematicians, and clinical scientists that are focused on exploring novel approaches in intrinsic and extrinsic skeletal repair;
- Building collaborations among team members which will increase through the acquisition of new trainees and grant support, and through the development of new knowledge that will be translated to both clinical and commercial entities;
- The development and validation of model systems for the transplantation of ESC-derived osteoblasts and chondrocytes into animal and human models.

For more information about CIHR-SRMT

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Message from the Project Manager

The Canadian Institutes of Health Research (CIHR) created an Emerging Team Grant: Regenerative Medicine and Nanomedicine in order to strengthen Canadian health research by funding research teams conducting multidisciplinary research. For a total of five years (2006-2011), the Skeletal Regenerative Medicine Team (SRMT) will receive funding in order to establish a multi-disciplinary approach to discover unifying principles, algorithms, and operational paradigms of stem cell biology that can be used to promote skeletal repair and regeneration. It is through the establishment of a unique, trans-disciplinary research program that this will be accomplished.

Since taking on the role as the Project Manager for the CIHR-SRMT in September 2008, I have witnessed the CIHR-SRMT evolve into a dynamic multidisciplinary and collaborative team. After its 1st Annual Meeting, held in Canmore, a team emerged. Participant enthusiasm and face-to-face communication sparked ideas and memories between investigators and trainees. Within the next few months, collaborations were forming among members, an online communication strategy was developing, trainees were participating in exchanges to various laboratories across the country to receive trans-disciplinary training, and members were re-engaging in online seminars. The team's research projects were re-defined with specific objectives, and team members now refer to these projects as the four research bins. In July 2009, the CIHR-SRMT teamed up with the Canadian Connective Tissue Conference (CCTC) and participated in the 15th Annual CCTC meeting in Calgary. This allowed CIHR-SRMT trainees to showcase some of their research accomplishments and to interact with other investigators and students conducting similar research.

This is the first report I have compiled about our team which highlights our accomplishments. I hope you enjoy reading about some of our recent changes and about our team successes and challenges. In order to remain a prosperous research group, we need to encourage each other, increase communication, and continue working TOGETHER as a team. The grant is currently within its fourth year, and it is my hope that we will be able to continue with our successes well into the future.

Sincerely,



Niccole Germscheid

Meeting the Members: 1st Annual SRMT Meeting – January 15-17, 2009

Niccole Germscheid

“This meeting was a great opportunity to network, exchange ideas, learn how to improve my poster presentation skills and think about how to work more collaboratively.” (UBC – PhD Candidate)

“Very helpful to find out what everyone is doing/ has to offer and to put faces to names.” (UofC – Investigator)



The CIHR-Skeletal Regenerative Medicine Team now referred to as ‘SRMT’ met face-to face for the first time at the Falcon Crest Lodges in Canmore, AB for their 1st Annual SRMT Meeting. Trainees and investigators came from the *University of Calgary, University of Alberta, and University of British Columbia*. In total, there were 9 investigators and 27 trainees who attended the event. The purpose of the meeting was to meet the team members – put a face to a name, learn about each other and their research interests/ technical expertise, and to begin developing research connections.

The meeting began with the unveiling of the new team logo; a powerful image of a phoenix and the orthopaedic broken tree limb and a few SRMT ice-breaker challenges.

The following day was filled with short biographical talks given by each SRMT member. This allowed members to learn about each others’ personal, academic and research backgrounds, and current research projects and interests. To further promote camaraderie and team work, the group participated in a unique and exciting outdoor winter activity: traditional dog-sledding. In the evening, over a dozen research posters were displayed and discussed. The evening concluded by awarding five SRMT members with a ‘fun’ prize for the following:

Category	Recipient
Most Enthusiastic Trainee	Nathan Corbett
Best Poster	Jaymi Taiani
Best Trainee Talk	Charlie Hsu
Best Investigator Talk	Ken Muldrew
Best Dog-sledder	Aki Yamashita

Throughout this day, there were several opportunities to network and converse with other SRMT members.

On the closing morning, the team participated in a discussion focused on the importance of collaboration versus competition and then discussed its future goals, potential communication strategies, and the dates/ objectives for the next SRMT meeting.

Ultimately, the event was an enjoyable and invaluable weekend of collaboration initiation and dynamic team-building.

A “Joint” Meeting: SRMT/ CCTC Combined Meeting – July 14-18, 2009

Niccole Germscheid

“This meeting was useful because I met potential collaborators, learned about new techniques, and exciting research projects.” (McGill – Investigator)

“I especially liked the multidisciplinary nature of the meeting. The keynote speakers gave eye-opening speeches.” (University of Toronto – PhD Candidate)

“I was made aware of the other connective tissue research conducted in Canada.” (University of Calgary – Investigator)

On July 14-18, 2009, the SRMT teamed up with the Canadian Connective Tissue Conference (CCTC) and participated in a “joint” meeting: 2009 SRMT Summer Meeting/ 15th Annual CCTC. The event was held in downtown Calgary at the Delta Bow Valley. Approximately 100 delegates attended the combined event – one of the largest turnouts for the CCTC and the first CCTC meeting held in Western Canada! There were trainees and investigators from all across the country including the *University of Calgary, University of Alberta, University of British Columbia, McGill University, Université de Montréal, École Polytechnique de Montréal, University of Toronto* and *University of Western Ontario*. The aim of the CCTC and this combined event was to bridge the gaps in the scientific and clinical knowledge of connective tissues. The topics of discussion ranged from the genetics of fracture repair and bone disease to clinical aspects of connective tissue disease. Keynote lecturers included the following world-class researchers in connective tissue biology: **Dr. Véronique Lefebvre**, *Lerner Research Institute*, **Drs. Cy Frank** and **Nick Mohtadi**, *University of Calgary*, **Drs. Jane Aubin** and **Craig Simmons**, *University of Toronto*, and **Dr. Michael Buschmann**, *École Polytechnique de Montréal*.

Congratulations to our SRMT trainees, **Jaymi Taiani** and **Vincent Stadelmann** who were each awarded for top podium presentations and **Helen Dranse** who won top poster. This meeting was an excellent opportunity for young trainees to disseminate their research and actively participate in a research conference.

Thank-you to the Canadian Arthritis Network (CAN), the McCaig Institute for Bone and Joint Health at the *University of Calgary*, and the Biomedical Engineering Student Society (BMESS) in the Schulich School of Engineering at the *University of Calgary* for their generous support.

The SRMT portion of the meeting took place from July 14 to 15, 2009 where a total of 9 investigators and 27 trainees from *University of Calgary, University of Alberta, University of British Columbia*, and *University of Toronto* reunited. The objectives of this meeting were to disclose the four core research focuses and initiate collaboration among team members. The meeting began with short biographical presentations from its new members and SRMT jeopardy – an entertaining and interactive activity to reacquaint the team. Four main research projects otherwise referred to as “the bins” were unveiled. Bin project leaders presented their objectives, what had been accomplished, and what they were hoping to achieve. Trainees had

an opportunity to practice their presentation skills and share 5-slides about their research progress. During the length of the meeting, four blank posters highlighting each of the bins were displayed around the room so team members could cover them with thoughts and suggestions as their ideas evolved.

On day two, after becoming reacquainted with the SRMT research, trainees and investigators participated in a collaborative written activity where they discussed and formally created written proposals of possible collaborative projects. Eight collaborative projects were proposed! **Sarah Manske, Sufeng Zhang, Laura Rose, and Roman Krawetz** shared their experiences of the new Trainee Exchange Program. The preliminary design and content for the new interactive website was also revealed and then discussed by team members. Business meetings and a team dinner at Divino - Wine and Cheese Bar concluded the day.

On July 16, participants had an opportunity to study connective tissues, in particular bones and cartilage, in a different light. **Dr. Donald Brinkman**, Director of Preservation and Research at the Royal Tyrrell Museum of Palaeontology lead conference participants on an extraordinary tour of Dinosaur Provincial Park (DPP), a UNESCO World Heritage Site. Participants saw and learned about the geology and palaeontology of the badlands and dinosaur bones! At the end of the tour, participants stopped at the Patricia Hotel where they engaged in a cook-your-own Alberta BBQ.



SRMT Website: Launch of an Online Communication Strategy

Niccole Germscheid

In October 2009, a new and interactive website was launched for the SRMT – <http://srmt.ca>. With the assistance Cornerstone Technologies (<http://www.cs-tech.com/>) this online platform was created in order to further unite our nation-wide research team and spark team communication. The website describes the CIHR-SRMT, its members, its current research projects, houses information and forms necessary for the CIHR-SRMT funding and training opportunities, provides updates on upcoming and past seminars, and provides links to other relevant websites and resources. Members have an opportunity to login to the secure area on the website. This area is intended for trainees and

investigators to upload documents and videos for other team members to see. There is also an interactive forum within this area where members can engage in discussions surrounding projects, current events, and whatever else they desire.

The CIHR-SRMT's online presence is also found on Facebook (<http://facebook.com>) at the Skeletal Regenerative Medicine Team Facebook Page and on Twitter (<http://twitter.com>) at SRMTeam.

Frequently asked questions:

Is the website forum secure?

Yes, the forum is only accessible to SRMT members.

Can we share documents through the website?

Yes, within the secured members' area, trainees and investigators can upload and share documents and videos with each other.

Can I remain anonymous when making a forum post on the website?

Yes. Create an unrecognizable "Forum Alias" instead of writing your name.

SRMT Seminar Series: A Re-introduction to Seminars Online

Niccole Germscheid

The SRMT was re-introduced to *Elluminate Live!*[®], an interactive online communication tool, in order to facilitate training seminars for all of the SRMT trainees and investigators across the country. The team was provided with head-sets and then seven online seminars were delivered over the course of 2009. Topics ranged from the building blocks of SRMT research to what to expect from a career in academia.

Prior to starting the seminar series, an overview of SRMT Research was given by **Dr. John Matyas, Roman Krawetz, Dr. Sufeng Zhang, Dr. Jeff Dunn, and Graeme Campbell** to ensure that all members were familiar with the main research areas of the team. In the following seminar, **Charlie Hsu** taught the trainees about qPCR and about the training program he took part in at *University of Alberta*. **Karen Thomas**, a media specialist from AHFMR at the *University of Calgary*, informed our team how to effectively discuss scientific research with media and non-scientists. **Dr. Heather Jamniczky** provided us with teaching tips and techniques that can be applied not only at an academic level, but to 5-year olds and their shoe laces. **Dr. Julia Boughner** shared her experience of the Communication Workshop she attended at the *Banff Arts Centre* in August 2009 and provided the team with direction on how to effectively communicate within various forms of media. **Dr. Derrick Rancourt** finished off the 2009 Seminar Series with an outlook of what an academic career involves and should encompass.

The majority of the seminars have been recorded and can be found either through the UCalgary Blackboard (<http://blackboard.ucalgary.ca>) or through the SRMT website (<http://srmt.ca>).

SRMT Outreach: Science Café/ Mentor Night

Niccole Germscheid

To commemorate the 200th anniversary of Charles Darwin's birth, a few University of Calgary SRMT trainees participated in a Science Café/ Mentor night organized by the Student Chapter of the TELUS World of Science on Tuesday, February 10, 2009. The evening began with a Science Café on LUNA – the origins of life followed by an informal session where trainees spoke to aspiring junior and senior high students as well as undergraduate university students about their career and research experiences. Thank-you to all of the trainees that participated and shared their stories.

SRMT Trainee Awards: A Renewal Form Has Been Created

Niccole Germscheid

Three times per year (January, May, and September), SRMT trainees are able to apply for funding from the SRMT. Trainees pursuing Master and Doctoral degrees are eligible for a \$10,000 stipend per year while Post-doctoral Fellows can earn upwards to \$20,000 per year. Trainees who have guaranteed external funding from another agency or organization may apply for a 'top-up' award in the amount of \$5,000 per year. To simplify the application process for returning trainees, a Renewal Form was created for the September 2009 competition. In 2009, approximately \$175,000 was allocated to trainee support.

Congratulations to the following trainees:

TRAINEE	PROGRAM	INSTITUTION	SUPERVISOR
Sarah Manske	PhD	UofC	Boyd/ Zernicke
May Taha	MSc	UofC	Dunn
Helen Dranse	MSc	UBC	Underhill
Giovanna Lara	MSc	UofC	Rinker
Trish Parsons	PhD	UofC	Hallgrimsson
Kelsey Mountain	PhD	UofC	Matyas
Tia Gareau	Undergraduate	UofC	Kallos
Guilin Wang	PhD	UofA	Uludag
Alex Scott	PDF	UBC	Underhill
Guoliang Meng	PDF	UofC	Rancourt
Tanya Zappitelli	MSc	UofT	Aubin
Sufeng Zhang	PDF	UofA	Uludag
Jaymi Taiani	PhD	UofC	Matyas/ Rancourt
Roman Krawetz	PDF	UofC	Rancourt
Akihiro Yamashita	PDF	UofC	Rancourt
Mehdi Shafa	PhD	UofC	Rancourt
Helen Buie	PhD	UofC	Boyd
Vincent Stadelmann	PDF	UofC	Boyd
Swathi Damaraju	MSc	UofC	Duncan

SRMT Training Program: An Exchange Award Replaces the SRMT Travel Award

Niccole Germscheid

In order to foster more inter-lab interaction and assist SRMT trainees develop interdisciplinary expertise and research collaborations; a SRMT Exchange Award was created and has replaced the SRMT Travel Award. This new training opportunity allows trainees to hobnob with their colleagues in SRMT investigators' laboratories, private industry, and government laboratories. In order to secure an exchange award, trainees must apply to the training committee and describe how their exchange will meet the mandate of multi-disciplinary research training in skeletal research. Applications are accepted throughout the year and cover transportation and accommodation costs of the short-term research exchange. Laura Rose, Helen Buie, Sarah Manske, Dr. Roman Krawetz, and Dr. Sufeng Zhang have successfully participated in this exciting training opportunity. Their exchange experiences will be showcased in the next few paragraphs.

Laura Rose took part in an exchange at UofC in Dr. Derrick Rancourt's laboratory. She learned most everything she needed to know about ESCs and how to culture them with the help of Eileen Rattner. Laura learned about karyotyping ESCs, flow cytometry, PCR, and methods of measuring pluripotency.

"This is an excellent way to see what other labs are working on and meet people working in similar areas. Hopefully their experiences will help me avoid problems that they have previously encountered." – Laura Rose

Helen Buie and **Sarah Manske** visited Dr. Underhill's laboratory at UBC. The exchange was to train the Underhill laboratory how to use their new VicaCT40 scanner and assist with troubleshooting. Helen and Sarah spend the majority of their time working closely with Arthur Sampaio and Alex Scott from the Underhill lab and also provided support for the Mackay laboratory.

"It was nice to be able to do the trainee exchange with another trainee from my own lab. We were able to bounce ideas off of each other and had some good discussions in the evenings after we had left the lab. I hope you would consider providing a similar opportunity in the future." – Sarah Manske

Dr. Roman Krawetz traveled to the UofA and UBC/ Stem Cell Technologies on two separate exchanges. At the UofA in Dr. Hasan Uludag's laboratory, Roman spent the first part of his exchange mingling with the Uludag Lab group, learning about their projects, and discussing future collaborations with the Rancourt lab. In the latter half of the exchange, Roman worked with Dr. Sufeng Zhang. Roman learned how to conjugate BMP-2 to Gelatin and is able to modify this technique and apply it to collagen.

At UBC in Dr. Underhill's laboratory, Roman learned how to undertake high throughput screening assays which can be applied in the Rancourt lab. He also learned how to obtain primary osteoblasts from mouse calvaria and primary chondrocytes from mouse embryos for control purposes. At the end of the exchange, Roman presented some research to Stem Cell Technologies.

"Overall, this was a very profitable and enjoyable exchange. The program in general is sound and I will and have recommended undertaking this exchange to others." – Dr. Roman Krawetz

Dr. Sufeng Zhang from the UofA engaged in an exchange to the Rancourt Lab at the UofC. Since Sufeng's background is in chemical engineering this opportunity allowed her the exposure to the basics of stem cell biology and stem cell culture conditions. Now she can see the possibilities of combining the engineering carrier systems with stem cell technology for effective regulation of stem cell differentiation.

"This exchange program has been a valuable experience" – Dr. Sufeng Zhang

A New SRMT Funding Opportunity: Collaborative Research Award

Niccole Germscheid

In order to promote and develop multidisciplinary research, the SRMT has recently created a collaborative research award. This award acknowledges SRMT trainees who develop new research collaborations with SRMT members and who contribute to the field of regenerative medicine by publishing a peer-reviewed journal article or presenting an abstract at national or international conferences. This award will be active from January 1, 2010 until December 31, 2011.

At the July 2009 meeting, trainees had an opportunity to pre-plan and propose potential collaborations by completing SRMT Collaborative Activity Sheets. If trainees follow through with their proposal, they will be eligible for a bonus in addition to the collaborative research award.

More information regarding this funding opportunity and application forms can be found under Funding Opportunities at <http://srmt.ca>

Publications

Below is a list of some of the SRMT's publication accomplishments for 2009 and early 2010. This list represents publications where the first author was a CIHR-SRMT sponsored or active trainee/ investigator.

2010

Buie HR and Boyd SK. (2010) Reduced bone mass accrual in swim-trained pre-pubertal mice. *Med Sci Sports Exerc.*

Manske SL, Boyd SK and Zernicke RF. (2010) Muscle and bone follow similar temporal patterns of recovery from muscle-induced disuse due to botulinum toxin injection. *Bone* 46(1):24-31.

Meng G, Liu S, Li X, Krawetz R and Rancourt DE. (2010) Extra-cellular matrix isolated from foreskin fibroblasts supports long term xeno-free human embryonic stem cell culture. *Stem Cell Dev.*

Nishiyama KK, Campbell GM, Klinck RJ and Boyd SK. (2010) Reproducibility of bone micro-architecture measurements in rodents by in vivo micro-computed tomography is maximized with three-dimensional image registration. *Bone* 46(1):155-61.

Schmidt EJ, Parsons TE, Jamniczky HA, Gitelman J, Trpkov C, Boughner JC, Logan CC, Sensen CW and Hallgrímsson B. (2010) Micro-computed tomography-based phenotypic approaches in embryology: procedural artifacts on assessments of embryonic craniofacial growth and development. *BMC Dev Biol.*

Wang G, Kucharski C, Lin X and Uludağ H. (2010) Bisphosphonate-coated BSA nanoparticles lack bone targeting after systemic administration. *J Drug Target.*

Zhang S, Kucharski C, Doschak MR, Sebald W and Uludağ H. (2010) Polyethyleneimine-PEG coated albumin nanoparticles for BMP-2 delivery. *Biomaterials* 31(5): 952-63.

2009

Clements BA, Hsu CY, Kucharski C, Lin X, Rose L and Uludağ H. (2009) Nonviral delivery of basic fibroblast growth factor gene to bone marrow stromal cells. *Clin Orthop Relat Res* 467(12): 3129-37.

Itoh S and Aubin JE. (2009) A novel purification method for multipotential skeletal stem cells. *J Cell Biochem* 108(2):368-77.

Krawetz RJ, Li X and Rancourt DE. (2009) Human embryonic stem cells: caught between a ROCK inhibitor and a hard place. *Bioessays* 31: 336-343.

Krawetz RJ, Wu E, Rancourt DE and Matyas JM. (2009) Osteoblasts suppress high bone turnover cause by osteolytic breast cancer in vitro. *Exp Cell Res.* 315:2333-42.

Krawetz RJ, Taiani JT, Liu SY, Meng G, Li X, Kallos MS and Rancourt DE. (2009) Large-scale expansion of pluripotent human embryonic stem cells in stirred suspension bioreactors. *Tissue Eng Part C Methods*.

Li X, Krawetz RJ, Liu SY, Meng G and Rancourt DE. (2009) ROCK inhibitor improves survival of cryopreserved serum/feeder-free single human embryonic stem cells. *Hum Reprod* 24(3): 580-9.

Taiani JT, Krawetz RJ, zur Nieden NI, Wu E, Kallos MS, Matyas, JR and Rancourt DE. (2009) Reduced differentiation efficiency of murine embryonic stem cells in stirred suspension bioreactors. *Stem Cell Dev*.

Yamashita A, Krawetz R and Rancourt DE. (2009) Loss of discordant cells during micro-mass differentiation of embryonic stem cells into the chondrocyte lineage. *Cell Death Differ* 16: 278-86.

Nishiyama KK, Macdonald HM, Buie HR, Hanley DA and Boyd SK. (2009) Postmenopausal women with osteopenia have higher cortical porosity and thinner cortices at the distal radius and tibia than women with normal aBMD: an in vivo HR-pQCT study. *J Bone Miner Res*.

Zhang S, Doschak MR and Uludağ H. (2009) Pharmacokinetics and bone formation by BMP-2 entrapped in polyethylenimine-coated albumin nanoparticles. *Biomaterials* 30(28): 5143-55.

Zhang S and Uludağ H. (2009) Nanoparticulate systems for growth factor delivery. *Pharm Res* 26(7): 1561-80.

Core Research Project Updates

Four research 'Bins' were created and were unveiled at the Summer 2009 meeting:

1. Bin1 – Micro-Environmental Influences upon ESC Pluripotency and Differentiation
2. Bin2 – Transplantation and Imaging
3. Bin3 – Strategies for Directing Multipotential Cells to a Skeletogenic Fate
4. Bin4 – Mechanobiology of Fracture Healing – Fracture in a Dish

Each bin consists of three to four primary objectives and has a team lead. The team lead is responsible for coordinating the research activities of their bin and reporting progress to the CIHR-SRMT Project Manager. To date, each research Bin has been provided with a research allowance of \$50,000.

Bin 1: Micro-Environmental Influences upon ES Cell Pluripotency and Differentiation

Derrick Rancourt

The Bin I project explores a variety of tissue culture approaches for supporting ES cell self renewal, pluripotency and differentiation to bone and/or cartilage. **Roman, Guoliang** and **Poh** have been exploring the role of ECM in supporting ES cells. **Guoliang's** work on developing ECM from xeno-free foreskin fibroblasts was recently ePub'd in *Stem Cells Dev*. In parallel, **Roman** developed a method for isolating ECM gel from placenta (i.e. Placentagel) which supports xeno-free hES cell growth. As an undergrad, **Poh** developed "Cardiogel", which supports cardiomyocyte differentiation. **Roman** also has demonstrated nicely that a collagen I gel can promote osteoblast formation without the need for exogenous growth factors. If these gels are supplemented with chondroitin sulfate, then the output is cartilage. **Roza** has submitted a paper demonstrating that ES cells can form osteoblasts when differentiated on Collagen I coated microcarriers in suspension culture. The team hopes to investigate the influence of conjugating growth factor molecular cages to ECM constructs. However this plan has been waylaid by **Sufeng's** decision to take up a PDF at UC Berkeley instead of at the U of C.

Prior to returning to China, **Yun** demonstrated that hES cells display severe membrane blebbing, which explains their sensitivity to enzymatic passaging and cryopreservation. This observation is controversial and we have had a hell of a time publishing the paper but it has led to an industrial research grant to develop a new media formulation which suppresses membrane blebbing. Recently, **Guoliang** has developed a formulation, which suppresses blebbing and is investigating mechanism together with **Roman**. On a similar note, while bFGF plays important roles in promoting hES cell pluripotency and osteoblast differentiation **Laura** and **Roman** have a manuscript demonstrating that bFGF plays no similar role in mouse ES cells.

Another nice environmental vignette is captured in **Aki's** recently accepted PLoS One paper. Previously, **Aki** developed a novel way to differentiate ES cells to cartilage using micromass culture. He demonstrated that upon growth factor addition, cells left the micromass and formed cartilaginous aggregates. In this paper, focused on bone differentiation, **Aki** demonstrated that the fate of the resulting aggregates was influenced by culture conditions: adhesion culture promoted adipocyte formation, static suspension culture formed bone, while suspension culture suppressed differentiation in favor of pluripotent stem cells. This latter result supports **Jaymi's** *Stem Cells Dev* paper (in press), which demonstrates that suspension culture suppresses osteoblast and chondrocyte differentiation by maintaining pluripotency. **Jaymi's** working hypothesis is that shear forces in the suspension bioreactor influence the expression of pluripotency genes. This idea is supported by **Olesja's** preliminary data demonstrating that ES experiencing shear forces in flow chamber upregulate several pluripotency related genes.

Jaymi calls suspension bioreactors "pluripotency machines" and there has been considerable interest in the paper that she and **Roman** published in *Tiss Eng* demonstrating that human ES

cells also maintain their pluripotency when cultured as aggregates in suspension. Coming full circle, about a year ago **Roman** attempted to culture **Guoliang's** human foreskin fibroblasts in suspension in order to generate enough cells to biochemically prepare ECM the bioreactor. While that was a failure, **Roman** observed that the cells formed aggregates which resembled ES cell aggregates and also expressed many of the pluripotency genes. Based upon this initial observation, **Mehdi** has compelling preliminary data suggesting that fibroblasts can be efficiently reprogrammed in suspension. In addition to working with Roman, **Mehdi** is working with **Laura** to include the transient transfection of reprogramming genes into his protocols.

Bin 2: Transplantation and Imaging

John Matyas

(1) CT and MR contrast imaging of cartilage

Considerable progress in developing the contrast agents for CT imaging of articular cartilage and verifying that they do not unduly influence MRI signals. Trainees **Kelsey Mountain**, **Trish Parsons**, and **Erika Kristensen** (along with Tad Foniuk of the Experimental Imaging Centre) have been working diligently developing and implementing the optimal imaging sequences to allow valid comparisons of osteochondral samples using both imaging modalities.

(2) Stem cell tracking by MRI

The protocol for safely labeling stem cells for transplantation has been developed for MRI using superparamagnetic iron oxides, and preliminary findings suggest this labeling does not unduly influence the capacity of undifferentiated cells to differentiate into cells of bone lineage. This was verified in vitro. Initial microMR studies reveal it is possible to image burr-hole fractures as they heal using high-field MRI. The next challenge is to track the cells implanted into burr-hole defects ex vivo, and in vivo. Trainee **May Taha** is working on this project.

(3) Burr-hole fractures in OVX mice

One of the challenges of using small, stable fractures to study bone healing is that they heal quite well without intervention. To improve the “dynamic range” of experimental stem cell therapies to promote bone healing in this model, trainees **Jaymi Taiani**, **Helen Buie**, **Yves Pauchard**, and **Graeme Campbell** have been using mice in which bone formation is impaired, viz., mice that have been ovariectomized and are now estrogen deficient. Leave the lights on in the lab for repeated microCT examinations.

(4) Developing novel contrast agents for MR and CT imaging

Tetracycline analogs for labeling bone apposition would be valuable for monitoring the effects of bone forming and degrading agents. As microCT is unable to resolve cell-level activities, cell and matrix labeling agents that might be used in microCT and microMRI would have considerable utility. Moreover, the efficiency of dynamically tracking skeletal activity when exposed to anabolic molecules such as BMP2 would be enhanced if it were possible to label such molecules with contrast agents. These projects have been designed and we hope to

exploit them in the coming year.

Bin 3: Strategies for Directing Multipotential Cells to a Skeletogenic Fate

T. Michael Underhill

The central focus of BIN3 is to develop improved methods for directing multi-potent cells to a skeletogenic fate. Over the past year, significant progress has been made on this goal and this is highlighted below under the corresponding specific aim.

Specific Aim 1: Characterization of the developmental signals that regulate commitment of multipotent cells (stem or progenitor) to a chondrogenic or osteogenic fate.

The **Aubin group** has demonstrated that expression of FGF23 directly inhibits osteoblast differentiation, and thus modulation of this pathway may have some utility in regulating the expansion and differentiation of osteoprogenitors (11). The **Rancourt group** has recently shown that embryonic stem cells (ESCs) can be efficiently differentiated into chondrocytes using a micro-mass culture approach that does not involve embryo body formation (13). This methodology in combination with the small molecules identified in aim 4 is expected to improve the efficiency of cartilage formation from ESCs.

To better understand the mechanisms that regulate pro-chondrogenic responsiveness to bone morphogenetic proteins (BMPs) in limb skeletal development, the **Underhill group** employed a screen in early limb mesenchymal cells to identify factors that regulate BMP activity. These analyses showed that prior exposure to TGF β /Activin signals was required for subsequent induction of chondrogenesis by BMPs (5). Furthermore, mutations in BMP-related factors the growth and differentiation factors (GDF) 3 and 6, negatively impact skeletogenesis, suggesting that these factors are important in skeletal development and may be useful for directing multipotent cells to a chondrogenic and/or osteogenic fate (1, 14).

Specific Aim 2: Comparison of multipotent mesenchymal cells from embryonic and adult sources.

To date, direct comparisons between embryonic- and adult-derived mesenchymal cells have not been made, as up until recently it has been challenging to prospectively purify embryonic mesenchymal cells with skeletogenic potential (2). The **Aubin group** has developed a novel purification strategy to enrich for multipotential cells from bone marrow. These cells have the potential to not only contribute to bone formation, but also the establishment of bone microenvironments to support hematopoiesis (4). Furthermore, the **Aubin group** has recently reported that precursor cells derived from the bone marrow and calvaria exhibit differential responsiveness to PPAR γ agonists, with bone nodule formation being reduced in the former but not in the latter cell population (3).

Specific Aim 3: Development of methods to enhance differentiation or “re-differentiation” of expanded cells from primary tissue.

This aim is still very much a work in progress. However, the newly identified molecules, factors and treatment regimens from aims 1 and 4 will soon be applied to enhancing differentiation of tissue-derived primary cells. To this end, the **Aubin group** has shown that bone sialoprotein (BSP) is required for efficient bone growth and mineralization, and also for repair (6-8, 10). Thus, manipulation of BSP expression may prove to be useful for enhancing bone growth and/or repair *in vivo*. The Underhill group has found that antagonists of the retinoic acid receptors (RARs) enhance expression of chondrogenic markers in cells from human articular cartilage, and these studies are ongoing.

Specific Aim 4: Identification of pro-chondrogenic and/ or pro-osteogenic small molecules.

Both the **Aubin** and **Underhill groups** have been actively engaged in identifying new chondrogenic and osteogenic-modulatory factors and/or characterizing the activity of existing molecules. In recent studies, the **Aubin group** has examined the role of the various prostaglandin receptors (EP1-EP4) in prostaglandin-induced bone formation, and found that p38 MAPK and ERK signaling pathway are important in EP2 and EP4-mediated osteogenesis, respectively (9). Over the last couple of years, the **Underhill group** has screened ~ 5,000 compounds for pro-chondrogenic activity and this has yielded ~ 60 compounds with “pro-chondrogenic activity”. From this group, potassium channel modulators have been found to regulate chondrogenesis (Garcha et al., in preparation), and animal studies will be initiated shortly to examine their efficacy in a arthritis animal model (12).

Bin 4: Mechanobiology of Fracture Healing – Fracture in a Dish

Neil Duncan

Background

Bone heals well in young individuals, however, in the elderly, fracture repair is often slow and incomplete. Bone can optimize its mechanical properties to different physical conditions, including fracture repair, yet the mechanisms driving this mechanobiological adaptation are largely unknown. Tissue engineering is a promising treatment of fractures, as combinations of cells and substrates are used to promote bone healing. Due to their regenerative capacity, we use stem cells as a source of transplantable cells for bone and cartilage tissue engineering. Our overall aim is to understand the role of physical factors driving stem cell differentiation in fracture callous, which includes both cartilage and bone tissue. In this project, the mechanobiology of mouse ESC derived osteoblasts will be investigated in cell/gel constructs and in an *ex vivo* tibial fracture model.

Knowledge of mechanotransduction mechanisms and the response of stem cells to mechanical load has been investigated within static culture by growing the cells in monolayer in two-dimensional environments. However, it is well accepted that cells reside in a three-dimensional environment and respond very differently in three-dimensional scaffolds to mechanical

stimulation. Therefore, we will use a three-dimensional collagen scaffold seeded with ESC derived osteoblasts to explore the effects of mechanical load on stem cell differentiation.

Project Goals

- I. Investigation of the effect of applied static and cyclic compression on osteoblast differentiation in cell-seeded collagen scaffolds. The optimal mechanical and scaffold parameters to enhance ESC differentiation will be established.
- II. Examination of the effect of dynamic mechanical stimulation on mineralization in an ex vivo fracture model with transplanted cell seeded collagen scaffolds.
- III. Development of computational models to better understand the mechanobiological environment of implanted cells with the fracture callus.
- IV. Investigation in an ovariectomized (OVX) mouse model the role of mechanical loading on the ESC derived osteoblasts to treat fracture in weakened osteoporotic bone.

Progress – Fall 2009

- I. Loading systems have been developed to apply static and cyclic compression to cell-gel constructs in culture.
 - a. Static: A loading device was developed to apply static compression (0, 2, 6 and 10% strain) to cylindrical cell-gel constructs.
 - b. Cyclic: A loading device was developed to apply cyclic compression (2-10% strain) to cylindrical cell-gel constructs.
- II. A loading device was also developed to apply cyclic compression (2-4%) to mouse tibia-femur specimens seeded with differentiated osteoblasts in a burr-hole fracture model.

The research in projects I and II were conducted by **Olesja Hazenbiller** for her Bachelor Thesis as an exchange student from Germany. The feasibility of the loading devices was demonstrated and preliminary data generated on the biosynthetic activity of the osteoblasts in all three loading systems. Baseline gene expression of the cells in the gels and within the fracture models were examined. These systems will allow us to explore the effects of mechanical load on various cell-gel constructs in an efficient manner. The optimal mechanical loading conditions to augment differentiation and thereby mechanically pre-condition the cells prior to implantation can now be examined. These projects will be conducted in collaboration with **Drs. Duncan** and **Matyas**.

Olesja Hazenbiller will begin her MSc in May 2010 as a direct continuation of this project. She was also awarded a Nanotechnology scholarship to incorporate growth factors encapsulated in nanoparticles into these cell-gel systems. This project will be conducted in collaboration with **Drs. Duncan, Rancourt** and **Uludag**.

- III. **Swathi Damaraju** (MSc) wrote two term papers on the role of intercellular communication in the mechanobiological behaviour of stem cells. **Geoff Buckley-Herd**

(BME undergrad) wrote one term paper on the role of mechanical factors driving differentiation of stem cells. Together, these provide the background on the state of the literature on the role of mechanical factors in regulation and differentiation of stem cells. **Swathi** will now be examining intercellular communication and the micro-mechanical environment of the osteoblasts in the various gels being considered.

We also have ongoing studies examining the micro-mechanical environment of fibroblasts seeded in collagen gels using confocal microscopy. This technology will also be used on the osteoblast seeded gels to better understand their mechanical environment that is needed to develop mechanobiology algorithms.

Future – Winter 2010 & Summer 2010

- Optimization of the collagen gels and refinement of the prototypes for static and cyclic loading to enable mechanical loads to be applied at earlier time points.
- Development and set-up of a perfusion chamber system to incorporate into the burr-hole ex vivo fracture model.

The mechanobiology modelling algorithms will be implemented into Matlab and finite element models of the systems to better understand the micro-scale cellular environment and the mechanotransduction pathways that may be active in the ex vivo model. Incorporation of MR and μ CT imaging to non-invasively evaluate mineralization in the burr-hole fracture model will now be explored with **Drs. Boyd, Duncan and Dunn**.

Tibia-femur specimens from the ovariectomized (OVX) mouse model will be examined using the re-developed mechanical loading system to examine the mechanobiology of stem cells implanted in weakened osteoporotic bone in collaboration with **Drs. Duncan, Matyas and Rancourt**.

References

1. Asai-Coakwell, M., C. R. French, M. Ye, K. Garcha, K. Bigot, A. G. Perera, K. Staehling-Hampton, S. C. Mema, B. Chanda, A. Mushegian, S. Bamforth, M. R. Doschak, G. Li, M. B. Dobbs, P. F. Giampietro, B. P. Brooks, P. Vijayalakshmi, Y. Sauve, M. Abitbol, P. Sundaresan, V. van Heyningen, O. Pourquie, T. M. Underhill, A. J. Waskiewicz, and O. J. Lehmann. 2009. Incomplete penetrance and phenotypic variability characterize Gdf6-attributable oculo-skeletal phenotypes. *Hum Mol Genet* 18:1110-21.
2. Chan, C. K., C. C. Chen, C. A. Luppen, J. B. Kim, A. T. DeBoer, K. Wei, J. A. Helms, C. J. Kuo, D. L. Kraft, and I. L. Weissman. 2009. Endochondral ossification is required for haematopoietic stem-cell niche formation. *Nature* 457:490-4.
3. Hasegawa, T., K. Oizumi, Y. Yoshiko, K. Tanne, N. Maeda, and J. E. Aubin. 2008. The PPARgamma-selective ligand BRL-49653 differentially regulates the fate choices of rat calvaria versus rat bone marrow stromal cell populations. *BMC Dev Biol* 8:71.
4. Itoh, S., and J. E. Aubin. 2009. A novel purification method for multipotential skeletal stem cells. *J Cell Biochem* 108:368-77.
5. Karamboulas, K., H. J. Dranse, and T. M. Underhill. Regulation of BMP-dependent chondrogenesis in early limb mesenchyme by TGF β signals. *J Cell Sci.* (in revision)
6. Malaval, L., J. E. Aubin, and L. Vico. 2009. Role of the small integrin-binding ligand N-linked glycoprotein (SIBLING), bone sialoprotein (BSP) in bone development and remodeling. *Osteoporos Int* 20:1077-80.
7. Malaval, L., L. Monfoulet, T. Fabre, L. Pothuau, R. Bareille, S. Miraux, E. Thiaudiere, G. Raffard, J. M. Franconi, M. H. Lafage-Proust, J. E. Aubin, L. Vico, and J. Amedee. 2009. Absence of bone sialoprotein (BSP) impairs cortical defect repair in mouse long bone. *Bone* 45:853-61.
8. Malaval, L., N. M. Wade-Gueye, M. Boudiffa, J. Fei, R. Zirngibl, F. Chen, N. Laroche, J. P. Roux, B. Burt-Pichat, F. Duboeuf, G. Boivin, P. Jurdic, M. H. Lafage-Proust, J. Amedee, L. Vico, J. Rossant, and J. E. Aubin. 2008. Bone sialoprotein plays a functional role in bone formation and osteoclastogenesis. *J Exp Med* 205:1145-53.
9. Minamizaki, T., Y. Yoshiko, K. Kozai, J. E. Aubin, and N. Maeda. 2009. EP2 and EP4 receptors differentially mediate MAPK pathways underlying anabolic actions of prostaglandin E2 on bone formation in rat calvaria cell cultures. *Bone* 44:1177-85.
10. Monfoulet, L., L. Malaval, J. E. Aubin, S. R. Rittling, A. P. Gadeau, J. C. Fricain, and O. Chassande. 2009. Bone sialoprotein, but not osteopontin, deficiency impairs the mineralization of regenerating bone during cortical defect healing. *Bone*.
11. Wang, H., Y. Yoshiko, R. Yamamoto, T. Minamizaki, K. Kozai, K. Tanne, J. E. Aubin, and N. Maeda. 2008. Overexpression of fibroblast growth factor 23 suppresses osteoblast differentiation and matrix mineralization in vitro. *J Bone Miner Res* 23:939-48.

12. Welch, I. D., M. F. Cowan, F. Beier, and T. M. Underhill. 2009. The retinoic acid binding protein CRABP2 is increased in murine models of degenerative joint disease. *Arthritis Res Ther* 11:R14.
13. Yamashita, A., R. Krawetz, and D. E. Rancourt. 2009. Loss of discordant cells during micro-mass differentiation of embryonic stem cells into the chondrocyte lineage. *Cell Death Differ* 16:278-86.
14. Ye, M., K. M. Berry-Wynne, M. Asai-Coakwell, P. Sundaresan, T. Footz, C. R. French, M. Abitbol, V. C. Fleisch, N. Corbett, W. T. Allison, G. Drummond, M. A. Walter, T. M. Underhill, A. J. Waskiewicz, and O. J. Lehmann. Mutation of the bone morphogenetic protein GDF3 causes ocular and skeletal anomalies. *Hum Mol Genet* 19:287-98.